UNIVERSITÉ DE STRASBOURG

Habilitation à Diriger des Recherches

Année 2018

Chirurgie endoscopique fœtale : identification des candidats et développement des techniques

Nicolas SANANÈS

Membres du jury :

Pr Christian Debry (Université de Strasbourg)	Garant
Pr Alexandra Benachi (Université Paris Sud)	Rapporteur
Pr Patrick Rozenberg (Université Versailles Saint-Quentin)	Rapporteur
Pr Olivier Morel (Université de Lorraine)	Rapporteur
Pr Romain Favre (Université de Strasbourg)	Examinateur
Pr Bruno Langer (Université de Strasbourg)	Examinateur

"Qui augmente sa connaissance, augmente son ignorance"

Friedrich Schlegel

Remerciements

Plus je grandis, plus je me rends compte à quel point mes Maîtres sont grands. Je mesure la chance et le privilège de vous avoir comme modèles, comme guides. Et puis il faut dire qu'au fond, peu de choses sont plus belles et émouvantes que la transmission. Alors merci infiniment.

Professeur Christian Debry
Merci de m'avoir fait confiance depuis le début et de m'avoir accompagné à chaque étape avec enthousiasme. Votre extraordinaire passion de l'innovation donne des ailes.

Professeur Alexandra Benachi
Tu es un exemple d'excellence. Merci de me faire à nouveau l'honneur de te pencher sur mes travaux. J'espère que nous pourrons collaborer plus avant, ça serait pour moi une fantastique opportunité.

Professeur Patrick Rozenberg
Tu es un exemple de contre-exemple ! Merci de tout questionner avec malice et intelligence. "Les convictions sont des ennemis de la vérité plus dangereux encore que des mensonges".

Professeur Olivier Morel
Tu es un peu mon grand frère dans la région, un grand frère fin et pertinent. Merci d'avoir accepté de juger ce travail. J'espère que nous continuerons à construire ensemble une belle dynamique régionale.

Professeur Romain Favre
Cher Romain, c'est beaucoup grâce à toi que je suis le médecin que je suis aujourd'hui. Merci de m'avoir toujours accompagné avec concernement et sensibilité. Je tâcherai d'être à la hauteur.

Professeur Bruno Langer
Cher Bruno, merci d'avoir toujours été là, avec constance et bienveillance. Ton soutien a été déterminant et je t'en suis infiniment reconnaissant. C'est avec beaucoup d'émotion que je marcherai dans tes pas.

À Sarah, Axel, Romane et Charlie.

Pas besoin de chercher plus loin.

Introduction

La chirurgie endoscopique fœtale est actuellement en plein essor, en raison de l'amélioration de l'imagerie échographique et du diagnostic prénatal d'une part, et de l'amélioration des outils techniques, notamment la miniaturisation des fibres optiques, d'autre part. Cela est à mettre en regard des 2 questions fondamentales que pose toute chirurgie : Quelle est la bonne indication ? Quelle est la bonne technique ?

Ces deux questions sont évidemment intimement liées et nécessitent d'être abordées selon une approche à la fois clinique et technique. C'est pourquoi l'objectif de mon travail de recherche est de tenter d'apporter des réponses ou de contribuer à la recherche de solutions, concernant l'identification des candidats et le développement des techniques.

Ma thématique de recherche principale est la hernie de coupole diaphragmatique, avec en particulier le développement d'un ballonnet innovant pour l'occlusion trachéale fœtale. Cependant, je m'intéresse également à d'autres thématiques de chirurgie endoscopique fœtale, car les questions cliniques et techniques ont des problématiques communes, font appel aux mêmes outils diagnostiques, recouvrent des considérations techniques proches, et peuvent être étudiées sur des modèles animaux semblables.

Ce manuscrit est composé de quatre parties :

- Première partie : Curriculum Vitae avec notamment le cursus universitaire et hospitalier.

- Deuxième partie : Activité de soin, d'enseignement et de recherche.

- Troisième partie : Mémoire de recherche qui décrit la structuration de la recherche concernant la chirurgie endoscopique fœtale, et résume les travaux réalisés ainsi que les perspectives pour chaque thématique.

- Quatrième partie : Détail des articles et des travaux évoqués dans le mémoire de recherche.

- Première partie : Curriculum Vitae -

- Deuxième partie : Activité de soin, d'enseignement et de recherche -

- Troisième partie : Mémoire de recherche -

- Quatrième partie : Articles et travaux réalisés -

- Première partie -

CURRICULUM VITAE

1. CURRICULUM VITAE CONDENSÉ

Nicolas SANANÈS

N° d'inscription à l'Ordre : 67 / 9842
N° RPPS : 10100029544

Praticien Hospitalier au sein des HUS
Pôle de Gynécologie – Obstétrique
Hôpitaux Universitaires de Strasbourg
BP 426 – 67091 Strasbourg, cedex
Téléphone : 03 88 12 78 39

Attaché à l'unité INSERM UMR-S 1121
Inserm 1121 « Biomatériaux et Bioingénierie »
11, rue Humann
67000 Strasbourg
Téléphone : 03 68 85 33 78

ÉTAT CIVIL

Né le 27 avril 1978 à Boulogne Billancourt (92)
Nationalité française
Situation familiale : marié, trois enfants

ADRESSE PERSONNELLE

16, rue du Commandant Reibel
67000 Strasbourg
nicolas.sananes@gmail.com

CURSUS

- 2003 - 2008 : **Interne DES (spécialité gynécologie obstétrique)** / Hôpitaux Universitaires de Strasbourg
- 2008 - 2010 : **Chef de Clinique des Universités – Assistant des Hôpitaux** / Hôpitaux Universitaires de Strasbourg

DIPLOMES UNIVERSITAIRES

- 2011 : **Diplôme Universitaire de pédagogie médicale** (Université de Strasbourg)
- 2013 : **Master 2 Santé Publique et Environnement**, spécialité ERCE (Université de Lorraine)
- 2017 : **Thèse d'Université de « Physique – Chimie Physique »** (École Doctorale 182, Université de Strasbourg)

MOBILITÉ

- 01/07/2014 - 30/06/2015 : ***Research Fellowship*** au Texas Children's Fetal Center, Houston, Texas, USA

PRIX, DISTINCTIONS ET FINANCEMENTS

- 2014 : Lauréat d'une bourse de la commission franco-américaine « Fulbright » (36 000 dollars)
- 2015 : Appel à projet de Recherche Médicale Appliquée de la Fondation de l'Avenir (50 000 euros)
- 2016 : Prix "CASDEN" du jeune chercheur (20 000 euros)
- 2016 : Partenariats Régionaux d'Innovation, en collaboration avec *BS Medical Tech Industry* (120 000 euros)
- 2017 : Prix « La Javaness » à l'occasion du Hackaton du « Hacking Health Camp » de Strasbourg

CNU

- Pré-audition en 2017 en vue des fonctions de PU-PH : Avis très favorable

ACTIVITE DE SOIN

Mon activité clinique est centrée autour de l'obstétrique (surveillance des grossesses à risque, accouchements) et de la médecine fœtale (échographie de référence, échographie interventionnelle, thérapie anténatale).

Je suis le responsable du secteur de l'Obstétrique du Centre Médico-Chirurgical et Obstétrical (3246 accouchements en 2017) mais je travaille également sur le site de Hautepierre, ce qui permet d'assurer à la fois la cohésion des équipes et la cohérence des soins.

ENSEIGNEMENT

Mon activité d'enseignement est beaucoup axée sur la simulation, étant engagé dans ce type de pédagogie depuis ma participation au comité de pilotage de l'unité de simulation pédagogique de Strasbourg en 2009.
Je dispense également des cours magistraux, en particulier au sein des DIU que je coordonne.

- Responsable du projet de pôle sur l'enseignement du pôle de gynécologie obstétrique des H.U.S.
- Coordonnateur de l'enseignement de gynécologie obstétrique par simulation à l'UNISIMES
- Coordonnateur du DIU d'échographie en gynécologie et en obstétrique
- Coordonnateur du DIU de médecine fœtale

ENCADREMENT

- Thèses de doctorat : 7
- Masters : 3
- Mémoires de DU et DIU : 17
- Mémoires de fin d'études de sage-femme : 3

RECHERCHE

Mon activité de recherche porte essentiellement sur la thérapie anténatale : identification des candidats et développement des techniques. Nous travaillons en particulier sur la thématique de la hernie diaphragmatique congénitale avec 3 axes de recherche (fondamental, translationnel et clinique). Nous développons le modèle du singe pour l'endoscopie fœtale sur la plateforme *Simian Laboratory Europe* (SILABE) de l'Université de Strasbourg.

Nous avons mis au point un dispositif breveté (FR 1653954) pour la prise en charge prénatale de la hernie diaphragmatique. La phase actuelle d'industrialisation est assurée par la société *BS Medical Tech Industry*.

Thèmes de recherche en thérapie anténatale :

- Hernie de coupole diaphragmatique
- Grossesses monochoriales
- Uropathies obstructives

Principales collaborations :

- Hôpital Antoine Béclère, Université Paris Sud
- Université Catholique de Louvain, Belgique
- *Texas Children's Hospital, Houston, USA*

PUBLICATIONS

- Articles scientifiques référencés Pubmed : 68
- Score SIGAPS : 788 / h-index : 9
- Citations : 221 (201 en excluant auto-citations)
- Nombre moyen de citations par article : 2,3

COMMUNICATIONS

- Séance plénière sur invitation : 45
- Séance plénière sans invitation : 32
- Session libre : 30
- Communications affichées : 20

2. CURRICULUM VITAE

2.1. État Civil

Nicolas SANANÈS

Né le 27 avril 1978 à Boulogne – Billancourt (92)
Nationalité : Française
Situation familiale : marié, 3 enfants

Adresse personnelle :
16, rue du Commandant Reibel
67000 Strasbourg

email : nicolas.sananes@gmail.com
Téléphone : 06 31 93 76 08

2.2. Situation professionnelle actuelle

Praticien Hospitalier au sein du Pôle de Gynécologie – Obstétrique des Hôpitaux Universitaires de Strasbourg.

Pôle de Gynécologie – Obstétrique
Hôpitaux Universitaires de Strasbourg
BP 426 – 67091 Strasbourg, cedex
Téléphone : 03 69 55 33 97
Fax : 03 88 12 83 08
email : nicolas.sananes@chru-strasbourg.fr

Attaché à l'unité INSERM UMR-S 1121 « Biomatériaux et Bioingénierie ».

INSERM UMR-S 1121 « Biomatériaux et Bioingénierie »
11, rue Humann
67000 Strasbourg
Téléphone : 03 68 85 33 78

N° d'inscription au Conseil National de l'Ordre des Médecins : 67 / 9842

N° RPPS : 10100029544

2.3. Cursus universitaire et hospitalier

2.3.1. Cursus et fonctions exercées

2015-2019 : **Praticien Hospitalier** aux Hôpitaux Universitaires de Strasbourg.

2014-2015 : ***Fellowship*** d'un an au Texas Children's Fetal Center - *Baylor College of Medicine*, Houston, Texas, USA.

2010-2014 : **Praticien Hospitalier** aux Hôpitaux Universitaires de Strasbourg.

2008-2010 : **Chef de Clinique des Universités – Assistant des Hôpitaux** aux Hôpitaux Universitaires de Strasbourg.

2003-2008 : **Interne DES (spécialité gynécologie obstétrique)** aux Hôpitaux Universitaires de Strasbourg.

1999-2003 : **Deuxième cycle des études médicales et externat** à la faculté de Necker – Enfants Malades à Paris (Université René Descartes, Paris V).

1996-1999 : **Premier cycle des études médicales** à la faculté de Necker – Enfants Malades à Paris (Université René Descartes, Paris V).

2.3.2. Diplômes universitaires

2018 : **Habilitation à Diriger des Recherches** (Université de Strasbourg).
Chirurgie endoscopique fœtale : identification des candidats et développement des techniques.

2017 : **Thèse d'Université de « Physique – Chimie Physique »** (École Doctorale 182, Université de Strasbourg).
Thèse sur le développement d'un dispositif médical innovant pour la prise en charge prénatale de la hernie de coupole diaphragmatique.

2013 : **Diplôme Inter-Universitaire de médecine foetale** (Université de Strasbourg).
Mémoire sur la chirurgie endoscopique fœtale chez le singe, faisabilité et intérêt dans l'entraînement à l'occlusion trachéale.

2012 : **Master 2 Santé Publique et Environnement « Épidémiologie, Recherche Clinique, Évaluation »** (Université de Lorraine).
Mémoire sur la prédiction de l'accouchement prématuré, à partir des caractéristiques maternelles, des antécédents obstétricaux et de biomarqueurs, au premier trimestre de la grossesse.

2011 : **Master 1 Santé Publique et Environnement « Épidémiologie, Recherche Clinique, Évaluation »** (Université de Lorraine).

2011 : **Diplôme Universitaire de pédagogie médicale** (Université de Strasbourg).
Mémoire sur les aptitudes et les connaissances qu'évaluent les Épreuves Nationales Classantes (ECN) de médecine.

2008 : **Thèse de Doctorat en médecine, spécialité gynécologie-obstétrique** (Université de Strasbourg).
« Mise en place de la chirurgie robotique dans un service de gynécologie. Expérience du CHU de Strasbourg ».
Président de thèse : Pr Israël Nisand. Directeur de thèse : Dr Olivier Garbin.
Mention très honorable avec félicitations du jury.

2008 : **Diplôme d'Études Spécialisées de gynécologie-obstétrique** (Université de Strasbourg).
Mémoire sur l'intérêt de l'utilisation de Z-scores dans l'évaluation des courbes de références de biométries fœtales.

2008 : **Diplôme Inter-Universitaire de chirugie vaginale** (Université Claude Bernard, Lyon).

2007 : **Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale** (Université de Strasbourg).
Mémoire sur l'intérêt de la mesure de la clarté nucale au 1er trimestre de la grossesse dans le dépistage des cardiopathies.

2006 : **Diplôme Inter-Universitaire de Mécanique et Techniques Obstétricales** (Université de Franche-Comté, Besançon).
Mémoire sur la version du siège par acupuncture.

2005 : **Diplôme Universitaire de gynécologie médicale** (Université de Strasbourg).
Mémoire sur l'intérêt de la cytologie péritonéale dans la prise en charge des cancers de l'endomètre, avec l'analyse rétrospective d'une série de 150 cas.

2003 : **Reçu au concours de l'internat en médecine (inter-région nord), nommé interne de gynécologie-obstétrique au CHU de Strasbourg.**

2003 : **Maîtrise de sciences biologiques et médicales (MSBM).**

2003 : **Certificat de synthèse clinique et thérapeutique (CSCT).**

1999 : **Certificat MSBM de génétique** (responsable : Pr Munich).

2000 : **Certificat MSBM d'éthique médicale** (responsable : Pr Hervé).

2.3.3. Autres formations

2016 : **Formation aux Bonnes Pratiques Cliniques** du Groupement Interrégional de Recherche Clinique (GIRCI) de l'Est

2015 : **Enseignement du *Collaborative Institutional Training Initiative*** *: Basic/Refresher, Conflict of interest, Responsible conduct of Research, Basic course in laboratory animal welfare for investigators, Introduction to working with animals in biomedical research.*

2015 : **Enseignement de l'*Animal Care and Use in Research and Education (American Association for Laboratory Animal Science)*** *: 8th Edition of the guide for the care and use of laboratory animals, Animal welfare act regulations, Aseptic technique for rodent survival surgery, Common compliance issues, Health risks and safety procedures for working with nonhuman primates, Inhalation anesthesia systems for rodents, Introduction to nonhuman primates, Introduction to rabbits, Introduction to sheep and goats, mouse breeding colony management, Pain recognition and alleviation in laboratory animals, Post-procedure care of mice and rats in research: minimizing pain and distress, Public health service policy on human care and use of laboratory animals, Semiannual facility inspection, working with the laboratory mouse, working with the laboratory rat.*

2014 : **Enseignement au sein du *Baylor College of Medicine*** : *Fundamentals of Clinical Investigation Course, Responsible Conduct of Research.*

2008 : **Formation universitaire en Gynécologie Obstétrique Humanitaire** (Faculté Libre de Médecine de Lille et Faculté de Médecine de Nantes).

2007 : **Formation pratique de chirurgie endoscopique en gynécologie au Centre International de Chirurgie Endoscopique (CICE)**, Clermont-Ferrand.

2005-2008 : **Cours avancés à l'Institut de Recherche contre les Cancers de l'Appareil Digestif – European Institute of Telesurgery (IRCAD-EITS), Strasbourg** : chirurgie robotique, techniques avancées en endoscopie gynécologique, approche laparoscopique des cancers gynécologiques, techniques du traitement de l'endométriose sévère, sutures en laparoscopie.

2.3.4. Mobilité

***Research Fellowship* d'un an (01/07/14 - 30/06/15) au Texas Children's Fetal Center - *Baylor College of Medicine*, Houston, Texas, USA. Mentor : Pr Rodrigo Ruano.**

Ce centre de chirurgie fœtale de Houston était alors le seul centre aux États-Unis à avoir la pratique de l'occlusion trachéale fœtale par endoscopie en cas de hernie de coupole diaphragmatique congénitale. Cette mobilité a été l'occasion d'assister à ces interventions ainsi qu'à d'autres procédures de chirurgie fœtale non réalisées en France, telles que le traitement endoscopique *in utero* des myéloméningocèles.

Un protocole de recherche fondamentale sur la hernie de coupole diaphragmatique a également été élaboré et devrait donner lieu à une collaboration entre la *Mayo Clinic* à Rochester (où travaille désormais le Pr Rodrigo Ruano) et l'équipe du *Lucile Packard Children's Hospital* à Stanford.

Plusieurs articles médicaux, en particulier sur la hernie diaphragmatique mais aussi sur d'autres thématiques de chirurgie fœtale, ont été rédigés en collaboration avec l'équipe de Houston à l'occasion de cette mobilité.

Enfin, cette mobilité a été l'occasion de suivre des enseignements de recherche cliniques et fondamentale au travers de différents programmes : *Baylor College of Medicine, Collaborative Institutional Training Initiative, Animal Care and Use in Research and Education (American Association for Laboratory Animal Science).*

2.3.5. Thèse d'Université

Titre : **Développement d'un dispositif médical innovant pour la prise en charge prénatale de la hernie de coupole diaphragmatique.**

École doctorale ED 182 « Physique – Chimie Physique » de l'Université de Strasbourg.

Directeur de thèse : Pr Christian Debry (Université de Strasbourg).
Co-directeur : Pr Rodrigo Ruano (Mayo Clinic, Rochester, États-Unis).
Rapporteurs : Pr Alexandra Benachi (Université Paris Sud).
Pr Jan Deprest (Katholieke Universiteit, Louvain, Belgique).
Jury : Pr Romain Favre, Pr François Becmeur et Pr Pierre Kuhn (Université de Strasbourg).

Ce travail de thèse a permis la mise au point d'un nouveau ballonnet breveté pour l'occlusion trachéale fœtale par endoscopie. Les tests in vitro sont concluants, ainsi que des travaux préliminaires chez le singe.

2.4. Activités et responsabilités collectives

2.4.1. Sociétés savantes

Membre du Collège National des Gynécologues et Obstétriciens Français (CNGOF).
Implication au sein du CNGOF :

- Membre de la commission échographie du CNGOF depuis 2017.
- Co-rédacteur des Recommandations pour la Pratique Clinique (RPC) sur l'Herpès et la grossesse en 2017.
- Co-rédacteur des Recommandations pour la Pratique Clinique (RPC) sur l'accouchement du siège en 2019.

Membre du Collège des Gynécologues-Obstétriciens d'Alsace (CGOA) depuis 2008.

Membre du Club Francophone de Médecine Fœtale depuis 2012.

Membre de l'*International Fetal Medicine and Surgery Society* (IFMSS) depuis 2013.

2.4.2. Réseaux

Membre et coordonnateur régional du réseau Gynéco-Obstétrical des Centres d'Investigation Clinique (GO-CIC) depuis 2013.

Membre du Groupe de Recherche en Obstétrique et Gynécologie (GROG) depuis 2013.

Membre du comité scientifique du Centre de Référence des hernies de coupole diaphragmatique depuis 2016.

Membre du "Club de Médecine Foetale" depuis 2016.

2.4.3. Vie associative

GALCE (Groupe Alsacien de Lutte Contre les Excisions et les mutilations sexuelles féminines) : secrétaire depuis 2009.

APSIM (Association de Promotion et de développement de la Simulation dans le domaine de la santé) : membre fondateur, depuis 2016.

RISE (Association de promotion de la recherche dans le domaine de la santé environnementale) : membre fondateur, depuis 2017.

2.5. Distinctions honorifiques, prix et financements

Prix « La Javaness » à l'occasion du Hackaton du « Hacking Health Camp » de Strasbourg en 2017. Le projet présenté était celui d'un réseau social intra-hospitalier, accessible sur smartphone et sur le web, ayant pour but d'améliorer la communication et la cohésion d'équipe au sein d'un pôle hospitalier. Cette application « Rhésus » est actuellement en cours de développement, en collaboration avec Alcatel Lucent Entreprise, La Javaness et les Hôpitaux Universitaires de Strasbourg.

Financement des premières étapes de l'industrialisation d'un ballonnet innovant pour la prise en charge anténatale de la hernie de coupole diaphragmatique par un dispositif Partenariats Régionaux d'Innovation (PRI) 2016 (120 000 euros), en collaboration avec la société *BS Medical Tech Industry*.

Prix « CASDEN » du jeune chercheur en 2016 (20 000 euros). Ce prix de la banque populaire a été remis pour encourager les travaux en cours concernant le développement d'un ballonnet innovant pour la prise en charge anténatale de la hernie de coupole diaphragmatique.

Financement sur l'appel à projet de Recherche Médicale Appliquée 2015 de la Fondation de l'Avenir, pour la réalisation d'un prototype de ballonnet innovant pour la prise en charge anténatale de la hernie de coupole diaphragmatique (51 500 euros).

Lauréat d'une bourse de la commission franco-américaine « Fulbright » en 2014 pour soutenir le travail de recherche à l'occasion de la mobilité au *Texas Children's Fetal Center* de Houston (36 000 dollars).

- Deuxième partie -

ACTIVITÉ DE SOIN, DE RECHERCHE ET D'ENSEIGNEMENT

3. ACTIVITÉ DE SOIN

3.1. Description du pôle de gynécologie - obstétrique du CHU de Strasbourg

3.1.1. Activités et structures de soin

Le pôle de Gynécologie – Obstétrique regroupe deux structures de soin : l'Hôpital de Hautepierre à Strasbourg et le Centre Médico-Chirurgical et Obstétrical (CMCO) à Schiltigheim. Les principales activités sont les suivantes : Obstétrique, Médecine Fœtale, Orthogénie, Procréation Médicalement Assistée, Gynécologie, Chirurgie Gynécologique et Sénologique.

Seules les activités du pôle en termes d'obstétrique et de médecine fœtale seront détaillées.

La maternité de l'Hôpital de Hautepierre est de niveau III, tandis que celle du CMCO est de niveau IIb. Les données ne seront pas différenciées les deux sites. Le pôle de Gynécologie Obstétrique des Hôpitaux Universitaires de Strasbourg accueille la majorité des grossesses à haut-risque de la région Alsace, ainsi que certaines patientes des départements voisins. Il existe une étroite collaboration avec les services de réanimation et de chirurgie pédiatrique au sein du CHU. Le CHU héberge également et participe à la coordination du réseau périnatal "Naître en Alsace".

Le Centre Pluridisciplinaire de Diagnostic Prénatal (CPDPN) de Strasbourg est organisé autour d'une réunion hebdomadaire en visioconférence avec les différents centres de médecine fœtale de la région. Un total de 908 dossiers ont été traités en 2017 et concernaient majoritairement des patientes en provenance de la région (78%) mais également des régions voisines (19%) et de l'étranger (3%).

Le département de médecine fœtale du pôle est également l'un des 12 centres de compétence pour la prise en charge des syndromes transfuseur-transfusé.

Activité de soin du pôle (année 2017) - <u>Obstétrique</u>

Accouchements (total)	6 200
Césariennes	1 169
Extractions instrumentales	651
Consultations de surveillance intensive de grossesse	10 068
Hospitalisations pendant la grossesse	1 591

Activité de soin du pôle (année 2017) - Médecine fœtale

Echographies de dépistage	11 139
Echographie de référence	8 362
Amniocentèses et choriocentèses	448
Cordocentèses et transfusions *in utero*	42
Poses de drains	8
Foetoscopies (lasers)	28
Cystoscopies	2
Interruptions médicales de grossesse	157
Foeticides	25

Structures de soin du pôle

Salles d'accouchement	22
Lits d'obstétrique	96
Salles d'échographie	8

3.1.2. Effectifs du service

Le pôle de Gynécologie – Obstétrique dispose de 5 postes de PU-PH :

- Philippe Déruelle : obstétrique
- Bruno Langer : obstétrique
- Jean-Jacques Baldauf : chirurgie gynécologique
- Chérif Youssef Akladios : chirurgie gynécologique
- Carole Mathelin : pathologie mammaire
- (Arnaud Wattiez - en disponibilité : chirurgie gynécologique)

S'y ajoute le poste de Professeur Associé de Romain Favre : médecine fœtale.

Au total au sein du pôle, il y a 5 PU-PH, 1 PHU, 5 CCA, 18 PH, 2 assistants spécialistes, 27 internes de gynécologie - obstétrique, 3 internes de gynécologie médicale et 8 internes de médecine générale. Il y a également chaque mois environ 20 externes réalisant un stage intégré.

Les activités d'obstétrique et de médecine fœtale sont majoritairement assurées par 2 PU-PH, 7 PH, 3 CCA et 2 assistants spécialistes.

3.2. Activités et responsabilités personnelles

3.2.1. Activités personnelles

Activité clinique centrée autour de l'obstétrique et de la médecine fœtale :

- Animation des staffs d'obstétrique quotidiens.
- Gestion de la salle d'accouchement et des consultations de surveillance intensive de grossesse : une à deux journée(s) par semaine en moyenne.
- Animation / participation aux réunions hebdomadaires du CPDPN.
- Consultations d'échographie interventionnelle et de référence : 3 vacations par semaine en moyenne.
- Gestes de thérapeutique fœtale en fonction de l'activité.
- Participation à la continuité des soins : 64 gardes et 18 astreintes opérationnelles au cours de l'année 2016.

3.2.2. Responsabilité clinique personnelle

Responsable de l'obstétrique du Centre Médico-Chirurgical et Obstétrical (CMCO) :

- Salle d'accouchement (UF 9564)
- Obstétrique - Hospitalisation complète (UF 9562)
- Surveillance intensive de grossesse (UF 9569)
- Préparation à la naissance (UF 9565)

4. ACTIVITÉ D'ENSEIGNEMENT

4.1. Formation personnelle, responsabilités et publications

4.1.1. Formation personnelle (synthèse)

Diplôme Universitaire de pédagogie médicale (Université de Strasbourg) en 2010.
Mémoire sur les aptitudes et les connaissances qu'évaluent les Épreuves Nationales Classantes (ECN) de médecine.

4.1.2. Responsabilités

2011-2018 Responsable du projet de pôle sur l'enseignement du pôle de Gynécologie Obstétrique des Hôpitaux Universitaires de Strasbourg

2012-2018 Coordonnateur de l'enseignement de Gynécologie Obstétrique par simulation au sein de l'Unité de Simulation Européenne en Santé (UNISIMES) de l'Université de Strasbourg.

2009-2012 Membre du comité de pilotage pour la création d'une unité de simulation pédagogique au sein de l'Université de Strasbourg.

2015-2018 Coordonnateur du DIU d'échographie en gynécologie et en obstétrique (Université de Strasbourg).

2015-2018 Coordonnateur du DIU de Médecine Fœtale (Université de Strasbourg).

4.1.3. Publications pédagogiques (synthèse)

Andres E, **Sananes N**, Langer B, Pottecher T. [National exam for the validation of the second medicine cycle in France: What does this examination evaluates?]. Presse Med. 2012;41(6 Pt 1):e245-9.

N. Sananès, E. Andres. L'examen national classant : quelles clés pour les étudiants et quelles pistes de réfllexion pour les enseignants ? Médecine thérapeutique 2012 ; 18(1) : 36-9.

N. Sananès, B. Langer, M. Patris, T. Pottecher, E. Andres. E. Andres. Analyse de la validité de contenu et de la qualité formelle des épreuves classantes nationales administrées en France de 2004 à 2011. Pédagogie Médicale 2013 ; 14(4) : 1-13.

N. Sananès, E. Andres. L'examen national classant : quelles clés pour les étudiants et quelles pistes de réflexion pour les enseignants ? Médecine thérapeutique 2012 ; 18(1) : 36-9.

4.2. Activité de formation initiale

4.2.1. Aperçu de l'activité pédagogique de 2008 à 2018

S. I. A. P. S.

Score Individuel d'Aptitudes Pédagogiques en Santé

Conférence des Doyens des facultés de Médecine — SANTÉ FORMATION RECHERCHE

Nom :	**SANANES**	**Faculté de Médecine de :**	**Strasbourg**
Prénom :	**NICOLAS**	**Candidature à :**	**PU-PH**
Naissance :	**27/04/1978**		
Section :	**5403 - Gynécologie-obstétrique ; gynécologie Médicale (2 options)**		

Version 4.2 - 09/2015
D. GOSSET

[1] CHARGES D'ENSEIGNEMENT

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Coefficient	TOTAL
[1a] PACES : nb d'heures de cours magistraux	0	0	0	0	0	0	0	0	0	0	2	0
[1b] MED2 à MED6 : nb d'heures de cours magistraux	0	0	0	0	0	0	0	0	0	0	1	0
[1c] PACES à MED6 : nb d'heures d'ED	18	18	17	17	0	0	0	0	0	0	1	70
[1d] PACES à MED6 : nb d'heures de TP	0	0	0	0	0	0	0	0	0	0	0,5	0
[1e] MED2 à MED6 : nb d'heures en centre de simulation	0	0	0	0	0	47	0	61	68	28	1	204
[1f] Conférences d'internat au sein de la Faculté de médecine : nombre de conférences	0	0	3	0	0	0	0	0	0	0	2	6
[1g] 3° cycle et autres formations médicales (DES, DESC, DU, DIU) : nb d'heures de cours	0	2	2	2	1	19	0	20	21	22	1	89
[1h] Masters, thèses d'université : nb d'heures de cours	0	0	0	0	0	0	0	0	0	0	1	0
[1i] Formations paramédicales facultaires : nb d'heures de cours	0	0	0	0	0	0	0	0	0	0	1	0
[1j] Maïeutique : nb d'heures de cours	0	5	5	5	5	0	0	4	4	0	0,5	14
[1k] Formations paramédicales extra-facultaires : nb d'heures de cours	0	0	0	0	0	0	0	0	0	0	0,5	0
											Sous-total [1] CHARGES D'ENSEIGNEMENT	383

[2] FORMATION PEDAGOGIQUE

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Coefficient	TOTAL
[2a] Possession d'un DU ou DIU de pédagogie, ou équivalent (1=oui)	0	1	0	0	0	0	0	0		0	20	20
[2b] Nombre de journées de formation en pédagogie	0	0	0	0	0	0	0	0		0	1	0
[2c] Titulaire de la certification SIDES (1=oui)	0	0	0	0	0	0	0	0		1	3	3
											Sous-total [2] FORMATION PEDAGOGIQUE	23

[3] ACTIVITÉS PÉDAGOGIQUES et SERVICES RENDUS

	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15	2015-16	2016-17	2017-18	Coefficient	TOTAL
[3a] Production pédagogique numérique, TICE : nb d'heures de cours (hors mise à jour)	0	0	0	0	0	0	0	0	0	0	3	0
[3b] Publications en pédagogie ou didactique (nombre)	0	0		2	1	0	0	0	0	0	2	6
[3c] Participation à la rédaction d'un polycopié national ou livre (nombre, hors mise à jour)	0	0	0	0	0	0	0	0	0	0	10	0
[3d] Participation à une commission pédagogique de la Faculté (nombre)	0	0	0	0	0	0	0	0	0	0	10	0
[3e] Rédaction de sujets d'examen de fin d'année (1 par dossier ou pour 15 QCM)	1	1	1	1	0	0	0	0		0	2	8
[3f] Responsabilité d'UE 1er ou 2ème cycle études médicales (1 par UE)	0	0	0	0	0	0	0	0	0	0	10	0
[3g] Responsabilité d'autre UE (1 par UE)	0	0	0	0	0	0	0	2	2	2	5	30
[3h] Contributions ECNi validées (Faculté, SIDES) (1 par dossier ou pour 15 QRM)	0	0	0	0	0	0	0	0	0	2	2	4
[3i] Nombre de copies corrigées	0	0	0	0	0	0	0	0	0	0	0,02	0
											Sous-total [3] ACTIVITÉS PÉDAGOGIQUES et SERVICES RENDUS	48
									0			
											Score SIAPS total	**454**

4.2.2. Détail de l'activité d'enseignement universitaire en 2017-2018

Total de 50 heures de cours : 16h de Cours Magistral (CM) et 34h de TD/TP.

Apprenants	Intitulé de la formation	Date	Type de formation	Volume horaire
Étudiants du DIU d'échographie en gynécologie et en obstétrique (Université de Strasbourg)	Travaux dirigés d'échographie sur bandes vidéo	22/11/17	TD	1h30
	Dépistage prénatal non invasif	24/11/17	CM	1h00
	Les prélèvements sur le fœtus	06/02/17	CM	1h30
	Travaux dirigés d'échographie sur bandes vidéo	06/02/18	TD	1h30
	Doppler ombilical, aortique et cérébral	07/02/18	CM	2h00
Étudiants du DIU de médecine fœtale (Université de Strasbourg)	Dépistage prénatal non invasif	27/10/17	CM	1h00
	Pathologies discordantes	27/10/17	CM	1h30
	Gestes invasifs	18/04/18	CM	1h00
	Endoscopie fœtale	18/04/18	CM	2h00
	Retard de croissance intra-utérin	18/04/18	CM	1h00
Étudiants du DU de stérilité (Université de Strasbourg)	TP sur simulateur d'échographie	11/01/18	TP	1h30
	TP sur simulateur d'échographie	11/01/18	TP	1h30
	Suivi des grossesses gémellaires	21/02/18	CM	1h00
Internes DES de gynécologie obstétrique	Dépistage des anomalies chromosomiques	12/01/18	CM	2h00
	TP sur simulateur d'échographie	13/02/18	TP	2h00
	Annonce, communication, savoir-être	13/02/18	TP	2h00
	TP sur simulateur d'échographie	27/03/18	TP	2h00
	Annonce, communication, savoir-être	27/03/18	TP	2h00
	Prise en charge du terme dépassé	06/04/18	CM	2h00
	TP sur simulateur d'échographie	17/04/18	TP	2h00
	Annonce, communication, savoir-être	17/04/18	TP	2h00
	TP sur simulateur d'échographie	19/06/18	TP	2h00
	Annonce, communication, savoir-être	19/06/18	TP	2h00
Internes de médecine générale	TP sur mannequin. Examen gynécologique et sénologique	02/08/18	TP	2h00
	TP sur simulateur d'échographie	02/08/18	TP	2h00
Externes DCEM3	TP sur mannequin. Examen gynécologique et sénologique	06/06/18	TP	2h00
	TP sur mannequin. Examen gynécologique et sénologique	06/06/18	TP	2h00
	TP sur mannequin. Examen gynécologique et sénologique	06/06/18	TP	2h00
	TP sur mannequin. Examen gynécologique et sénologique	06/06/18	TP	2h00

4.2.3. Formation initiale des étudiants en médecine (jusqu'à 2017)

Années	Type et sujet de l'enseignement	Volume horaire
2013-2017	Travaux pratiques sur mannequins : apprentissage de l'examen gynécologique et sénologique	96 h
2008-2012	Travaux dirigés : stérilité du couple, procréation médicalement assistée, aménorrhée, contraception, interruption volontaire de grossesse, problèmes posés par les maladies génétiques (syndrome de l'X fragile, trisomie 21, mucoviscidose)	70 h
2010-2011	Séminaires de méthodologie et de préparation aux Épreuves Nationales Classantes	3 h
2007	Réalisation de la partie gynécologique d'un CD d'imagerie de préparation aux Épreuves Nationales Classantes.	NA
2004-2007	Conférences de préparation aux Épreuves Nationales Classantes (gynécologie – obstétrique, urologie et méthodologie générale)	60 h

4.2.4. Formation initiale des internes en médecine (jusqu'à 2017)

Années	Type et sujet de l'enseignement	Volume horaire
2013-2017	Travaux pratiques sur mannequins : apprentissage de l'examen gynécologique et sénologique	16 h
2013-2017	Travaux pratiques sur mannequins : apprentissage des extractions instrumentales et des manœuvres obstétricales	28 h
2015-2017	Travaux pratiques sur simulateur d'échographie : apprentissage de l'échographie gynécologique et obstétricale de base	16 h
2015-2017	Mise en situation sur simulateur haute-fidélité : apprentissage des urgences obstétricales	12 h
2016-2017	Travaux pratiques sur simulateur : apprentissage des prélèvements invasifs en médecine foetale	8 h

4.2.5. Enseignement dans le cadre du DES de gynécologie Obstétrique (jusqu'à 2017)

Années	Type et sujet de l'enseignement	Volume horaire
2009-2017	Cours sur le dépassement de terme, le retard de croissance intra-utérin, le dépistage des aneuploïdies	10 h

4.2.6. Diplômes Universitaires et Inter-Universitaires (jusqu'à 2017)

Années	Type et sujet de l'enseignement	Volume horaire
2013-2017	Cours dans le cadre du DIU d'échographie en gynécologie et en obstétrique (Université de Strasbourg) : dépistage des aneuploïdies, dépistage prénatal non invasif, maladie trophoblastique, dopplers, les prélèvements foetaux	26 h
2013-2017	Travaux dirigés sur vidéos dans le cadre du DIU d'échographie en gynécologie et en obstétrique (Université de Strasbourg)	8 h
2013-2017	Cours dans le cadre du DU de médecine fœtale (Université de Strasbourg) : dépistage prénatal non invasif, prélèvements fœtaux, chirurgie fœtale, surveillance échographique des grossesses gémellaires, RCIU sélectif, TAPS, TRAP, Interruption sélective de grossesse, réduction embryonnaire	22 h
2017	Cours dans le cadre du DU de Biologie, Clinique et Thérapeutique en stérilité (Université de Strasbourg) : surveillance, risques et pathologies obstétricales des grossesses multiples	1 h
2003-2004	Cours dans le cadre du DU de gynécologie médicale (Université de Strasbourg) : pathologies bénignes de l'endomètre	2 h

4.2.7. Enseignement à l'École de sage-femmes (jusqu'à 2017)

Années	Type et sujet de l'enseignement	Volume horaire
2009-2013	Cours sur la contraception, les douleurs pelviennes, les tumeurs bénignes de l'ovaire	20 h
2015-2017	Travaux pratiques sur mannequins : apprentissage des extractions instrumentales et des manœuvres obstétricales	8 h

4.3. Encadrement

4.3.1. Thèses de doctorat

1. « Les hernies de coupole diaphragmatique droites sont-elles vraiment de mauvais pronostic ». Anne Pinton. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2017.

2. « Césarienne à 38 semaines d'aménorrhée après corticothérapie contre césarienne à 39 semaines : résultats de l'étude César n Co ». Antoine Koch. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2016.

3. « Intérêt de l'échographie périnéale pour prédire la difficulté d'extraction instrumentale ». Sidi Kasbaoui. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2016.

4. « Evaluation de l'intérêt de la procalcitonine pour le diagnostic de chorioamniotite dans les ruptures prématurées des membranes. Etude prospective observationnelle ». Valentine Dubost. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2015.

5. « Aspects diagnostiques et prise en charge prénatale du TAPS (Twin Anemia Polycythemia Sequence ». Marine Veujoz. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2014.

6. « Extractions instrumentales : peut-on diminuer leur taux ? ». Aline Host. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2013.

7. « Hématome rétro-placentaire : épidémiologie, diagnostic, prise en charge et évolution obstétricale ». Thomas Boisramé. Thèse pour l'obtention du diplôme de Docteur en Médecine (Université Louis Pasteur, Strasbourg), 2013.

4.3.2. Masters

1. Master 2 de Santé Publique et Environnement « Épidémiologie, Recherche Clinique, Évaluation » (Université de Lorraine), 2017-2018. Floriane Jochum. Mémoire intitulé « score prédictif de la réussite d'un déclenchement ».

2. Master 2 de Santé Publique et Environnement « intervention en promotion de la santé» (Université de Lorraine), 2017-2019. Virginie Hamann. Mémoire sur l'éducation thérapeutique en cas de diabète gestationnel.

3. Master 2 de Santé Publique – Recherche (Université Paris Sud), 2015. Anne Pinton. Mémoire intitulé « Analyse prédictive de l'accouchement prématuré en situation de menace d'accouchement prématuré »

4.3.3. Mémoires de Diplômes Universitaires

1. « Évaluation à long terme du développement neurologique en cas de syndrome transfuseur-transusé traité par laser ». Victor Gabriele. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2016.

2. « Twin Reversed Arterial Perfusion Sequence. Aspects diagnostiques, pronostiques et thérapeutiques ». Hélène Brossat. Mémoire pour l'obtention du Diplôme Universitaire de médecine foetale (Université Louis Pasteur, Strasbourg), 2016.

3. « Diagnostic et traitement en cas de retard de croissance intra-utérin sélectif ». Antoine Koch. Mémoire pour l'obtention du Diplôme Universitaire de médecine foetale (Université Louis Pasteur, Strasbourg), 2015.

4. « Malformations cloacales : quels examens pour quel diagnostic ? ». Anne Pinton. Mémoire pour l'obtention du Diplôme Universitaire de médecine foetale (Université Louis Pasteur, Strasbourg), 2015.

5. « Diagnostic prénatal et pronostic des mégalourètres. A propos de 3 cas et revue de la littérature. » François Stoll. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2014.

6. « Pronostic des hernies diaphragmatiques droites. A propos de notre expérience au CHU de Hautepierre. » Valentine Dubost. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2014.

7. « Étude de cohorte évaluant l'intérêt de l'échographie transpérinéale dans le diagnostic d'engagement de la présentation avant extraction. Sidi Kasbaoui. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2014.

8. « Malformations cloacales et examens pour le diagnostic anténatal ». Anne Pinton. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2014.

9. « Association entre mesure échographique du col utérin au premier trimestre, caractéristiques maternelles et accouchement prématuré spontané. Étude d'une cohorte de 2 319 patiente et revue de la littérature ». Elodie Schuller. Mémoire pour

l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2012.

10. « Développement neurologique à long terme après laser pour syndrome transfuseur-transfusé. Notre expérience strasbourgeoise ». Stéphanie Andrès. Mémoire pour l'obtention du Diplôme Inter-Universitaire d'échographie gynécologique et obstétricale (Université Louis Pasteur, Strasbourg), 2012.

11. « Pertinence des critères diagnostiques de chorio-amniotitte dans les cas de rupture prématurée des membranes ». Valentine Dubost. Mémoire pour l'obtention du Diplôme Universitaire de Mécanique et Techniques Obstétricales (Université Joseph Fourier, Grenoble 1), 2011.

12. « Hématome rétro-placentaire : diagnostic, prise en charge et évolution obstétricale. Étude rétrospective de 100 cas au CHU de Strasbourg ». Thomas Boisramé. Mémoire pour l'obtention du Diplôme Inter-Universitaire de Mécanique et Techniques Obstétricales (Université de Franche-Comté, Besançon), 2011.

13. « Étude avant-après de la prise en charge du dépassement de terme : déclenchement ou expectative ». Aline Host. Mémoire pour l'obtention du Diplôme d'Études Spécialisées en gynécologie-obstétrique (Université Louis Pasteur, Strasbourg), 2011.

14. « Prise en charge des volumineux kystes ovariens ». Thomas Boisramé. Mémoire pour l'obtention du Diplôme Universitaire de chirurgie gynécologique (Université Louis Pasteur, Strasbourg), 2010.

15. « Intérêt du repos dans la prise en charge des menaces d'accouchement prématuré ». Elise Knopf. Mémoire de fin d'études (École de Sages-Femmes, Strasbourg), 2010.

16. « La pose d'un stérilet immédiatement après IVG médicamenteuse : un moyen de diminuer les récidives ? ». Stéphanie Andres. Mémoire pour l'obtention du Diplôme Universitaire de gynécologie médicale (Université Louis Pasteur, Strasbourg), 2009.

17. « Extractions instrumentales : résultats d'un audit interne ». Aline Felder. Mémoire pour l'obtention du Diplôme Inter-Universitaire de Mécanique et Techniques Obstétricales (Université de Franche-Comté, Besançon), 2009.

4.3.4. Mémoires de fin d'études de sage-femme

1. « Transferts maternels du CMCO vers l'Hôpital de Hautepierre pour hémorragie sévère du post-partum entre 2011 et 2015. Analyses des pratiques ». Aurélia Zitvogel. Mémoire de fin d'études (École de Sages-Femmes, Strasbourg), 2017.

2. « La contraception par dispositif intra-utérin chez la femme nullipare : revue de la littérature et enquête de pratiques en Alsace ». Muriel Geschwind. Mémoire de fin d'études (École de Sages-Femmes, Strasbourg), 2011.

3. « Le drainage placentaire lors de la troisième phase du travail ». Sophie Hohl. Mémoire de fin d'études (École de Sages-Femmes, Strasbourg), 2011.

4.4. Enseignement « grand public »

4.4.1. Prévention dans les Collèges et Lycées

2008-2014 Interventions dans les Collèges et les Lycées pour donner des informations en matière de contraception et de sexualité, avec l'association « Info Ado ».

2008-2014 Réponses aux questions sur le site web dédié de l'association « Info Ado ».

4.4.2. Vulgarisation médicale

Rédaction d'un livre : « Plus de 100 questions sur la grossesse et l'accouchement ». Israël Nisand, Nicolas Sananès, Adrien Gaudineau. MA Éditions, Paris 2016.

Expert sur le site web « Laurence Pernoud » depuis 2017 : laurencepernoud.com.

Participation au développement d'une application de vulgarisation médicale disponible sur smartphone, centrée sur la gynécologie, la grossesse et la pédiatrie (en cours).

4.5. Projet d'enseignement

4.5.1. Introduction

La simulation en santé est devenue un outil pédagogique incontournable, tant dans l'enseignement des gestes techniques, que dans l'apprentissage du savoir-être. Je me suis engagé depuis 2009 dans cette voie, en tant que membre du comité de pilotage pour la création d'une unité de simulation pédagogique au sein de l'Université de Strasbourg. Je coordonne aujourd'hui l'enseignement de gynécologie - obstétrique au sein de l'Unité de Simulation Européenne en Santé (UNISIMES). Mon projet pédagogique s'articule principalement autour de la simulation en santé.

L'enseignement par simulation s'avère particulièrement pertinent en gynécologie - obstétrique car c'est une discipline dont la pratique implique la connaissance de nombreux gestes techniques : examen gynécologique et sénologique, mécanique de l'accouchement, chirurgie gynécologique, échographie gynécologique et obstétricale, diagnostic prénatal. L'UNISIMES dispose de simulateurs de tâches pour l'apprentissage de chacun de ces gestes techniques : bassins gynécologiques, simulateurs pour examen du sein, bassin d'accouchement, simulateurs d'entraînement à la laparoscopie, simulateurs d'échographie (génératif et interpolatif).

De plus, l'obstétrique peut donner lieu à des situations d'urgences extrêmes qui nécessitent des facultés de gestion de crise et une collaboration multidisciplinaire optimale. L'UNISIMES dispose d'un mannequin d'accouchement haute-fidélité permettant la pratique de simulation pleine échelle, en impliquant dans une seule mise en situation plusieurs professionnels et étudiants : sage-femmes, gynécologues - obstétriciens, anesthésistes - réanimateurs et pédiatres néonatologues.

Enfin, en urgence ou non, la dimension humaine est tout à fait centrale en gynécologie - obstétrique : annonce d'une maladie grave, urgence obstétricale, diagnostic prénatal. Les jeux de rôles tels que pratiqués à l'UNISIMES paraissent particulièrement adaptés à cet enseignement.

Au final, l'enseignement par simulation offre un apprentissage pertinent des nombreuses facettes de la gynécologie - obstétrique et ce, quel que soit le niveau de formation. L'UNISIMES dispose à la fois des ressources techniques et des ressources humaines permettant une approche multidisciplinaire inhérente au travail en salle d'accouchement. Mon objectif est de continuer à développer l'enseignement par simulation, quantitativement et qualitativement, en formation initiale et en formation continue.

Mon projet pédagogique inclut par ailleurs une modernisation des DIU que je coordonne, en intégrant la simulation mais également des nouveaux outils informatiques tels que le Système Informatique Distribué d'Évaluation en Santé (SIDES). Enfin, un de mes objectifs est de créer des vocations en transmettant la passion de l'enseignement

4.5.2. Formation initiale

Nous avons mis en place une formation initiale de l'examen gynécologique et sénologique, sur bassins et simulateurs de palpation mammaire, à destination de tous les étudiants de deuxième cycle en stage en gynécologie - obstétrique.
Notre projet est de dispenser également cette formation de façon systématique aux étudiants "check-list" et d'acquérir un simulateur avancé de toucher pelvien permettant une expérience plus réaliste et un repérage dans l'espace en trois dimensions. Ce type de formation paraît tout à fait indispensable, après les scandales récents qui ont éclaté à juste titre, concernant le non consentement des patientes qui étaient soumises à leur insu à des examens répétés par des étudiants, à l'occasion d'une anesthésie générale.

Tous les nouveaux internes de spécialité et de médecine générale bénéficient également de cette formation lors de leurs premiers jours de stage en gynécologie obstétrique. De plus, ils bénéficient d'une formation initiale à l'échographie gynécologique et obstétricale sur simulateur, avec l'utilisation en particulier de sondes endovaginales.

Nous avons mis en place pour cette année universitaire, pour chacune des cinq années d'internat, un programme d'enseignement par simulation de deux jours adapté au niveau et incluant chaque fois différents outils pédagogiques : apprentissage des extractions instrumentales et manœuvres obstétricales sur bassin d'accouchement, séances de simulation pleine échelle sur mannequin haute-fidélité avec équipe pluridisciplinaire, enseignement de la chirurgie sur simulateurs de laparoscopie, apprentissage de l'échographie sur simulateurs d'échographie, enseignement de la communication et du savoir-être par jeux de rôles.
Notre projet est de renforcer cette offre en proposants encore plus de jours de formation. Aussi, nous pensons développer la combinaison de plusieurs outils de simulation pédagogique, comme par exemple le diagnostic d'une malformation fœtale sur simulateur d'échographie et l'annonce à la patiente avec la gestion du doute diagnostique.

4.5.3. Formation continue

Je participe en tant que formateur à un cycle de formations dans les différentes maternités du réseau "Naître en Alsace", et qui consiste à faire de la simulation pleine échelle avec les différentes équipes, dans leur propre environnement (simulation "in situ"). Cette approche offre la possibilité de mises en situations encore plus réaliste et permet d'aborder des questions très pratiques en termes d'organisation, de gestion d'équipe, ou encore de matériel.
Notre projet est de proposer ce type de formation très enrichissante à tous les professionnels du pôle de gynécologie obstétrique des Hôpitaux Universitaires de Strasbourg. Le mannequin haute-fidélité appartenant au réseau "Naître en Alsace" pourrait être utilisé à cet objet.

4.5.4. Diplômes Inter-Universitaires

Le DIU d'échographie en gynécologie et en obstétrique consistait jusqu'à présent en des cours théoriques et des séances de travaux dirigés sur bandes vidéo. L'examen théorique consiste en des dossiers rédactionnels et l'examen pratique en une échographie obstétricale sur une patiente enceinte volontaire.

Depuis cette année universitaire, nous avons mis en place une séance d'autoformation sur simulateur d'échographie. Cela permet de s'entraîner à la pratique de l'échographie gynécologique et obstétricale, par voie abdominale et endovaginale. Notre projet est de rendre cette auto-formation obligatoire l'année universitaire prochaine et de mettre en place, en plus de l'examen pratique sur patiente enceinte volontaire, un examen pratique sur simulateur d'échographie. Cela permet d'évaluer des situations cliniques différentes mais aussi d'évaluer par exemple la pratique de l'échographie par voie endovaginale.

Concernant l'examen écrit, nous mettons en place cette année une épreuve blanche sur la plateforme SIDES. Notre projet est, qu'après ce test, l'examen du DIU d'échographie en gynécologie et en obstétrique soit dématérialisé sur tablettes tactiles. L'intégration aisée de clichés échographiques et de boucles vidéo dans un examen portant sur une technique d'imagerie représenterait évidemment un progrès majeur.

Enfin, pour rendre plus attractifs encore le DIU d'échographie et surtout le DIU de médecine fœtale, notre projet est de proposer de coupler ces deux formations, d'y adjoindre un stage pratique avec des objectifs définis, afin de valider un Master en diagnostic prénatal.

4.5.5. Création d'un cercle vertueux autour de l'enseignement

Si l'enseignement par simulation paraît tout à fait pertinent dans l'apprentissage de la gynécologie - obstétrique, il faut dire aussi que les apprenants sont eux-mêmes très demandeurs de ce type d'enseignement et qu'ils prennent un véritable plaisir à assister à ce type de formation.

C'est sans doute pour toutes ces raisons que beaucoup de formateurs adhèrent sans réserve à cette démarche d'enseignement et que cela représente un véritable levier de motivation pour créer des vocations. Par ailleurs, l'enseignement par simulation ouvre à de nombreuses voies de recherche car il implique de nouveaux outils et de nouvelles stratégies qui restent à évaluer. De plus, certains simulateurs permettent de recueillir des données quantitatives, ce qui ouvre des perspectives d'analyses intéressantes.

Au final, l'enseignement par simulation est pertinent pour les étudiants et, fait tout aussi important, l'enseignement par simulation est attractif pour les formateurs.

5. ACTIVITÉ DE RECHERCHE

5.1. Formation personnelle et responsabilités

5.1.1. Formation personnelle concernant la recherche (synthèse)

2016 : **Formation aux Bonnes Pratiques Cliniques** du Groupement Interrégional de Recherche Clinique (GIRCI) de l'Est

2015 : **Enseignement du *Collaborative Institutional Training Initiative*** : *Basic/Refresher, Conflict of interest, Responsible conduct of Research, Basic course in laboratory animal welfare for investigators, Introduction to working with animals in biomedical research.*

2015 : **Enseignement de l'*Animal Care and Use in Research and Education (American Association for Laboratory Animal Science)*** : *8th Edition of the guide for the care and use of laboratory animals, Animal welfare act regulations, Aseptic technique for rodent survival surgery, Common compliance issues, Health risks and safety procedures for working with nonhuman primates, Inhalation anesthesia systems for rodents, Introduction to nonhuman primates, Introduction to rabbits, Introduction to sheep and goats, mouse breeding colony management, Pain recognition and alleviation in laboratory animals, Post-procedure care of mice and rats in research: minimizing pain and distress, Public health service policy on human care and use of laboratory animals, Semiannual facility inspection, working with the laboratory mouse, working with the laboratory rat.*

2012 : **Master 2 Santé Publique et Environnement « Épidémiologie, Recherche Clinique, Évaluation »** (Université de Lorraine).
Mémoire sur la prédiction de l'accouchement prématuré, à partir des caractéristiques maternelless, des antécédents obstétricaux et de biomarqueurs, au premier trimestre de la grossesse.

2011 : **Master 1 Santé Publique et Environnement « Épidémiologie, Recherche Clinique, Évaluation »** (Université de Lorraine).

5.1.2. Responsabilités concernant la recherche (synthèse)

Membre et coordonnateur régional du réseau Gynéco-Obstétrical des Centres d'Investigation Clinique (GO-CIC) depuis 2013.

Membre du Groupe de Recherche en Obstétrique et Gynécologie (GROG) depuis 2013.

5.2. Recherche clinique

5.2.1. Participation en tant qu'investigateur principal de protocoles de recherche

« César n Co » : Étude randomisée multicentrique en ouvert évaluant l'intérêt de la programmation des césariennes à 38 semaines d'aménorrhée en association avec une cure de corticoïdes par rapport à une programmation à 39 semaines d'aménorrhée.
Financement API des Hôpitaux Universitaires de Strasbourg. 2007-2013.

« Version acu » : Étude randomisée contre placebo évaluant l'intérêt de l'acupuncture dans la version du siège. 2008-2014.

5.2.2. Participation en tant qu'investigateur principal régional

« TUB » : Évaluation de l'efficacité du tamponnement intra-utérin par ballonnet de Belfort-Dildy dans le traitement des hémorragies sévères du post-partum immédiat (Pr Patrick Rozenberg). Financement PHRC national. Depuis 2018 (en cours).

« e-POPP » : Programme personnalisé utilisant les nouvelles technologies pour la prise en charge de l'obésité au cours de la grossesse afin d'améliorer les conditions de l'accouchement (Pr Philippe Deruelle). Financement PHRC national. Depuis 2018 (en cours).

« TRAAP 2 » : L'acide tranexamique en prévention de l'hémorragie du post-partum après césarienne (Pr Loïc Sentilhes). Financement PHRC national. Depuis 2018 (en cours).

« HPAG » : Hauteur utérine et petits poids pour l'âge gestationnel (Dr Anne Ego). Financement PHRC national. Depuis 2017 (en cours).

« ICAR » : Étude de l'intérêt des cellules T et Th17 plasmatiques maternelles comme marquer de chorioamniotite en cas de rupture prématurée des membranes (Pr Marc Bardou). Financement PHRC interrégional. 2013-2016.

« JUMODA » : Étude nationale prospective sur le mode d'accouchement des jumeaux (Pr Thomas Schimtz). Financement PHRC national. 2014-2015.

5.2.3. Participation en tant qu'investigateur associé

« PARTODYS » : Étude randomisée en ouvert comparant l'effet de deux partogrammes sur le taux de césariennes (Dr Adrien Gaudineau). Depuis 2016 (en cours).

« URANIC » : Étude randomisée en aveugle comparant l'Urapidil et la Nicardipine dans le traitement de la pré-éclampsie sévère (Pr Pierre Diemunsch). Depuis 2016 (en cours).

« PESSAR'ONE » : Étude randomisée en ouvert évaluant l'intérêt du pessaire pour la prévention de la prématurité dans les grossesses gémellaires à col court (Pr Christophe Vayssière). Depuis 2014 (en cours).

Étude randomisée en ouvert évaluant l'intérêt de la Metformine dans la prise en charge du diabète gestationnel chez les femmes non-obèses (Pr Nathalie Jeandidier). Financement PHRC interrégional. 2011-2017.

« PHENIX » : Évaluation de l'efficacité de la 17 alpha-hydroxyprogestérone caproate dans la prévention de l'accouchement prématuré (Pr Patrick Rozenberg). Financement PHRC national. 2009-2012.

Étude préliminaire d'équivalence entre l'Urapidil et la Nicardipine dans le traitement de l'hypertension artérielle sévère an cas de pré-éclampsie (Pr Pierre Diemunsch). 2007-2010.

5.3. Publications scientifiques

5.3.1. Synthèse des publications Pubmed

Total de 67 articles médicaux référencés Pubmed.

Score SIGAPS : 788.

Divers index et citations :

- h-index : 9.
- Total des citations : 221 (201 en excluant les auto-citations).
- Nombre moyen de citations par article : 2,3.

Impact factor (après exclusion des *abstracts*, *case reports* et lettres) :

	1er, 2ème ou dernier rang Nb / Total IF	**Autre rang Nb / Total IF**	**Total IF**
Publications en Anglais	30 / 75,545	6 / 14,423	89,968
Publications en Français	10 / 6,797	5 / 3,350	10,147
Total	40 / 82,342	11 / 17,773	51 / 100,115

5.3.2. Liste des publications référencées Pubmed

1. Kasbaoui S, Severac F, **Sananes N**. Reply. Am J Obstet Gynecol. 2018;218(1):150.

2. Ducellier-Azzola G, Pontvianne M, Weingertner AS, Kohler M, Viville B, Weil M, **Sananes N**, Favre R. [Outcome of in utero transfusion in case of foetomaternal red blood cell incompatibility]. Gynecol Obstet Fertil Senol. 2018;46(1):14-9.

3. Senat MV, Anselem O, Picone O, Renesme L, **Sananes N**, Vauloup-Fellous C, Sellier Y, Laplace JP, Sentilhes L. Gynecol Obstet Fertil Senol. 2017;45(12):705-14.

4. Schneider A, Koob M, **Sananes N**, Senger B, Hemmerle J, Becmeur F. Computed Tomographic Study of the Pediatric Diaphragmatic Growth: Application to the Treatment of Congenital Diaphragmatic Hernia. Eur J Pediatr Surg. 2017;27(2):177-80.

5. **Sananes N**, Koch A, Escande B, Aissi G, Fritz G, Roth E, Weil M, Bakri A, Bolender C, Meyer N, Vayssiere C, Gaudineau A, Nisand I, Favre R, Kuhn P, Langer B. Pilot randomised controlled trial comparing the risk of neonatal respiratory distress in elective caesarean section at 38 weeks' gestation following a course of corticosteroids versus caesarean at 39 weeks. Eur J Obstet Gynecol Reprod Biol. 2017;212:54-9.

6. **Sananes N**, Kasbaoui S, Severac F. Reply. Am J Obstet Gynecol. 2017;217(3):382.

7. **Sananes N**. [Management of pregnant women with first episode of genital herpes. Guidelines for clinical practice from the French college of gynecologists and obstetricians (CNGOF)]. Gynecol Obstet Fertil Senol. 2017;45(12):664-76.

8. Pinton A, Severac F, Meyer N, Akladios CY, Gaudineau A, Favre R, Langer B, **Sananes N**. A comparison of vaginal ultrasound and digital examination in predicting preterm delivery in women with threatened preterm labor: a cohort study. Acta Obstet Gynecol Scand. 2017;96(4):447-53.

9. Lecointre L, **Sananes N**, Weingertner AS, Gaudineau A, Akladios C, Cavillon V, Langer B, Favre R. [Fetoscopic laser coagulation in 200 consecutive monochorionic pregnancies with twin-twin transfusion syndrome]. J Gynecol Obstet Hum Reprod. 2017;46(2):175-81.

10. Koch A, Favre R, Viville B, Fritz G, Kohler M, Guerra F, Lecointre L, Gaudineau A, Langer B, Weingertner AS, **Sananes N**. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod. 2017;46(10):731-6.

11. Kasbaoui S, Severac F, Aissi G, Gaudineau A, Lecointre L, Akladios C, Favre R, Langer B, **Sananes N**. Predicting the difficulty of operative vaginal delivery by ultrasound measurement of fetal head station. Am J Obstet Gynecol. 2017;216(5):507 e1- e9.

12. **Sananes N**, Roth GE, Aissi GA, Meyer N, Bigler A, Bouschbacher JM, Helmlinger C, Viville B, Guilpain M, Gaudineau A, Akladios CY, Nisand I, Langer B, Vayssiere C, Favre

R. Acupuncture version of breech presentation: a randomized sham-controlled single-blinded trial. Eur J Obstet Gynecol Reprod Biol. 2016;204:24-30.

13. **Sananes N**, Rodo C, Peiro JL, Britto IS, Sangi-Haghpeykar H, Favre R, Joal A, Gaudineau A, Silva MM, Tannuri U, Zugaib M, Carreras E, Ruano R. Prematurity and fetal lung response after tracheal occlusion in fetuses with severe congenital diaphragmatic hernia. J Matern Fetal Neonatal Med. 2016;29(18):3030-4.

14. **Sananes N**, Javadian P, Schwach Werneck Britto I, Meyer N, Koch A, Gaudineau A, Favre R, Ruano R. Technical aspects and effectiveness of percutaneous fetal therapies for large sacrococcygeal teratomas: cohort study and literature review. Ultrasound Obstet Gynecol. 2016;47(6):712-9.

15. **Sananes N**, Gabriele V, Weingertner AS, Ruano R, Sanz-Cortes M, Gaudineau A, Langer B, Nisand I, Akladios CY, Favre R. Evaluation of long-term neurodevelopment in twin-twin transfusion syndrome after laser therapy. Prenat Diagn. 2016;36(12):1139-45.

16. **Sananes N**, Cruz-Martinez R, Favre R, Ordorica-Flores R, Moog R, Zaloszy A, Giron AM, Ruano R. Two-year outcomes after diagnostic and therapeutic fetal cystoscopy for lower urinary tract obstruction. Prenat Diagn. 2016;36(4):297-303.

17. **Sananes N**, Britto I, Akinkuotu AC, Olutoye OO, Cass DL, Sangi-Haghpeykar H, Lee TC, Cassady CI, Mehollin-Ray A, Welty S, Fernandes C, Belfort MA, Lee W, Ruano R. Improving the Prediction of Neonatal Outcomes in Isolated Left-Sided Congenital Diaphragmatic Hernia by Direct and Indirect Sonographic Assessment of Liver Herniation. J Ultrasound Med. 2016;35(7):1437-43.

18. Ruano R, **Sananes N**, Wilson C, Au J, Koh CJ, Gargollo P, Shamshirsaz AA, Espinoza J, Safdar A, Moaddab A, Meyer N, Cass DL, Olutoye OO, Olutoye OA, Welty S, Roth DR, Braun MC, Belfort MA. Fetal lower urinary tract obstruction: proposal for standardized multidisciplinary prenatal management based on disease severity. Ultrasound Obstet Gynecol. 2016;48(4):476-82.

19. Ruano R, Britto IS, **Sananes N**, Lee W, Sangi-Haghpeykar H, Deter RL. Growth Patterns of Fetal Lung Volumes in Healthy Fetuses and Fetuses With Isolated Left-Sided Congenital Diaphragmatic Hernia. J Ultrasound Med. 2016;35(6):1159-66.

20. Ronzino-Dubost V, **Sananes N**, Lavaux T, Youssef C, Gaudineau A, Lecointre L, Langer B. [Evaluation of the interest of procalcitonin in the diagnosis of chorioamnionitis in preterm premature rupture of membranes. An observational and prospective study]. J Gynecol Obstet Biol Reprod (Paris). 2016;45(7):745-53.

21. Kellenberger F, Akladios CY, **Sananes N**, Gaudineau A, Langer B. [The practice of neonatal umbilical blood gas analysis in the "Alsace" regional French perinatal network]. J Gynecol Obstet Biol Reprod (Paris). 2016;45(8):835-40.

22. Daney de Marcillac F, Moliere S, Pinton A, Weingertner AS, Fritz G, Viville B, Roedlich MN, Gaudineau A, **Sananes N**, Favre R, Nisand I, Langer B. [Accuracy of placenta

accreta prenatal diagnosis by ultrasound and MRI in a high-risk population]. J Gynecol Obstet Biol Reprod (Paris). 2016;45(2):198-206.

23. Daney de Marcillac F, Lecointre L, Guillaume A, **Sananes N**, Fritz G, Viville B, Boudier E, Nisand I, Gaudineau A, Langer B, Akladios CY. [Maternal morbidity and mortality associated with conservative management for placenta morbidly adherent (accreta) diagnosed during pregnancy. Report of 15 cases]. J Gynecol Obstet Biol Reprod (Paris). 2016;45(8):849-58.

24. Bonne S, Sauleau E, **Sananes N**, Akaladios C, Rongieres C, Pirrello O. Influence of medically assisted reproduction techniques on crown-rump length and biochemical markers of trisomy 21 in the first trimester of pregnancy. Fertil Steril. 2016;105(2):410-6.

25. Bel S, **Sananes N**, Gaudineau A, Akladios C, Favre R. Treatment of a Fetal Tracheal Obstruction by Fetoscopy and Laser. Fetal Diagn Ther. 2016;40(1):63-6.

26. Veujoz M, **Sananes N**, Severac F, Meyer N, Weingertner AS, Kohler M, Guerra F, Gaudineau A, Nisand I, Favre R. Evaluation of prenatal and postnatal diagnostic criteria for twin anemia-polycythemia sequence. Prenat Diagn. 2015;35(3):281-8.

27. **Sananes N**, Veujoz M, Severac F, Barthoulot M, Meyer N, Weingertner AS, Kohler M, Guerra F, Gaudineau A, Nisand I, Favre R. Evaluation of the Utility of in utero Treatment of Twin Anemia-Polycythemia Sequence. Fetal Diagn Ther. 2015;38(3):170-8.

28. **Sananes N**, Ruano R, Weingertner AS, Regnard P, Salmon Y, Kohler A, Miry C, Mager C, Guerra F, Schneider A, Becmeur F, Leroy J, Dimarcq JL, Debry C, Favre R. Experimental fetal endoscopic tracheal occlusion in rhesus and cynomolgus monkeys: nonhuman primate models. J Matern Fetal Neonatal Med. 2015;28(15):1822-7.

29. **Sananes N**, Favre R, Koh CJ, Zaloszyc A, Braun MC, Roth DR, Moog R, Becmeur F, Belfort MA, Ruano R. Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves: surgical technical aspects. Ultrasound Obstet Gynecol. 2015;45(2):183-9.

30. Ruano R, **Sananes N**, Sangi-Haghpeykar H, Hernandez-Ruano S, Moog R, Becmeur F, Zaloszyc A, Giron AM, Morin B, Favre R. Fetal intervention for severe lower urinary tract obstruction: a multicenter case-control study comparing fetal cystoscopy with vesicoamniotic shunting. Ultrasound Obstet Gynecol. 2015;45(4):452-8.

31. Moaddab A, **Sananes N**, Hernandez-Ruano S, Werneck Britto IS, Blumenfeld Y, Stoll F, Favre R, Ruano R. Prenatal Diagnosis and Perinatal Outcomes of Congenital Megalourethra: A Multicenter Cohort Study and Systematic Review of the Literature. J Ultrasound Med. 2015;34(11):2057-64.

32. Lecointre L, Gaudineau A, Hild C, **Sananes N**, Langer B. [Squamous cell carcinoma of the vulva and pregnancy: Tough choices]. Gynecol Obstet Fertil. 2015;43(9):625-7.

33. Guillaume A, **Sananes N**, Poirier V, Gaudineau A, Fritz G, Boudier E, Viville B, Aissi G, Favre R, Nisand I, Langer B. Benefits of cord blood collection in the prevention of post-partum hemorrhage: a cohort study. J Matern Fetal Neonatal Med. 2015;28(17):2111-4.

34. Gassmann AS, Koch A, Boudier E, Averous G, **Sananes N**, Nisand I, Schneider F, Langer B. Toxic Shock Syndrome detected at 21 weeks' gestation complicating acute chorioamnionitis with intact sac. Gynecol Obstet Fertil. 2015;43(5):400-2.

35. Britto IS, **Sananes N**, Olutoye OO, Cass DL, Sangi-Haghpeykar H, Lee TC, Cassady CI, Mehollin-Ray A, Welty S, Fernandes C, Belfort MA, Lee W, Ruano R. Standardization of Sonographic Lung-to-Head Ratio Measurements in Isolated Congenital Diaphragmatic Hernia: Impact on the Reproducibility and Efficacy to Predict Outcomes. J Ultrasound Med. 2015;34(10):1721-7.

36. Bonne S, **Sananes N**, Rongieres C. [Recurrent early pregnancy loss: What the life-birth chances are? A 10-years retrospective study]. J Gynecol Obstet Biol Reprod (Paris). 2015;44(5):419-25.

37. Boisrame-Helms J, Meziani F, **Sananes N**, Boisrame T, Langer B, Schneider F, Ragot T, Andriantsitohaina R, Tesse A. Detrimental arterial inflammatory effect of microparticles circulating in preeclamptic women: ex vivo evaluation in human arteries. Fundam Clin Pharmacol. 2015;29(5):450-61.

38. Akladios CY, **Sananes N**, Gaudineau A, Boudier E, Langer B. [Cervical cerclage]. J Gynecol Obstet Biol Reprod (Paris). 2015;44(8):771-5.

39. **Sananes N**, Rodriguez M, Stora C, Pinton A, Fritz G, Gaudineau A, Aissi G, Boudier E, Viville B, Favre R, Nisand I, Langer B. Efficacy and safety of labour induction in patients with a single previous caesarean section: a proposal for a clinical protocol. Arch Gynecol Obstet. 2014;290(4):669-76.

40. **Sananes N**, Langer B, Gaudineau A, Kutnahorsky R, Aissi G, Fritz G, Boudier E, Viville B, Nisand I, Favre R. Prediction of spontaneous preterm delivery in singleton pregnancies: where are we and where are we going? A review of literature. J Obstet Gynaecol. 2014;34(6):457-61.

41. Lecointre L, **Sananes N**, Weingertner AS, Kohler M, Guerra F, Fritz G, Viville B, Langer B, Nisand I, Favre R. Fetoscopic laser coagulation for twin-twin transfusion syndrome before 17 weeks' gestation: laser data, complications and neonatal outcome. Ultrasound Obstet Gynecol. 2014;44(3):299-303.

42. Koch A, Aissi G, Gaudineau A, **Sananes N**, Murtada R, Favre R, Nisand I. [Klippel-Trenaunay syndrome and pregnancy: difficult choice of delivery from a case and a review of the literature]. J Gynecol Obstet Biol Reprod (Paris). 2014;43(7):483-7.

43. Gapp-Born E, **Sananes N**, Weingertner AS, Guerra F, Kohler M, Fritz G, Viville B, Gaudineau A, Langer B, Sauleau E, Nisand I, Favre R. Predictive value of cardiovascular

parameters in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol. 2014;44(4):427-33.

44. Gapp-Born E, **Sananes N**, Guerra F, Kohler M, Weingertner AS, Fritz G, Viville B, Langer B, Sauleau E, Nisand I, Favre R. Predictive value of cardiovascular parameters in stages 1 and 2 of twin-to-twin transfusion syndrome. Prenat Diagn. 2014;34(9):908-14.

45. Boisrame T, **Sananes N**, Fritz G, Boudier E, Viville B, Aissi G, Favre R, Langer B. [Abruptio placentae. Diagnosis, management and maternal-fetal prognosis: a retrospective study of 100 cases]. Gynecol Obstet Fertil. 2014;42(2):78-83.

46. Boisrame T, **Sananes N**, Fritz G, Boudier E, Aissi G, Favre R, Langer B. Placental abruption: risk factors, management and maternal-fetal prognosis. Cohort study over 10 years. Eur J Obstet Gynecol Reprod Biol. 2014;179:100-4.

47. Bel S, Gaudineau A, Zorgnotti L, **Sananes N**, Fritz G, Langer B. [Survey on cervical ripening practices in France]. Gynecol Obstet Fertil. 2014;42(5):301-5.

48. Akladios CY, **Sananes N**, Gaudineau A, Boudier E, Langer B. [Secondary postpartum hemorrhage]. J Gynecol Obstet Biol Reprod (Paris). 2014;43(10):1161-9.

49. Aissi G, Gaudineau A, Trieu NT, **Sananes N**, Boisrame T, Stoll F, Koch A, Favre R, Nisand I. [Exceptional hemoperitoneal third trimester of pregnancy]. Gynecol Obstet Fertil. 2014;42(6):441-3.

50. **Sananes N**, Schuller E, Gaudineau A, Kohler M, Guerra F, Weingertner AS, Fritz G, Viville B, Langer B, Nisand I, Favre R. What is predictive of preterm delivery in the first trimester: isthmus or cervical length? Prenat Diagn. 2013;33(9):894-8.

51. **Sananes N**, Meyer N, Gaudineau A, Aissi G, Boudier E, Fritz G, Viville B, Nisand I, Langer B, Favre R. Prediction of spontaneous preterm delivery in the first trimester of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2013;171(1):18-22.

52. Langer B, Gaudineau A, **Sananes N**, Fritz G. [Management of patients with a history of late abortion or very premature delivery]. Gynecol Obstet Fertil. 2013;41(2):123-9.

53. Guillaume A, **Sananes N**, Akladios CY, Boudier E, Diemunsch P, Averous G, Nisand I, Langer B. Amniotic fluid embolism: 10-year retrospective study in a level III maternity hospital. Eur J Obstet Gynecol Reprod Biol. 2013;169(2):189-92.

54. Gaudineau A, Doray B, Schaefer E, **Sananes N**, Fritz G, Kohler M, Alembik Y, Viville B, Favre R, Langer B. Postnatal phenotype according to prenatal ultrasound features of Noonan syndrome: a retrospective study of 28 cases. Prenat Diagn. 2013;33(3):238-41.

55. Favre R, Koch A, Weingertner AS, **Sananes N**, Trieu NT, Kohler M, Guerra F, Nisand I. Vascular pattern in monochorionic placentas with spontaneous TAPS and TTTS with residual anastomoses after laser: a case-control study. Prenat Diagn. 2013;33(10):979-82.

56. Aissi G, **Sananes N**, Veujoz M, Felder A, Kasbaoui SM, Trieu NT, Favre R, Nisand I. [Vasa previa: Of the diagnosis to neonatal prognosis]. J Gynecol Obstet Biol Reprod (Paris). 2013;42(6):591-5.

57. Andres E, **Sananes N**, Langer B, Pottecher T. [National exam for the validation of the second medicine cycle in France: What does this examination evaluates?]. Presse Med. 2012;41(6 Pt 1):e245-9.

58. **Sananes N**, Garbin O, Hummel M, Youssef C, Vizitiu R, Lemaho D, Rottenberg D, Diemunsch P, Wattiez A. Setting up robotic surgery in gynaecology: the experience of the Strasbourg teaching hospital. J Robot Surg. 2011;5(2):133-6.

59. Zacharopoulou C, **Sananes N**, Baulon E, Garbin O, Wattiez A. [Robotic gynecologic surgery: state of the art. Review of the literature]. J Gynecol Obstet Biol Reprod (Paris). 2010;39(6):444-52.

60. **Sananes N**, Vayssiere C, Helmlinger C, Viville B, Kohler M, Aissi G, Trieu NT, Langer B, Favre R. Acupuncture for breech version: principles, technique, mode of action and utility--a literature review. J Matern Fetal Neonatal Med. 2010;23(5):455-8.

61. **Sananes N**, Meyer C, Straub P. Merkel cell carcinoma of the breast: CT-scan and histologic finding. Breast J. 2010;16(4):429-31.

62. **Sananes N**, Guigue V, Vayssiere C, Kohler M, Girard-Lemaire F, Flori E, Carelle-Calmels N, Boehm N, Samama B, Doray B, Favre R. Contribution of 3D ultrasound and fetal face studies to the prenatal diagnosis of Pallister-Killian syndrome. J Matern Fetal Neonatal Med. 2010;23(6):558-62.

63. **Sananes N**, Guigue V, Kohler M, Bouffet N, Cancellier M, Hornecker F, Hunsinger MC, Kohler A, Mager C, Neumann M, Schmerber E, Tanghe M, Nisand I, Favre R. Nuchal translucency and cystic hygroma colli in screening for fetal major congenital heart defects in a series of 12,910 euploid pregnancies. Ultrasound Obstet Gynecol. 2010;35(3):273-9.

64. **Sananes N**, Gaudineau A, Langer B. [Residency in obstetrics and gynaecology in Alsace (France)]. Gynecol Obstet Fertil. 2010;38(1):72-3.

65. **Sananes N**, Gaudineau A, Favre R. [Choose a fetal biometric reference curve using Z-scores]. Gynecol Obstet Fertil. 2010;38(1):71-2.

66. Gaudineau A, **Sananes N**, Langer B. [PubMed for the hopeless!]. Gynecol Obstet Fertil. 2010;38(1):72.

67. **Sananes N**, Guigue V, Kohler M, Bouffet N, Cancellier M, Hornecker F, Hunsinger MC, Kohler A, Mager C, Neumann M, Schmerber E, Tanghe M, Nisand I, Favre R. Use of Z-scores to select a fetal biometric reference curve. Ultrasound Obstet Gynecol. 2009;34(4):404-9.

5.3.3. Liste des publications non référencées Pubmed

1. M.-V. Sénat, O. Anselem, O. Picone, L. Renesme, **N. Sananès**, C. Vauloup-Fellous, Y. Sellier, J.-P. Laplace, L. Sentilhes. Prévention et prise en charge de l'infection herpétique au cours de la grossesse et de l'accouchement : recommandations pour la pratique clinique - texte des recommandations (texte court). La revue sage-femme 2018 ; 17 (1) : 36-47.

2. R. Favre, N. Sananès. Place de la cystoscopie en cas d'obstacle sous-vésical. Mises à jour du Collège des Gynécologues Obstétriciens Français 2017 – Obstétrique.

3. **N. Sananès**, R. Favre. Place de l'échographie du premier trimestre dans les nouvelles pratiques comportant l'ADN fœtal. Mises à jour du Collège des Gynécologues Obstétriciens Français 2016 – Obstétrique.

4. **N. Sananès**, R. Favre. Interruption sélective de grossesse. Rev. Méd. Périnat. 2015 ; 7 : 5-12.

5. **N. Sananès**, B. Langer, M. Patris, T. Pottecher, E. Andres. E. Andres. Analyse de la validité de contenu et de la qualité formelle des épreuves classantes nationales administrées en France de 2004 à 2011. Pédagogie Médicale 2013 ; 14(4) : 1-13.

6. M. Veujoz, A. Gaudineau, **N. Sananes**, G Fritz, B Langer. Folates et grossesse. Médecine thérapeutique 2013 ; 19(4) : 275-84.

7. **N. Sananès**, E. Andres. L'examen national classant : quelles clés pour les étudiants et quelles pistes de réflexion pour les enseignants ? Médecine thérapeutique 2012 ; 18(1) : 36-9.

8. **N Sananès**, O Garbin, M Hummel, C Youssef, R Vizitiu, D Lemaho, D Rottenberg, P Diemunsch, A Wattiez. Setting up robotic surgery in gynaecology: the experience of the Strasbourg teaching hospital. J Robotic Surg. 2011 Jun 5:133-6.

9. **Sananès N**, Garbin O, Hummel M, Youssef C, Thoma V, Baulon E, Le Maho D, Vizitiu R, Rottenberg D, Diemunsch P, Baldauf JJ, Nisand I, Wattiez A. Chirurgie robotique : expérience initiale du pôle de gynécologie du CHU de Strasbourg. À propos de 5 cas d'hystérectomies par coelioscopie robot-assistée. Pelvimag. 2009 Mars 65 :14-7.

10. **N. Sananès**, B. Romain. Autour de la cancérologie digestive. Alsamed, 2006 ; 51 : 32-34.

11. **N. Sananès**, C. Meyer. Améliorer la prise en charge des personnes atteintes de cancer. Alsamed, 2006 ; 53 : 18-22.

5.3.4. Abstracts publiés

1. **Sananes N**, Debry C, Regnard P, Miry C, Fellman L, Haelewyn L, Delaine M, Gaudineau A, Mottet N, Schneider A, Langer B, Favre R. New balloon for fetal endoscopic tracheal occlusion. In vitro tests and evaluation in the non-human primate model. Am J Obstet Gynecol, jan 2018.

2. **Sananes N**, Kasbaoui S, Severac F, Gaudineau A, Aïssi G, Favre R, Langer B. Predictive operative vaginal delivery by ultrasound measurement of the fetal head station. Am J Obstet Gynecol, jan 2017.

3. B Langer, G Roth, G Aïssi, N Meyer, A Bigler, JM Bouschbacher, C Helmlinger, B Viville, M Guilpain, A Gaudineau, C Akladios, I Nisand, C Vayssière, R Favre, **N Sananes.** Acupuncture version of breech presentation: a randomized sham-controlled single-blinded trial. Am J Obstet Gynecol, jan 2016: S15

4. I Britto, **N Sananes**, D Cass, C Cassady, A Mehollin-Ray, S Welty, J Mastrobattista, O Olutoye, W Lee, M Belfort, R Ruano. Standardization of the Lung-To-Head Ratio in the prediction of prognosis in isolated congenital diaphragmatic hernia: a single center experience. Ultrasound Med Biol, 2015 ; 4 : 84.

5. SM Kasbaoui, R Moog, I Lacreuse, **N Sananes**, R Favre. Complications d'un cas d'ablation de valves de l'urètre postérieur par photocoagulation laser par cystoscopie fœtale. Arch Pédiatr 2014 ; 21 (1) : 575

6. E Gapp-Born, **N Sananès**, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre. Valeur prédictive de paramètres cardiovasculaires dans les stades précoces du syndrome transfuseur-transfusé. Rev Méd Périnat 2013;5: 40.

7. E Schuller, **N Sananès**, R Favre. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré. Etude d'une cohorte de 2 319 patientes. Rev Méd Périnat, 2013 ; 5 : 41.

8. A Guillaume, **N Sananès**, V Poirier, B Langer. Etude de cohorte évaluant l'intérêt du don de sang placentaire dans la prévention de l'hémorragie du post-partum. Rev Méd Périnat, 2013 ; 5 : 57.

9. T Boisramé, **N Sananès**, B Langer. Hématome rétroplacentaire : facteurs de risque, prise en charge et pronostic materno-fœtal. Etude de cohorte sur 10 ans. Rev Méd Périnat, 2013 ; 5 : 80.

10. **N. Sananès**. Prédiction de l'accouchement prématuré à partir de facteurs maternels, de l'histoire obstétricale et de biomarqueurs, dès le premier trimestre de la grossesse. Revue d'Épidémiologie et de Santé Publique, 2013 ; 61 : 82-5.

11. T Boisramé, **N Sananès**, B Langer. Hématome rétro-placentaire : diagnostic, prise en charge et évolution obstétricale : étude rétrospective de 100 cas au CHU de Strasbourg. Rev Méd Périnat, 2012 ; 4 : 56.

12. V Dubost-Ronzino, **N Sananès**, G Fritz, A Gaudineau, E Boudier, B Langer. Critères diagnostiques de la chorioamniotite : étude rétrospective sur l'expérience du CHU de Strasbourg. Rev Méd Périnat, 2012 ; 4 : 88.

13. **N. Sananès**, N Meyer, A Gaudineau, B Langer, I Nisand, R Favre. Prédiction de l'accouchement prématuré à partir des caractéristiques maternelles, de l'histoire obstétricale et des biomarqueurs, dès le premier trimestre de la grossesse. Rev Méd Périnat, 2012 ; 4 : 115.

14. E Schuller-Dufour, **N Sananès**, R Favre. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré spontané. Étude de cohorte de 2319 patientes. Rev Méd Périnat, 2012 ; 4 : 139.

15. A Felder, **N Sananes**, R Favre, B Langer. Audit interne sur les extractions instrumentales. Peut-on diminuer leur taux ? Rev Méd Périnat, 2010 ; 2 : 67-8.

16. **N. Sananès**, E. Baulon, A. Wattiez. Laparoscopic management of large adnexal masses. Surgical procedure. Gynecol Surg, 2010 ; 1 : 140.

17. **Sananes N**, Garbin O, Wattiez A. Setting up of a robotic surgery service in gynecology. A study in Strasbourg teaching hospital, France. J Minim Invasive Gynecol 2009; 95-6.

5.3.5. Livre

Sananès N. Mise en place d'une activité de chirurgie robotique en gynécologie. Éditions Universitaires Européennes 2010.

5.3.6. Articles didactiques

1. B Langer, **N Sananès**, A Gaudineau, L Lecointre, E Boudier. Adaptation du fœtus au travail. Encyclopédie Médico-Chirurgicale – Obstétrique 2017.

2. **Sananes N**, Gaudineau A, Akladios CY, Lecointre L, Langer B. Hypertension artérielle et grossesse. EMC Gynécologie / obstétrique 2016 ;11(1) :1-14.

3. S Bel, A Gaudineau, V Thoma, O Garbin, **N Sananes**, E Baulon, A Wattiez, J-J Baldauf, C Akladios. Prose en charge des kystes de l'ovaire. Encyclopédie Médico-Chirurgicale – Obstétrique 2015;1-15.

4. B Langer, C Youssef-Akladios, **N Sananès**, A Gaudineau. Analyse informatisée du rythme cardiaque fœtal au cours de la grossesse. Encyclopédie Médico-Chirurgicale – Obstétrique 2015;1-8.

5. A Gaudineau, A Gorse, **N Sananes**, AS Korganow, B Langer. Accidents thromboemboliques veineux et grossesse. Encyclopédie Médico-Chirurgicale – Obstétrique/Gynécologie 2014;9(4):1-15.

6. J Pottecher, M Szczot, **N Sananès**, T Pottecher. Réanimation de la femme enceinte. Encyclopédie Médico-Chirurgicale – Obstétrique/Gynécologie 2014;9(2):1-11.

7. J.-J. Baldauf, G. Averous, E. Baulon, V. Thoma, A. Talha-Vautravers, **N. Sananes**, Y.C. Akladios. Néoplasies intra-épithéliales du col. Encyclopédie Médico-Chirurgicale – Gynécologie 2013;8(2):1-21.

8. **N. Sananes**, T. Boisrame, B. Langer. Hématome rétroplacentaire. Encyclopédie Médico-Chirurgicale – Obstétrique 2012;7(3):1-11.

5.3.7. Revue d'articles médicaux avant publication

Un total de 23 articles médicaux ont été revus avant publication :
- Journaux internationaux : 21.
- Journaux français : 1.

Journal	**Nombre de revues**
Prenatal Diagnosis	8
Journal of Obstetrics and Gynaecology	5
Fetal Diagnosis and Therapy	3
Ultrasound in Obstetrics and Gynecology	1
Acta Obstetricia et Gynecologica Scandinavica	1
American Journal of Perinatology	1
BMC Pregnancy and Childbirth	1
Journal of Obstetrics and Gynaecology Research	1
Journal of Medical Case Reports	1
BMJ Case Reports	1
Gynécologie Obstétrique Fertilité & Sénologie	2

5.4. Dépôt de brevet

Brevet déposé le 2 mai 2016 (numéro d'enregistrement national FR 1653954 – classification internationale des brevets A61B17/12) : «Ballonnet gonflable et détachable, destiné à être implanté dans une cavité corporelle, nécessaire de traitement et procédé de vidange associés».

5.5. Communications scientifiques

5.5.1. Organisation de congrès et de journées de formation

1. Comité d'organisation des journées "échofoetus" à Strasbourg, à raison de deux journées par an, depuis 2015.

2. Comité d'organisation des journées annuelles du CPDPN de Strasbourg, à raison d'une journée par an, depuis 2015.

3. Comité d'organisation des journées DPC sur la trisomie 21 à Strasbourg, à raison d'une journée par an, depuis 2017.

4. Comité d'organisation des "Rencontres Internationales Santé et environnement" (RISE), depuis 2016.

5. Organisation d'un cours pré-congrès d'une demi-journée à l'occasion des 41èmes journées du Collège National des Gynécologues Français (CNGOF), 2017, Lille. "Améliorer significativement ma pratique de l'écho en une demi-journée. Un session pratique pour la pratique". **Nicolas Sananès**, Philippe Bouhanna, Edwin Quarello.

6. Comité d'organisation du Club Francophone de Médecine Fœtale, 2016, Strasbourg.

5.5.2. Communications orales en séance plénière sur invitation

1. 17th World Congress in Fetal Medicine, 2018, Athènes. Developmennt of a new balloon for FETO. **N Sananès**, F Russo, D Basurto, R Favre, J Deprest.

2. 42èmes journées du Collège National des Gynécologues Français (CNGOF), 2018, Strasbourg. Développement d'un nouveau ballonnet pour la prise en charge prénatale de la hernie diaphragmatique congénitale. **N Sananès**, F Russo, D Basurto, R Favre, J Deprest.

3. Soirée multidisciplinaire du Diagnostic Anténatal CHBM, 2018, Belfort. Macrosomie : définition, épidémiologie et complications associées. **N Sananès**.

4. Journée nationale de l'AFOP, 2018, Strasbourg. Développement d'un dispositif médical innovant pour la prise en charge prénatale de la hernie diaphragmatique congénitale. **N Sananès**, C Debry, R Favre.

5. European Science Open Forum 2018, Toulouse. Prenatal management of congenital diaphragmatic hernia. **N Sananès.**

6. Journée Échofoetus : Nouveaux concepts en biométries foetales, 2018, Strasbourg. RCIU : définition et courbes individualisées. **N Sananès.**

7. Les conférences du jardin des sciences, 2018, Strasbourg. Des implants innovants. **N. Sananès**.

8. Journée Échofoetus : Actualités en thérapeutique foetale, 2018, Strasbourg. Prise en charge de la hernie diaphragmatique. **N Sananès.**

9. Journée nationale du centre de référence maladie rare de la hernie de coupole diaphragmatique, 2018, Paris. Les hernies droites sont-elles vraiment de mauvais pronostic ? A Pinton, **N Sananès**, A Benachi.

10. Journée du Cercle d'Etude des Gynécologues et Obstétriciens du Parc (CEGOP), 2018, Colmar. Est-il possible de définir un taux "idéal" de césariennes ? **N Sananès**.

11. Rencontres de Port-Royal, 2017, Paris. La macrosomie. Définition, épidémiologie, conséquences obstétricales et maternelles. **N. Sananès.**

12. 41èmes journées du Collège National des Gynécologues Français (CNGOF), 2017, Lille. Herpès et grossesse, recommandations pour la pratique clinique. Suspicion de lésion d'herpès génital chez une patiente sans antécédent connu d'herpès génital. **N Sananès.**

13. 41èmes journées du Collège National des Gynécologues Français (CNGOF), 2017, Lille. Place de la cystoscopie en cas d'obstacle sous-vésical. R Favre, **N. Sananès**.

14. Rencontres Haguenoviennes, 2017, Haguenau. Macrosomie. Définition et conséquences. **N. Sananès.**

15. Journée Bisontine de Mécanique et Techniques Obstétricales, 2017, Besançon. Macrosomie, un gros problème : définition, épidémiologie et facteurs de risque. **N Sananès**.

16. 45èmes Assises Nationales des sages-femmes, 2017, Strasbourg. Animation de deux ateliers d'échographie sur simulateurs. **N Sananès**, C Miry.

17. Journée « dynamique Cadres », 2017, Strasbourg. Hackaton Kesako ? C Tavernier, **N Sananès**.

18. Journée Échofoetus : Cas cliniques et communication, 2017, Strasbourg. RCIU : définition, diagnostic et annonce. **N Sananès.**

19. Journée du Cercle d'Etude des Gynécologues et Obstétriciens du Parc (CEGOP), 2017, Colmar. La pratique de l'obstétrique à bas-risque : quelques exemples de modèles européens. **N Sananès.**

20. Journée nationale du centre de référence maladie rare de la hernie de coupole diaphragmatique, 2017, Paris. Occlusion trachéale réversible par champ magnétique. **N Sananès**, C Debry, R Favre.

21. Journée nationale du centre de référence maladie rare de la hernie de coupole diaphragmatique, 2017, Paris. Rôle du comité scientifique. **N Sananès**.

22. 15th World Congress in Fetal Medicine, 2016, Palma de Majorque. Fetal cystoscopy. **N Sananes**, R Favre.

23. 15th World Congress in Fetal Medicine, 2016, Palma de Majorque. Ongoing development of a new balloon for fetal endoscopic tracheal occlusion. **N Sananes**, R Favre.

24. 40èmes journées du Collège National des Gynécologues Français (CNGOF), 2016, Montpellier. Place de l'échographie du premier trimestre dans les nouvelles pratiques comportant l'ADN foetal. **N Sananès**, R Favre.

25. Journée « Meet and Match » de l'Alsace Biovalley, 2016. Ongoing development of a new balloon for the prenatal management of the diaphragmatic congenital hernia. **N Sananès.**

26. Journée Échofoetus : Le doppler en obstétrique, 2016, Strasbourg. Artères utérines et dépistage de la pré-eclampsie. **N Sananès.**

27. Journée annuelle du CPDPN de Strasbourg, 2016, Strasbourg. Données d'une série de 200 lasers pris en charge à Strasbourg. L Lecointre, **N Sananès**, R Favre.

28. Journée annuelle du CPDPN de Strasbourg, 2016, Strasbourg. Diagnostic et prise en charge du TAPS. M Veujoz, **N Sananès**, R Favre.

29. Journée annuelle du CPDPN de Strasbourg, 2016, Strasbourg. Diagnostic et prise en charge du retard sélectif de croissance intra-utérin. **N Sananès**, A Koch, R Favre.

30. Journée annuelle du CPDPN de Strasbourg, 2016, Strasbourg. Diagnostic et prise en charge du TRAP. H Brossat, **N Sananès**, R Favre.

31. Journée annuelle du CPDPN de Strasbourg, 2015, Strasbourg. MOMS trial. **N Sananès.**

32. 17èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2016, Illkirch. Césarienne à 38 semaines d'aménorrhée après corticothérapie contre césarienne à 39 semaines d'aménorrhée : essai pilote randomisé contrôlé. **N. Sananès**.

33. 17èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2016, Illkirch. Version du siège par acupuncture : essai randomisé contrôlé contre placebo. **N. Sananès**.

34. Soirée multidisciplinaire du Diagnostic Anténatal CHBM, 2016, Belfort. Tests génétiques sur sang maternel et dépistage de la trisomie 21. **N Sananès**, R Favre.

35. Journée Échofoetus : Le cerveau fœtal normal et pathologique, 2016, Strasbourg. Les anomalies de la ligne médiane. **N Sananès.**

36. Journée du Cercle d'Etude des Gynécologues et Obstétriciens du Parc (CEGOP), 2016, Colmar. Le suivi de grossesse est actuellement optimal en France : Vrai ou faux. **N Sananès.**

37. Journée du réseau périnatal « Naître en Alsace », 2015, Strasbourg. Prise en charge de la menace d'accouchement prématuré. **N Sananès.**

38. 6ème Journée annuelle du CPDPN de Strasbourg, 2015, Strasbourg. MOMS trial. **N Sananès.**

39. Soirée multidisciplinaire du Diagnostic Anténatal CHBM, 2014, Belfort. Petits poids. **N Sananès**, R Favre.

40. 18èmes Journées de Médecine Fœtale, 2013, Morzine. Imagerie du col au premier trimestre. R Favre, **N Sananès**.

41. Journée du Cercle d'Etude des Gynécologues et Obstétriciens du Parc (CEGOP), 2013, Colmar. Prédiction de l'accouchement prématuré : scores, clinique et tocogramme. **N. Sananès**.

42. 13èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2012, Illkirch. Déclenchement sur utérus cicatriciel. **N. Sananès**, G. Fritz, B. Langer.

43. 36èmes journées du Collège National des Gynécologues Français (CNGOF), 2012, Paris. Prédiction de la prématurité par l'échographie du col au premier trimestre en population générale. **N. Sananès**, E. Schuller, R. Favre.

44. 37èmes journées nationales de Médecine Périnatale, 2007, Marseille. Administration de corticoïdes après 34 semaines d'aménorrhée : les arguments pour. B Langer, **N Sananès**.

45. 7èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2006 Illkirch. Intérêt d'une cure de corticoïdes dans les césariennes programmées à terme. **N. Sananès**, B. Langer.

5.5.3. Communications orales en séance plénière

1. Club Francophone de Médecine Fœtale, 2018, Clermont-Ferrand. Développement d'un nouveau ballon pour la prise en charge prénatale de la hernie diaphragmatqiue. Premiers tests animaux. **N Sananès**, F Russo, D Basurto, R Favre, J Deprest.

2. Club Francophone de Médecine Fœtale, 2018, Clermont-Ferrand. Place du laser interstitiel dans les grosses monochoriales. R Favre, **N Sananès**, AS Weingertner.

3. Club Francophone de Médecine Fœtale, 2018, Clermont-Ferrand. TAPS et grossesse gémellaire bichoriale biamniotique. M Zilliox, **N Sananès**, M Kohler.

4. Club Francophone de Médecine Fœtale, 2017, La Martinique. Développement d'un ballonnet à ouverture magnétique l'occlusion trachéale fœtale par endoscopie. **N Sananès**, R Favre, C Debry.

5. Club Francophone de Médecine Fœtale, 2017, La Martinique. Second stage screening. R Favre, **N Sananès**, AS Weingertner, M Kohler, F Guerra.

6. Club Francophone de Médecine Fœtale, 2017, La Martinique. Devenir des triples après syndrome transfuseur-transfusé. R Favre, AS Weingertner, **N Sananès**, M Kohler, A Kohler, C Mager, C Miry, V Richhard, C Drusch.

7. Club Francophone de Médecine Fœtale, 2017, La Martinique. Dépistage de la trisomie 21 chez les patients obèses. M Koler, R Favre, **N Sananès**.

8. 35th Annual Meeting of International Fetal Medicine and Surgery Society, 2016, Botswana. Ongoing development of a new balloon for fetal endoscopic tracheal occlusion. **N Sananes**, R Favre, C Debry.

9. Club Francophone de Médecine Fœtale, 2016, Strasbourg. Développement d'un ballonnet innovant pour l'occlusion trachéale fœtale par endoscopie. **N Sananès**, R Favre, C Debry.

10. Club Francophone de Médecine Fœtale, 2016, Strasbourg. Transfusion in utero pour allo-immunisation foeto-maternelle. R Favre, G Ducellier, M Kohler, AS Weingertner, **N Sananès**, F Guerra, B Viville, I Nisand.

11. 34th Annual Meeting of International Fetal Medicine and Surgery Society, 2015, Hersonissos. Fetal surgery for diaphragmatic hernia. Prediction of outcome by ultrasound. **N Sananes**, I Britto, A Akinkuotu, O Olutoye, D Cass, H Sangi-Hagheykar, T Lee, C Cassady, A Mehollin-Ray, S Welty, C Fernandes, M Belfort, W Lee, R Ruano.

12. 34th Annual Meeting of International Fetal Medicine and Surgery Society, 2015, Hersonissos. LUTO: outcome after fetal cystoscopic ablation of posterior urethral valves. **N Sananes**, R Favre, R Ruano.

13. 34th Annual Meeting of International Fetal Medicine and Surgery Society, 2015, Hersonissos. Fetal lower urinary tract obstruction – proposal of standardized multidisciplinary prenatal management based on the severity of the disease. R Ruano, **N Sananes**, C Wilson, J Au, C Koh, P Gargollo, A Shamshiraz, J Espinoza, N Meyer, D Cass, O Olutoye, S Welty, D Roth, M Braun, M Belfort.

14. 16èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2015, Illkirch. Césarienne à 38 semaines d'aménorrhée après corticothérapie contre césarienne à 39 semaines d'aménorrhée : les premiers résultats de l'étude César and Co. A Koch, **N Sananes**, P Kuhn, B Escande, G Aïssi, G Fritz, E Roth, M Weil, A Bakri, C Bolender, R Kutnahorsky, D Chognot, P Weber, Keller, A Gaudineau, C Vayssiere, I Nisand, R Favre, B Langer.

15. 16èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2015, Illkirch. Prédiction de l'accouchement prématuré en situation de menace d'accouchement prématuré. Intérêt du toucher vaginal. A Pinton, **N Sananes**, N Meyer.

16. 33rd Annual Meeting of International Fetal Medicine and Surgery Society, 2014, Cape Cod. Evaluation of Twin-Anemia Polycythemia Sequence's in utero treatment. **N Sananès**, M Veujoz, F Severac, N Meyer, R Favre.

17. 33rd Annual Meeting of International Fetal Medicine and Surgery Society, 2014, Cape Cod. Treatment of a fetal tracheal obstruction by fetoscopy and laser. **N Sananès**, S Bel, R Favre.

18. 33rd Annual Meeting of International Fetal Medicine and Surgery Society, 2014, Cape Cod. Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves – Surgical technical aspects. **N Sananès**, R Favre, CJ Koh, A Zaloszyc, MC Braun, DR Roth, R Moog, F Becmeur, MA Belfort, R Ruano.

19. Club Francophone de Médecine Fœtale, 2014, Marrakech. Considérations techniques de l'ablation laser des valves de l'urètre postérieur par cystoscopie foetale. Considérations techniques. **N Sananès**, R Ruano, I Nisand, R Favre.

20. Club Francophone de Médecine Fœtale, 2014, Marrakech. Valeur prédictive des paramètres cardiovasculaires dans le Syndrome Transfuseur-Transfusé. **N Sananès**, E Gapp-Born, E Sauleau, I Nisand, R Favre.

21. Club Francophone de Médecine Fœtale, 2014, Marrakech. Développement du modèle du singe dans l'occlusion trachéale par endoscopie fœtale. **N Sananès**, R Ruano, AS Weingertner, P Regnard, I Nisand, R Favre.

22. Club Francophone de Médecine Fœtale, 2014, Marrakech. Prise en charge in utero d'une obstruction trachéale fœtale. **N Sananès**, S Bel, I Nisand, R Favre.

23. Club Francophone de Médecine Fœtale, 2014, Marrakech. Devenir obstétrical et néonatal des syndromes transfuseur-transfusé traités par laser. À propos d'une série de 200 patientes. **N Sananès**, S Bel, I Nisand, R Favre.

24. 12th World Congress in Fetal Medicine, 2013, Marbella. Laser for TTTS : outcome and developmental follow-up. **N Sananes.**

25. 12th World Congress in Fetal Medicine, 2013, Marbella. Early TTTS : prediction of survival from fetal cardiac function. **N Sananes.**

26. 12th World Congress in Fetal Medicine, 2013, Marbella. 1st trimester prediction of preterm birth: isthmus and cervical length. **N Sananes.**

27. 32nd Annual Meeting of International Fetal Medicine and Surgery Society, 2013, Jerusalem. Predictive value of cardiovasculare parameters in early stages twin-to-twin transfusion syndrome. E Gapp-Born, **N Sananes**, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre.

28. 32nd Annual Meeting of International Fetal Medicine and Surgery Society, 2013, Jerusalem. Correlations between isthmus, cervical length and maternal characteristics, at first trimester of pregnancy. **N Sananes**, E Schuller, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre.

29. 32nd Annual Meeting of International Fetal Medicine and Surgery Society, 2013, Jerusalem. What is predictive of preterm delivery in the first trimester : isthmus or cervical length ? **N Sananes**, E Schuller, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre.

30. 32nd Annual Meeting of International Fetal Medicine and Surgery Society, 2013, Jerusalem. Fetal loss and developmental follow-up of infants who underwent laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. **N Sananes**, S Andres, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre.

31. Club Francophone de Médecine Fœtale, 2012, Saint-Omer. Mesure de l'endocol au premier trimestre. R Favre, AS Weingertner, N Trieu, M Kohler, **N Sananes**.

32. Club Francophone de Médecine Fœtale, 2012, Saint-Omer. Prédiction de l'accouchement prématuré dans les grossesses uniques, à partir de facteurs maternels et de l'histoire obstétricale dès le 1er trimestre de la grossesse. R Favre, AS Weingertner, N Trieu, M Kohler, **N Sananes**.

5.5.4. Communications orales en session libre

1. 23èmes journées de médecine fœtale, Marseille. Les hernies droites sont-elles vraiment de mauvais pronostic ? A Pinton, **N Sananès**, A Benachi.

2. Society for Maternal Fetal Medicine 37th Annual pregnancy meeting, 2017, Las Vegas. Predictive operative vaginal delivery by ultrasound measurement of the fetal head station. **N Sananes**, S Kasbaoui, F Severac, A Gaudineau, G Aïssi, R Favre, B Langer.

3. 24th EBCOG European Congress of Obstetrics and Gynaecology, 2016, Turin. Randomized controlled trial comparing the risk of neonatal respiratory distress in case of elective C-section at 38 WG following a course of corticosteroids versus C-section at 39 WG. A Koch, B Langer**, N Sananes**.

4. 24th EBCOG European Congress of Obstetrics and Gynaecology, 2016, Turin. A comparison of vaginal ultrasound and digital examination in predicting preterm delivery in women with threatened preterm labor. Pinton A, Severac F, Langer B**, Sananes N**.

5. 24th EBCOG European Congress of Obstetrics and Gynaecology, 2016, Turin. Spontaneous rupture of the unscarred uterus. Pinton A, Boudier E, Joal A, **Sananes N**, Severac F, Langer B**, Sananes N**.

6. 46ème journée nationale de la Société Française de Médecine Périnatale, 2016, Brest. Données d'une série de 200 lasers pris en charge à Strasbourg. V Cavillon, L Lecointre, **N Sananès**, AS Weingertner, C Akladios, A Gaudineau, B Langer, R Favre.

7. 40èmes journées du Collège National des Gynécologues Français (CNGOF), 2016, Montpellier. Intérêt de l'échographie périnéale pour prédire la difficulté d'extraction instrumentale. S Kasbaoui, F Severac, A Gaudineau, G Aïssi, R Favre, B Langer, **N Sananès**.

8. Society for Maternal Fetal Medicine 36th Annual pregnancy meeting, 2016, Atltanta. Acupuncture version of breech presentation: a randomized sham-controlled single-blinded trial. B Langer, G Roth, G Aïssi, N Meyer, A Bigler, JM Bouschbacher, C Helmlinger, B Viville, M Guilpain, A Gaudineau, C Akladios, I Nisand, C Vayssière, R Favre, **N Sananes.**

9. 39èmes journées du Collège National des Gynécologues Français (CNGOF), 2015, Paris. Césarienne à 38 semaines d'aménorrhée après corticothérapie contre césarienne à 39 semaines : essai randomisé contrôlé. **N Sananès**, A Koch, P Kuhn, B Escande, G Aissi, G Fritz, E Roth, M Weil, A Bakri, C Bolender, R Kutnahorsky, D Chognot, P Weber, L Keller, A Gaudineau, C Vayssiere, I Nisand, R Favre, B Langer.

10. Annual Convention of American Institute of Ultrasound in Medicine, 2015, Lake Buena Vista. Standardization of the Lung-To-Head Ratio in the prediction of prognosis in isolated congenital diaphragmatic hernia: a single center experience. I Britto, **N**

Sananes, D Cass, C Cassady, A Mehollin-Ray, S Welty, J Mastrobattista, O Olutoye, W Lee, M Belfort, R Ruano.

11. 24th Annual Congress of European Society of Gynaecological Endoscopy (ESGE), 2015, Budapest. Integrated Bigatti Shaver morcellatore (IBS®) versus standard hysteroscopic resection for endometrial polyps treatment: a prospective comparative study. F Stoll, L Schwartz, **N Sananes**, O Garbin.

12. 30th Annual Meeting of European Society of Human Reproduction and Biology, 2014, Munich. Assisted reproductive technologies (ART) have influence on first trimester usually used factors for Qown's syndrome screening. S Bonne, O Pirrello, E Sauleau, C Rongières, J Ohl, K Bettahar, L Ladureau-Fritsch, I Koscinski, I Nisand, R Favre, **N Sananes**.

13. World Federation for Ultrasound in Medicine and Biology, World Congress 2014, Sao Paulo. Standardization of the Lung-To-Head Ratio in the prediction of prognosis in isolated congenital diaphragmatic hernia: a single center experience. I Britto, **N Sananes**, D Cass, C Cassady, A Mehollin-Ray, S Welty, J Mastrobattista, O Olutoye, W Lee, M Belfort, R Ruano.

14. 43ème journée nationale de la Société Française de Médecine Périnatale, 2013, Monaco. Valeur prédictive de paramètres cardiovasculaires dans les stades précoces du syndrome transfuseur-transfusé. E Gapp-Born, **N Sananès**, M Kohler, F Guerra, AS Weingertner, G Fritz, B Viville, B Langer, E Sauleau, I Nisand, R Favre.

15. 43ème journée nationale de la Société Française de Médecine Périnatale, 2013, Monaco. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré. Etude d'une cohorte de 2 319 patientes. E Schuller, **N Sananès**, R Favre.

16. 43ème journée nationale de la Société Française de Médecine Périnatale, 2013, Monaco. Etude de cohorte évaluant l'intérêt du don de sang placentaire dans la prévention de l'hémorragie du post-partum. A Guillaume, **N Sananès**, V Poirier, B Langer.

17. 43ème journée nationale de la Société Française de Médecine Périnatale, 2013, Monaco. Hématome rétroplacentaire : facteurs de risque, prise en charge et pronostic materno-fœtal. Etude de cohorte sur 10 ans. T Boisramé, **N Sananès**, B Langer.

18. 14ème journée nationale des Gynécologues Obstétriciens en Formation (Jn'GOF), 2013, Paris. Efficacité et sécurité du déclenchement sur utérus cicatricel : étude de cohorte et revue de la littérature. **N Sananes**, M Rodriguez, C Stora, G Fritz, A Gaudineau, G Aïssi, E Boudier, B Viville, R Favre, I Nisand, B Langer.

19. 4èmes Assises de Gynécologie Obstétrique, 2013, Lille. Critères diagnostiques de la chorioamniotite : étude rétrospective sur l'expérience du CHU de Strasbourg. V Dubost-Ronzino, **N Sananès**, G Fritz, A Gaudineau, E Boudier, B Langer.

20. 4èmes Assises de Gynécologie Obstétrique, 2013, Lille. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré spontané. Étude de cohorte de 2319 patientes. E Schuller-Dufour, **N Sananès**, R Favre.

21. 42ème journée nationale de la Société Française de Médecine Périnatale, 2012, Montpellier. Hématome rétroplacentaire. Diagnostic, prise en charge et évolution obstétricale : étude rétrospective de 100 cas au CHU de Strasbourg. T Boisramé, **N Sananès**, B Langer.

22. 42ème journée nationale de la Société Française de Médecine Périnatale, 2012, Montpellier. Critères diagnostiques de la chorioamniotite : étude rétrospective sur l'expérience du CHU de Strasbourg. V Dubost-Ronzino, **N Sananès**, G Fritz, A Gaudineau, E Boudier, B Langer.

23. 13èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2012, Illkirch. Hématome rétroplacentaire. Diagnostic, prise en charge et évolution obstétricale : étude rétrospective de 100 cas au CHU de Strasbourg. T Boisramé, **N Sananès**, B Langer.

24. 13èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2012, Illkirch. Critères diagnostiques de la chorioamniotite : étude rétrospective sur l'expérience du CHU de Strasbourg. V Dubost-Ronzino, **N Sananès**, B Langer.

25. États Généraux de la Formation Médicale, 2011, Bobigny. Analyse de ce qu'évalue l'épreuve de dossiers de l'Examen National Classant de médecine : 8 ans d'annales de 2004 à 2011. **N. Sananès**, E Andrès.

26. 19th Annual Congress of European Society of Gynaecological Endoscopy (ESGE), 2010, Barcelone, Espagne. Laparoscopic management of large benign adnexal masses. **N. Sananès**, E. Baulon, A. Wattiez.

27. 40ème journée nationale de la Société Française de Médecine Périnatale, 2010, Deauville. Audit interne sur les extractions instrumentales. Peut-on diminuer leur taux ? **N. Sananès**, A Felder.

28. 38ème congrès de l'American Association of Gynecologic Laparoscopist (AAGL) : Global Congress on Minimally Invasive Gynecology, 2009, Orlando, Etats-Unis. Setting up a robotic surgery service in gynecology. **N. Sananès**, O. Garbin, A. Wattiez.

29. 132ème congrès de la Société de Gynécologie et d'Obstétrique du Rhin Supérieur, 2009, Belfort. Audit interne sur les extractions instrumentales. Peut-on diminuer leur taux ? A Felder, **N Sananès**.

30. 9èmes journées du Collège des Gynécologues Obstétriciens d'Alsace (CGOA), 2008 Illkirch. Intérêt des Z-scores dans le choix d'une courbe de référence de biométries fœtales. **N. Sananès**, B. Langer.

5.5.5. Communications affichées

1. Society for Maternal Fetal Medicine 38th Annual pregnancy meeting, 2018, Dallas. New balloon for fetal endoscopic tracheal occlusion. In vitro tests and evaluation in the non-human primate model. **Sananes N**, Debry C, Regnard P, Miry C, Fellman L, Haelewyn L, Delaine M, Gaudineau A, Mottet N, Schneider A, Langer B, Favre R.

2. 40èmes journées du Collège National des Gynécologues Français (CNGOF), 2016, Montpellier. Second stage screening. Intérêt dans le dépistage de la trisomie 21. AS Gassmann, **N Sananès**, R Favre.

3. 40èmes journées du Collège National des Gynécologues Français (CNGOF), 2016, Montpellier. Tocolyse d'urgence par salbumol pendant le travail au CHU de Strasbourg : série descriptive. M Tissot, **N Sananès**, B Langer.

4. 40èmes journées du Collège National des Gynécologues Français (CNGOF), 2016, Montpellier. Retard de croissance sélectif intra-utérin. À propos d'une série de cas. A Koch, **N Sananès**, R Favre.

5. 19th International Conference on Prenatal Doagnosis and Therapy, 2015, Washington DC. Lung-to-head ratio, ultrasound liver herniation-to-thoracic ratio and stomach position in isolated left congenital diaphragmatic hernia to predict outcome. **N Sananes**, I Britto, A Akinkuotu, O Olutoye, D Cass, H Sangi-Haghpeykar, T Lee, C Cassady, A Mehollin-Ray, S Welty, C Fernandes, M Belfort, W Lee, R Ruano.

6. 19th International Conference on Prenatal Diagnosis and Therapy, 2015, Washington DC. Longitudinal measurements of fetal lung volumes in healthy fetuses and fetuses with isolated left-sided congenital diaphragmatic hernia. R Ruano, I Britto, **N Sananes**, H Sangi-Haghpeykar, MM da Silva, M Belfort, W Lee, U Tannuri, R Deter.

7. 24th European Congress of Perinatal Medicine, 2014, Florence. Placental abruption: risk factors, management and materal-fetal prognosis. Cohort-study over 10 years. T Boisramé, **N Sananès**, B Langer.

8. 24th European Congress of Perinatal Medicine, 2014, Florence. Diagnostic criteria, management and prognosis of TAPS (Twin Anemia Polycythemia Sequence). M Veujoz, **N Sananes**, F Severac, N Meyer, B Langer, R Favre.

9. 24th European Congress of Perinatal Medicine, 2014, Florence. Benefits of cord blood collection in the prevention of post-partum haemorrhage: a cohort study. A Guillaume, **N Sananès**, V Poirier, A Gaudineau, G Fritz, E Boudier, B Viville, G Aïssi, R Favre, I Nisand, B Langer.

10. 24th European Congress of Perinatal Medicine, 2014, Florence. Fetoscopic laser coagulation for twin-twin transfusion syndrome before 17 weeks' gestation: laser data, complications and neonatal outcomes. L Lecointre, **N Sananès**, R. Favre.

11. Congrès de la Société Française de Chirurgie Pédiatrique, 2014, Lyon. Complications d'un cas d'ablation de valves de l'urètre postérieur par photocoagulation laser par cystoscopie fœtale. SM Kasbaoui, R Moog, I Lacreuse, **N Sananes**, R Favre.

12. 19èmes Journées de Médecine Fœtale, 2013, Morzine. Traitement d'une obstruction trachéale fœtale par trachéoscopie in utero : à propos d'un cas. S Bel, **N Sananès**, R Favre.

13. 19èmes Journées de Médecine Fœtale, 2013, Morzine. Complications d'un cas d'ablation de valves de l'urètre postérieur par photocoagulation laser par cystoscopie fœtale. SM Kasbaoui, **N Sananes**, R Favre.

14. 42ème journée nationale de la Société Française de Médecine Périnatale, 2012, Montpellier. Prédiction de l'accouchement prématuré à partir des caractéristiques maternelles, de l'histoire obstétricale et des biomarqueurs, dès le premier trimestre de la grossesse. **N. Sananès**, N Meyer, A Gaudineau, B Langer, I Nisand, R Favre.

15. 42ème journée nationale de la Société Française de Médecine Périnatale, 2012, Montpellier. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré spontané. Étude de cohorte de 2319 patientes. E Schuller-Dufour, **N Sananès**, R Favre.

16. 13ème journée nationale des Gynécologues Obstétriciens en Formation (Jn'GOF), 2012, Paris. Critères diagnostiques de la chorioamniotite : étude rétrospective sur l'expérience du CHU de Strasbourg. V Dubost-Ronzino, **N Sananès**, G Fritz, A Gaudineau, E Boudier, B Langer.

17. 13ème journée nationale des Gynécologues Obstétriciens en Formation (Jn'GOF), 2012, Paris. Association entre mesure échographique du col utérin au premier trimestre de la grossesse, caractéristiques maternelles et accouchement prématuré spontané. Étude de cohorte de 2319 patientes. E Schuller-Dufour, **N Sananès**, R Favre.

18. 10ème journée nationale des Gynécologues Obstétriciens en Formation (Jn'GOF), 2009, Paris. Audit interne sur les extractions instrumentales. Peut-on diminuer leur taux ? A. Felder, **N. Sananès**, R. Favre, B. Langer.

19. 14ème journées de médecine fœtale : diagnostic et prise en charge pré et post-natale des affections fœtales, 2008, Morzine. Apport de l'échographie 3D et de l'étude de la face fœtale dans le diagnostic prénatal du syndrome de Pallister-Killian. V. Guigue, **N. Sananes**, M. Kohler, E. Flori, F. Girard-Lemaire, B. Doray, C. Vayssiere, R. Favre

20. 8ème journée nationale des Gynécologues Obstétriciens en Formation (Jn'GOF), 2007, Paris. Clarté nucale dans le dépistage des cardiopathies fœtales. Étude d'une série de 12 910 grossesses euploïdes. **Sananès N**, Guigue V, Favre R

- Troisième partie -

MÉMOIRE DE RECHERCHE

6. MÉMOIRE DE RECHERCHE

Chirurgie endoscopique fœtale : Identification des candidats et développement des techniques

6.1. Introduction

La chirurgie du fœtus in utero s'adresse uniquement aux pathologies pour lesquelles il y a un intérêt à une prise en charge prénatale plutôt que postnatale, soit parce que ces pathologies compromettent la vie du fœtus, soit parce qu'elles entraînent des dommages définitifs d'un organe en développement.

La chirurgie fœtale est actuellement en plein essor, en raison de l'amélioration de l'imagerie par échographie et du diagnostic prénatal, mais aussi et surtout en raison de la miniaturisation des fibres optiques qui permet un abord endoscopique mini-invasif de la poche amniotique et du fœtus.

La chirurgie endoscopique fœtale pose 2 questions centrales :

1. Quelles sont les bonnes indications ? Cette question, incontournable pour tout type de traitement, est particulièrement cruciale en chirurgie fœtale. En effet, même en utilisant une approche mini-invasive par endoscopie, il persiste un risque important de rupture de la poche des eaux, de perte de la grossesse ou de prématurité induite. Par conséquent, le bénéfice potentiel pour un candidat donné se doit d'être évalué au mieux et mis en balance avec les risques de l'intervention.

2. Quelle est la bonne technique ? Faisant suite au questionnement précédent, l'objectif des développements techniques est d'augmenter le bénéfice de la chirurgie fœtale et/ou de limiter la morbidité induite. L'enjeu est majeur dans cette discipline récente dont l'avenir reste encore à écrire. Les champs d'innovation potentiels sont de différents ordre, mais concernent en particulier le développement matériel.

Mon travail de recherche portant sur la chirurgie endoscopique fœtale tente d'apporter des réponses ou de contribuer à la recherche de solutions, concernant l'identification des candidats et le développement des techniques.

Mes thématiques sont au nombre de quatre et concernent les principales indications de la chirurgie endoscopique fœtale :

1. Hernie de coupole diaphragmatique
2. Pathologies des grossesses monochoriales
3. Uropathies obstructives basses
4. Myéloméningocèle

6.2. Structuration de la recherche

6.2.1. Recherche fondamentale

Notre équipe a développé le modèle du singe macaque (rhésus et cynomolgus) pour la chirurgie endoscopique fœtale percutanée. Ce modèle est proche de l'humain au niveau physiologique et est donc utile en recherche fondamentale, notamment pour l'étude du développement pulmonaire fœtal.

Les travaux chez le singe sont réalisés sur la plateforme *Simian Laboratory Europe* (SILABE) de l'Université de Strasbourg, en collaboration avec l'équipe vétérinaire.

6.2.2. Recherche translationnelle

Le modèle du singe est également proche de l'humain au niveau anatomique. Il est donc pertinent en recherche translationnelle dans l'évaluation de nouvelles techniques ou l'usage de nouveaux matériels chirurgicaux.

Mon équipe de support est l'unité INSERM UMR-S 1121 "Biomatériaux et Bioingénierie", de l'Université de Strasbourg. Le traitement de la hernie diaphragmatique congénitale est l'un des 4 axes de recherche du projet 2018-2022 du laboratoire.

Mon travail sur cette thématique est centré sur le développement d'un nouveau dispositif médical pour la prise en charge prénatale de la hernie de coupole diaphragmatique. Ce projet s'inscrit dans le programme "Biomatériaux, imagerie et Robotique médicale" de la Fédération de Médecine Translationnelle de Strasbourg (FMTS).

6.2.3. Recherche clinique

Mon activité de soin au sein du service de diagnostic prénatal des Hôpitaux Universitaires de Strasbourg me permet d'analyser la question clinique de l'identification des candidats, mais aussi la pertinence des questionnements techniques.

6.3. Axes et thématiques de recherche

La prise en charge prénatale de la hernie de coupole diaphragmatique est ma thématique de recherche principale, pour laquelle j'ai développé un axe de recherche fondamentale, un axe de recherche translationnelle et un axe de recherche clinique.
Les travaux sur la prise en charge anténatale des pathologies des grossesses monochoriales et des uropathies obstructives basses portent essentiellement sur un axe de recherche clinique.
Enfin, la thématique des myélo-méningocèles est en cours de développement et est centrée sur un axe translationnel.

Les thématiques et les axes de recherche sont résumés dans le tableau suivant. Il rapporte, pour chaque thématique, le type d'axe de recherche qui est développé, mais aussi à quel(s) objectif(s) il répond : développement des techniques et/ou identification des candidats.

	Axe fondamental	Axe translationnel	Axe clinique
Hernie diaphragmatique	Développement des techniques	Développement des techniques	Développement des techniques Identification des candidats
Pathologies des grossesses monochoriales	/	/	Développement des techniques Identification des candidats
Uropathies obstructives basses	/	/	Développement des techniques Identification des candidats
Myéloméningocèles	/	Développement des techniques	/

6.4. Hernie de coupole diaphragmatique

L'existence d'une hernie diaphragmatique chez un fœtus va avoir pour conséquence l'irruption des viscères abdominaux dans la cage thoracique, ce qui entrave le développement normal des poumons et entraîne une mortalité néonatale de l'ordre de 30 %. L'occlusion de la trachée fœtale par la mise en place d'un ballonnet par voie endoscopique améliore la survie néonatale, en bloquant les sécrétions pulmonaires et en stimulant le développement des poumons par un mécanisme d'hyperpression. Deux études randomisées internationales en cours ("TOTAL") coordonnées par le Professeur Jan Deprest (Université Catholique de Louvain) ont pour but d'évaluer l'intérêt de l'occlusion trachéale dans les cas de hernies diaphragmatiques modérées et sévères.

6.4.1. Axe de recherche fondamentale

Problématique et objectif :

La question du mécanisme sous-jacent responsable de la croissance pulmonaire en cas d'occlusion trachéale fœtale est cruciale. Sa bonne compréhension pourrait permettre de développer des thérapies médicamenteuses adjuvantes au ballonnet, voire même de se passer de la mise en place celui-ci. C'est en cela que cet axe fondamental s'inscrit dans un travail de développement des techniques.

L'objectif est d'analyser les protéines en jeu dans le développement pulmonaire fœtal en cas d'occlusion trachéale.

Travaux effectués :

Nous avons développé le modèle du singe pour l'occlusion trachéale fœtale par endoscopie et nous sommes l'équipe avec l'expérience la plus importante de ce modèle - p 83.

Nous avons réalisé des prélèvements de liquide trachéal, avant et après occlusion, ainsi que les indispensables contrôles correspondants (ceux-ci ne pouvant justement pas être obtenus chez l'humain puisque cela nécessiterait de faire une endoscopie fœtale sans indication).

Perspectives :

Deux analyses sont prévues : la comparaison du protéome du liquide trachéal entre le singe et l'humain (pour évaluer leur similarité supposée), et l'analyse des modifications du protéome induites par l'occlusion trachéale chez le singe - p 89.

Moyens techniques :

Le développement du modèle du singe pour l'occlusion trachéale fœtale par endoscopie, ainsi que les prélèvements de liquide trachéal, ont été réalisés sur la plateforme *Simian Laboratory Europe* (SILABE) de l'Université de Strasbourg.

Les prélèvements de liquide trachéal sont stockés à une température de -80°C au sein de l'unité INSERM UMR-S 1121 « Biomatériaux et Bioingénierie ».

Les prélèvements de liquide trachéal seront analysés sur la plateforme protéomique CLIPP (Clinical Innovation Proteomic Plateform) à Dijon.

Acteurs et collaborations :

Les prélèvements humains seront réalisés par l'équipe de la *Mayo Clinic* (Rochester, Minessota, États-Unis) qui ont une pratique de l'occlusion trachéale chez l'humain.

Les analyses protéomiques seront analysées par l'équipe du *Lucile Packard Children's Hospital* (Stanford, Californie, États-Unis) qui ont une expertise sur le développement pulmonaire fœtal.

Financements :

Les travaux chez le singe ont été financés sur une Mission d'Intérêt Général et d'Aide à la Contractualisation (MIGAC).

Les analyses protéomiques seront financées sur un appel à projet de Recherche Médicale Appliquée de la Fondation de l'Avenir et le prix « jeune chercheur » de la CASDEN Banque populaire, obtenus en 2015 et en 2016.

6.4.2. Axe de recherche translationnelle

Problématique et objectif :

La principale limite de la technique d'occlusion trachéale fœtale par endoscopie en cas de hernie de coupole diaphragmatique est la question du retrait du ballonnet. Celui-ci doit se faire idéalement *in utero*, au cours d'une nouvelle endoscopie. Cette intervention est complexe et se fait dans un contexte d'urgence dans près de la moitié des cas, ce qui implique que les patientes restent à proximité d'un des rares centres d'expertises pendant les 6 semaines d'occlusion. Cela est souvent difficile en pratique et limite la diffusion de la technique. Aussi et surtout, cette deuxième intervention est à risque de morbidité induite, notamment en termes de prématurité.

L'objectif est de développer un ballonnet innovant, permettant une levée de l'occlusion simple, non invasive, par contrôle externe. Ce projet s'inscrit dans un travail de développement des techniques.

Travaux effectués :

Nous avons développé un prototype de ballonnet répondant à ce cahier des charges - p 96.

La solution technique est basée sur une valve à ouverture électromagnétique actionnable par le champ magnétique de fuite d'un appareil d'IRM. L'ouverture de la valve provoque la vidange du ballonnet, qui est ensuite expulsé dans la poche des eaux par les sécrétions pulmonaires.

Des tests in vitro de perméabilité, d'occlusion et de fonctionnement ont été réalisés avec succès.

Un brevet a été déposé le 2 mai 2016 (numéro d'enregistrement national FR 1653954 – classification internationale des brevets A61B17/12) : « Ballonnet gonflable et détachable, destiné à être implanté dans une cavité corporelle, nécessaire de traitement et procédé de vidange associés » - p 101.

Des tests pour évaluer le bon fonctionnement de la valve de vidange ont été réalisés sur simulateur ont été réalisés avec succès - p 124.

Des tests pour évaluer la faisabilité technique chez le singe ont été menés avec succès - p 129.

Perspectives :

Le modèle du singe est le modèle le plus adapté pour évaluer la faisabilité technique, ou encore pour des études physiologiques sur le développement pulmonaire comme nous en prévoyons. Cependant, certaines analyses ne peuvent pas être effectuées chez le singe, notamment car nous ne pratiquons en aucun cas d'euthanasie sur cette espèce.

Deux séries d'études expérimentales chez la brebis sont en cours ou prévues, tout d'abord pour analyser le caractère occlusif du ballonnet et les effets secondaires potentiels sur la trachée, puis ensuite pour évaluer la croissance pulmonaire induite par le ballonnet - p 135.

L'industrialisation de la fabrication du ballonnet et les démarches réglementaires nécessaires à la commercialisation sont en cours.

Moyens techniques :

Le dossier de valorisation et de transfert de technologie a été monté avec l'expertise de la Société d'Accélération du Transfert de Technologies (SATT) – Conectus Alsace et du pôle de compétitivité de la filière santé « Alsace Biovalley ».

Le développement du ballonnet et les tests in vitro ont été réalisés en partenariat avec la société *BS Medical Tech Industry*.

Les tests de faisabilité technique chez le singe ont été réalisés sur la plateforme *Simian Laboratory Europe* (SILABE) de l'Université de Strasbourg. L'actionnement de la vidange du ballonnet a été réalisé sur la plateforme de l'Institut Hospitalo-Universitaire (IHU).

Acteurs et collaborations :

La détermination de la solution technique a été faite en collaboration avec des acteurs de différentes institutions : Université de Strasbourg, INSERM UMR-S 1121 « Biomatériaux et Bioingénierie », Hôpitaux Universitaires de Strasbourg, Institut Hospitalo-Universitaire (IHU) de Strasbourg, Institut de Recherche contre les Cancers de l'Appareil Digestif (IRCAD) et *BS Medical Tech Industry*.

Le projet a été labellisé par le pôle de compétitivité de la filière santé « Alsace Biovalley ».

Les études expérimentales sur le modèle de la brebis, sont réalisées à l'Université Catholique de Louvain en Belgique, en partenariat avec le Professeur Jan Deprest.

Financement :

La réalisation d'un premier prototype et la preuve de concept ont été financés sur un appel à projet de Recherche Médicale Appliquée de la Fondation de l'Avenir et le prix « jeune chercheur » de la CASDEN Banque populaire, obtenus en 2015 et en 2016.

Le dépôt de brevet a été financé par la Société d'Accélération du Transfert de Technologies (SATT) – Conectus Alsace.

Les travaux chez le singe ont été financés sur une MIGAC.

Les premières étapes d'industrialisation en cours sont financées par un dispositif Partenariats Régionaux d'Innovation (PRI) obtenu en 2017, avec la société *BS Medical Tech Industry*.

6.4.3. Axe de recherche clinique

Problématiques et objectifs :

L'objectif principal est d'évaluer chez l'humain le ballonnet que nous avons mis au point, dans la continuité du travail de développement des techniques.

L'objectif secondaire consiste à identifier de façon la plus pertinente possible les candidats à qui une occlusion trachéale par foetoscopie pourrait véritablement bénéficier.

Travaux effectués :

Concernant la question de l'indication chirurgicale, plusieurs travaux de recherche clinique visant à identifier les candidats ont déjà été effectués et ont fait l'objet de 6 articles portant sur différents facteurs pronostiques : volume pulmonaire et moyens de l'évaluer - p 142, p 150 et p 157, position du foie et moyens de l'apprécier - p 152, évaluation de la vascularisation pulmonaire - p 169 et impact du côté de la hernie - p 181.

Perspectives :

Concernant le ballonnet que nous avons développé, la réalisation de tests de biocompatibilité sont prévus, car cela représente une étape réglementaire incontournable avant une première utilisation humaine.

Nous prévoyons ensuite de tester chez l'humain ce ballonnet que nous avons mis au point. Il est envisagé que le dispositif soit posé chez dix patientes afin de vérifier le caractère occlusif, le bon fonctionnement du système de vidange et l'absence d'effet indésirable inattendu.

Acteurs et collaborations :

La majorité des travaux cliniques effectués sur les aspects pronostiques ont été réalisés à l'occasion d'un *Research Fellowship* au *Texas Children's Fetal Center - Baylor College of Medicine*, Houston, Texas, États-Unis.

Le travail règlementaire et les tests de biocompatibilité seront sous la responsabilité de l'industriel BS - *Medical Tech Industry*.

Il est prévu que les premiers tests du ballonnet chez l'humain soient réalisés en collaboration avec le Professeur Alexandra Benachi (Université Paris Sud) et le Professeur Jan Deprest (Université Catholique de Louvain).

Financements :

Le *Research Fellowship* à Houston a été financé par une bourse de la commission franco-américaine « Fulbright ».

Concernant les tests de biocompatibilité et les premiers tests du ballonnet chez l'humain, un financement Horizon 2020 "Actions de recherche et d'innovation" est envisagé.

Au cas où ce financement n'est pas obtenu, d'autres sources de financement seront activées en parallèle : une candidature au prix Matmut de l'innovation a été déposée (pour les tests de biocompatibilité), une demande de CRC Innovation (Contrat de Recherche Clinique) sera faite pour financer l'étude clinique à l'APHP, et un financement interne de l'Université Catholique de Louvain sera demandé pour assurer la partie belge de l'étude clinique.

6.5. Autres thématiques de recherche

6.5.1. Pathologies des grossesses monochoriales

Syndrome transfuseur-transfusé :

Le syndrome transfuseur transfusé (STT) est la complication des grossesses monochoriales dont la prise en charge est la mieux codifiée. Celle-ci consiste à en une photocoagulation laser des anastomoses vasculaires entre les deux circulations fœtales, par voie endoscopique. Cependant, certaines questions techniques et d'indication chirurgicale restent encore à préciser.

Une sélection appropriée des candidats à la chirurgie implique une bonne connaissance du pronostic des grossesses et des enfants après photocoagulation laser, à court et à long terme. Nous avons rapporté notre expérience sur une série de 200 lasers, en se focalisant en particulier sur les paramètres pronostiques - p 194. Par ailleurs, nous avons analysé le pronostic neurologique des enfants à long terme - p 201.

Concernant toujours la question de la bonne indication, la classique classification de Quintero est probablement insuffisante car elle n'intègre que très peu de données hémodynamiques. Nous avons étudié l'intérêt pronostique de la fonction cardiaque fœtale - p 220, en particulier dans les stades précoces - p 227.

Enfin, pour ce qui est des aspects techniques, la question de la réalisation d'une photocoagulation laser précocement dans la grossesse est intéressant à explorer car l'essai randomisé qui avait démontré l'intérêt du laser n'incluait pas de grossesse avant 16 semaines. Nous avons donc étudié spécifiquement nos cas où un laser avait été réalisé tôt dans la grossesse - p 234.

Twin Anemia Polycythemia Sequence :

La *Twin Anemia Polycythemia Sequence* (TAPS) est une entité qui n'a été décrite que récemment, en 2017, et sa prise en charge n'est pas encore bien codifiée.

La question de la sélection des candidats implique avant tout une juste identification des critères diagnostiques. Nous avons donc étudié la pertinence et la fiabilité des critères diagnostiques de TAPS - p 239.

Par ailleurs, la question de la technique de prise en charge est également importante. En effet, une photocoagulation laser est techniquement plus délicate qu'en cas de STT (pas d'hydramnios et anastomoses minuscules) et la transfusion in utero n'est qu'un traitement symptomatique. C'est pourquoi nous avons tenté d'évaluer la faisabilité et l'intérêt d'une intervention prénatale - p 247.

Retard de croissance intra-utérin sélectif :

La prise en charge prénatale du retard de croissance intra-utérin sélectif (RCIUs) n'est pas bien codifiée non plus. Non seulement la question de l'indication chirurgicale est controversée, mais le type d'intervention l'est également. En particulier la photocoagulation laser est techniquement difficile (pas d'hydramnios), elle ne permet pas toujours de sauver le fœtus en restriction de croissance et peut s'avérer à risque pour le fœtus eutrophe.

Nous avons donc rapporté notre expérience dans la prise en charge des RCIUs, en fonction de la sévérité du RCIU et de la prise en charge réalisée - p 256.

Perspectives :

Le projet le plus ambitieux est celui de l'analyse des données de suivi neurodévelopmental pour les enfants issus de grossesses monochoriales. La constitution d'un réseau de suivi exhaustif des nouveau-nés vulnérables est planifié dans le PRS périnatalité 2018-2022 de la région Grand Est. Sa mise en place est initiée au sein de la COordination PErinatale du Grand Est (COPEGE) et incombe aux Réseaux périnatals de chaque territoire. Cette mise en place sera opérationnelle en 2019 et permettra le recueil exhaustif et harmonisé des données du suivi des enfants jusqu'à l'âge de 7 ans sur une base de données sécurisée informatique. Il s'agit là d'une opportunité très précieuse de chaîner la prise en charge prénatale au devenir des enfants et ainsi de répondre à certaines de nos questions de recherche sur la chirurgie endoscopique fœtale.

6.5.2. Uropathies obstructives basses

Problématique et travaux effectués :

En cas d'obstruction urinaire basse, la dérivation des urines par la mise en place d'un drain vésico-amniotique pourrait permettre d'améliorer la survie néonatale et potentiellement la fonction rénale. L'intérêt de cette prise en charge est controversé et la sélection adéquate des candidats est particulièrement cruciale. C'est pourquoi nous avons proposé une standardisation de la prise en charge prénatale - p 262. De plus, nous avons également évalué l'intérêt de la réalisation itérative de ponctions vésicales fœtales pour analyse biochimique, dans le but d'améliorer la sélection des candidats (article en cours de rédaction).

La prise en charge prénatale des uropathies obstructives basses soulève également des questions techniques car le drain vésico-amniotique peut se boucher et se déplacer. La cystoscopie fœtale est une technique potentiellement prometteuse car elle permet de faire un diagnostic étiologique en visualisant l'urètre postérieur et, en cas de valves, il est alors possible de les effondrer au laser et d'obtenir un drainage physiologique des urines. Nous avons analysé la survie et la fonction rénale après dérivation vésico-amniotique - p 286 ou cystoscopie - p 293. Cependant, la réalisation d'une ablation par laser des valves est à risque de fistule et pose de véritables questions techniques - p 300.

Perspectives :

Concernant les considérations techniques de la cystoscopie fœtale, nous sommes en train d'évaluer l'intérêt d'un endoscope flexible permettant de brûler les valves sans prendre un risque trop important de fistules. D'autres techniques sont envisagées, telles que l'effondrement mécanique des valves.

Enfin, des données à long terme sur la fonction rénale des enfants présentant une obstruction urinaire basse (pris en charge ou non) seraient très utiles pour guider la prise en charge prénatale. Nous envisageons d'intégrer ces enfants dans le réseau de suivi des nouveau-nés vulnérables prévu dans le PRS périnatalité 2018-2022 de la région Grand Est.

6.5.3. Myéloméningocèle

Problématique :

L'intérêt de la prise en charge prénatale par chirurgie ouverte de la myéloméningocèle a été démontrée dans un essai randomisé. Cependant, ce type de chirurgie invasive présente une morbidité certaine. La foetoscopie est une alternative intéressante mais est techniquement difficile car elle implique la réalisation de dissections et de sutures en milieu liquidien. C'est pourquoi il a été proposé de remplacer une partie du liquide amniotique par du CO_2, le temps de la chirurgie endoscopique. Cette nouvelle approche constitue potentiellement un nouveau paradigme dans le domaine de la chirurgie fœtale, mais la sécurité de l'insufflation d'un gaz dans l'utérus pendant la grossesse doit encore être démontrée. Des expérimentations animales sont nécessaires pour évaluer les effets de la foetoscopie au gaz sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal. Certaines évaluations ont déjà été conduites chez la brebis gestante mais elles ne sont malheureusement pas extrapolables à l'humain car la placentation chez la brebis est syndesmo-choriale et non hémo-choriale, c'est-à-dire que, contrairement à l'humain, il n'y a pas de contact entre le sang maternel et l'épithélium chorial.

Perspectives :

Nous envisageons une approche avant-tout translationnelle pour cette nouvelle thématique de recherche, en nous focalisant sur la question du développement des techniques. Pour cela, nous utiliserons notre modèle de foetoscopie chez le macaque, dont la placentation est de type hémo-choriale et très proche de celle de l'humain. Nous travaillerons avec l'équipe du Texas Children's Fetal Center de Houston, qui a une expérience clinique de la foetoscopie au gaz. L'objectif est d'évaluer les effets de la fœtoscopie au gaz (CO2 et Hélium) sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal, dans le modèle du macaque - p 307.

- Quatrième partie -

ARTICLES
ET TRAVAUX RÉALISÉS

7. HERNIE DE COUPOLE DIAPHRAGMATIQUE

7.1. Modèle du singe

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE

http://informahealthcare.com/jmf
ISSN: 1476-7058 (print), 1476-4954 (electronic)

J Matern Fetal Neonatal Med, 2015; 28(15): 1822–1827
 DOI: 10.3109/14767058.2014.969234

informa healthcare

ORIGINAL ARTICLE

Experimental fetal endoscopic tracheal occlusion in rhesus and cynomolgus monkeys: nonhuman primate models

Nicolas Sananès[1,2], Rodrigo Ruano[3], Anne-Sophie Weingertner[1], Pierrick Regnard[4], Yves Salmon[4], Anne Kohler[1], Claire Miry[1], Cécile Mager[1], Fernando Guerra[1], Anne Schneider[5], François Becmeur[5], Joël Leroy[6], Jean-Luc Dimarcq[7], Christian Debry[8], and Romain Favre[1]

[1]Department of Fetal Medicine, Strasbourg Teaching Hospital, France, [2]INSERM, UMR-S, "Biomatériaux et Bioingénierie", Strasbourg Cedex, France, [3]Texas Children's Fetal Center and Baylor College of Medicine, USA, [4]SILABE – ADUEIS Platform, Niederhausbergen, France, [5]Department of Pediatric Surgery, Strasbourg Teaching Hospital, France, [6]IRCAD – EITS, Strasbourg Teaching Hospital, France, [7]Scientific foundation IHU Strasbourg, France, and [8]Department of Ear, Nose and Throat, Strasbourg Teaching Hospital, France

ABSTRACT

Objective: The monkey model is the best model to investigate some physiological response to the fetal transitory tracheal occlusion but it has never been described in *Macaca* monkeys. The aim of this study was to evaluate the feasibility of fetal endoscopic tracheal occlusion (FETO) in a non-human primate model.

Methods: Pregnant rhesus monkeys and cynomolgus were tested as a potential experimental model for FETO in the third trimester of pregnancy, by performing fetal tracheoscopies with and without tracheal occlusion.

Results: A total of 22 pregnancies were followed in 16 monkeys and underwent fetal surgery. Percutaneous endoscopic access to the uterine cavity was possible in 20 cases (91%). Of these 20 pregnant monkeys, fetal tracheoscopy could be achieved in 15 cases (75%). In rhesus monkeys, the time between the onset of endoscopy and tracheal penetration decreases as operator experience increases. Neither maternal morbidity nor mortality was related to surgery. Two fetal losses were possibly due to the procedure.

Conclusion: FETO is feasible in the non-human primate, which closely reflects procedures in humans. The non-human primate model for FETO, specially the rhesus monkeys, may be useful for future studies concerning the mechanisms related to the lung growth after transitory fetal tracheal occlusion.

Keywords

Cynomolgus monkey, endoscopic surgery, fetal endoscopic tracheal occlusion, non-human primate, rhesus monkey

History

Received 24 May 2014
Accepted 22 September 2014
Published online 9 October 2014

Introduction

Congenital diaphragmatic hernia (CDH) is one of the most common isolated fetal malformations. CDH occurs in 1/3000 to 1/5000 live births [1]. CDH is associated with hypoplasia of both lungs, which affects airways and vessels with subsequent pulmonary hypertension. This leads to approximately 30% neonatal mortality in tertiary centres, even though CDH is a surgically correctable defect [2]. Promoting lung growth during fetal life is therefore of benefit.

Fetal tracheal occlusion (TO) increases airway pressure and prevents egress of lung fluid, leading to improved growth of the alveolar airspace and maturation of the pulmonary vasculature [3]. A minimally invasive approach has been developed which is known as fetal endoscopic tracheal occlusion (FETO) [4,5]. A thin endoscope is percutaneously introduced into the fetal trachea in order to position a detachable balloon between the carina and vocal cords at between 27 and 30 weeks. The balloon is ideally removed prenatally by fetal tracheoscopy or punctured by ultrasound-guided needle at around 34 weeks. Several studies have shown an improvement of neonatal survival in severe cases [6,7].

Different animal models have been used to study the effect of TO on lung growth: sheep (*Ovis aries*), rabbits (*Oryctolagus cuniculus*), rats (*Rattus norvegicus*) and mice (*Mus musculus*). Every model has strengths and weaknesses which vary depending on ethical issues, cost, animal and lung size, surgical difficulty, abortion rates, alveolarization timing, genome knowledge, and availability of genes and protein sequences [3]. For this purpose, the sheep model has been used for endoscopic TO, but unfortunately this model does not represent entirely the same condition observed in humans [8,9]. A non-human primate model may be a relevant translational model for fetal surgery, especially FETO, because both anatomy and physiology are closed to humans [3,10]. There are only few studies evaluating fetal surgery in monkeys and only three cases of FETO in baboons (*Papio* spp) have been recently reported [11–14]. In addition, to our

Address for correspondence: Dr. Nicolas Sananès, Service de Gynécologie Obstétrique, Hôpitaux Universitaires de Strasbourg, 67091 Strasbourg cedex, France. Tel: +33 3 88 12 78 39. E-mail: nicolas.sananes@chru-strasbourg.fr

knowledge, there is no study evaluating different types of monkey foetuses would be adequate as a FETO experimental model. Therefore, the objective of this study was to evaluate the feasibility of fetal surgery in non-human primates using exclusively percutaneous fetoscopy by testing two different models.

Methods

The monkeys (16 females from 6 reproduction groups) were group-housed in indoor/outdoor facilities in SILABE plateform (Simian Laboratory Europe). They lived in social groups (1 or 2 adult males and 4 to 10 females) in indoor/outdoor facilities of approximately 40 to 85 m^3 for the indoor facility (depending on the animal facility and group) and 40 to 50 m^3 for the outdoor facility. Water and food were distributed *ad libitum*. Animals were captured regularly in order to perform ultrasound to detect pregnancy and establish the gestational age. The timing of surgery was determined by ultrasound measurement of the biparietal diameter of the fetus in early pregnancy (Voluson E GE®). Pregnant rhesus monkeys (*Macaca mulata*) and cynomolgus (*Macaca fascicularis*) underwent fetal surgery in the last third trimester of pregnancy . Pregnant female were captured on the day of the surgery. After surgery, they were isolated until the following morning and were then returned to their social group. The Regional Ethics Committee on Animal Experimentation (CREMEAS) at Strasbourg University, France (number AL/02/03/03/09) approved this study. Guidelines for the care and use of the monkeys approved by this institution were followed.

A total of 22 pregnancies (12 in rhesus and 10 in cynomolgus monkeys) were followed in 16 monkeys and subsequently underwent fetal surgery. Gestational dates were interpreted by ultrasound measurement of the biparietal diameter on average at 66 ± 15 days' gestation (40 to 110). Surgery took place on average at 133 ± 5 days' gestation (124 to 144): 135 days in rhesus and 131 days in cynomolgus, representing 81.8% of the gestational period in rhesus and 79.9% in cynomolgus monkeys, assuming gestational lengths of 165 and 164 days respectively [15].

The pregnant monkey was first sedated with ketamine (10 mg/kg, IM) before general anesthesia with propofol (5 mg/kg, IV) and 1 to 2.5% isoflurane after tracheal intubation. Ventilation was controlled by assisted mechanical respiration after tracheal intubation. Maternal breath frequency, electrocardiogram, PO_2/PCO_2 measurement, halogenated gas and temperature were continually monitored and a heating blanket was applied to maintain body temperature. Maternal blood pressure was monitored with a sphygmomanometer. A single dose of 2% marbocyl was given (Marbofloxacin 2 mg/kg, IM) prior to surgery. No tocolytic was administered.

Before surgery, fetal monkey curarization was performed by an intra-muscular injection of 0.15 to 0.20 mg cisatracurium in the fetal leg, under ultrasound guidance. After localization of the fetal head and placenta in order to determine the optimal entry site, 5 ml 2% xylocaine was injected percutaneously into the myometrium of the pregnant monkey in order to provide additional local anesthesia. Endoscopic access was performed using the modified Seldinger technique under continuous ultrasound guidance [16,17]. A 17G needle was first introduced into the uterine cavity and an amnioinfusion of 200 to 300 mL saline administered. A guide wire was then introduced through the needle and a 10 French-gauge arterial introducer set placed over this guide. Instrument specifications for FETO procedure followed the usual recommendations [5]. A 1.3 mm fetal tracheoscope was inserted through a 3.3 mm sheath 30°-precurved with 3 side ports (11540AA and 11540KE, Karl Storz, Tuttlingen, Germany). A Goldbal 2 detachable balloon (Balt, Montmorency, France) was used for TO. Retrieval forceps (11510C, Karl Storz) and adjustable puncture stylet (11506P, Karl Storz) were used for balloon removal. Amnioinfusion of saline was performed during the procedure in order to open the fetal mouth and vocal cords.

Surgical procedures involved fetal tracheoscopies, with or without TO. When TO was performed, balloon removal was done in the same surgery session. A single operator performed all surgical procedures. Excessive amount of the infused fluid was drained through the trocar after surgery, under ultrasound guidance.

Results

Percutaneous endoscopic access inside the uterine cavity could be performed in 20 cases (91%). Of these 20 pregnant monkeys, fetal tracheoscopy was feasible in 15 cases (75%): 9 rhesus (90%) and 6 cynomolgus monkeys (60%). TO was attempted in 3 rhesus monkeys; it took place without incident, as well as the unplugging procedure.

The duration between onset of endoscopy and tracheal penetration was on average 19 ± 9 min (4 to 34): 17 ± 10 min in rhesus (4 to 35) and 22 ± 5 min in cynomolgus (13 to 28) monkeys. In rhesus monkeys, this duration decreased with increasing operator experience (Figure 1). This correlation was not replicated in the cynomolgus monkey (Figure 2).

The fetal monkey model reflects very closely human fetal anatomy. Figure 3 shows endoscopic views of the fetal monkey.

Gestational age at delivery was on average 158 ± 14 days (131 to 203). One mother died during delivery because of fetal malpresentation (arm dystocia). No other maternal mortality or morbidity was observed. There were 6 cases of

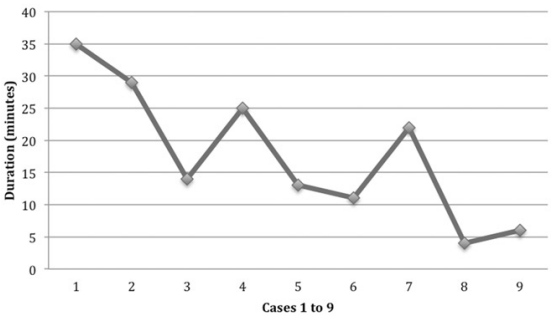


Figure 1. Duration between the beginning of endoscopy and the penetration into the trachea in fetal rhesus monkeys (first to ninth case). In rhesus monkeys, the duration between onset of endoscopy decreased with increasing operator experience.

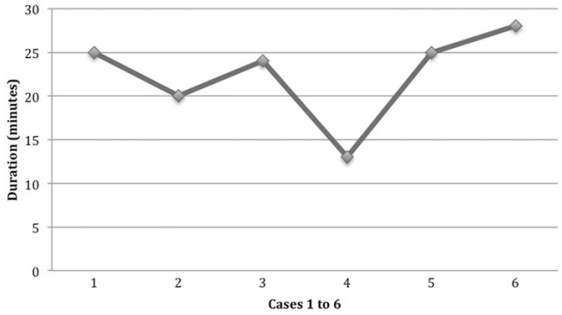
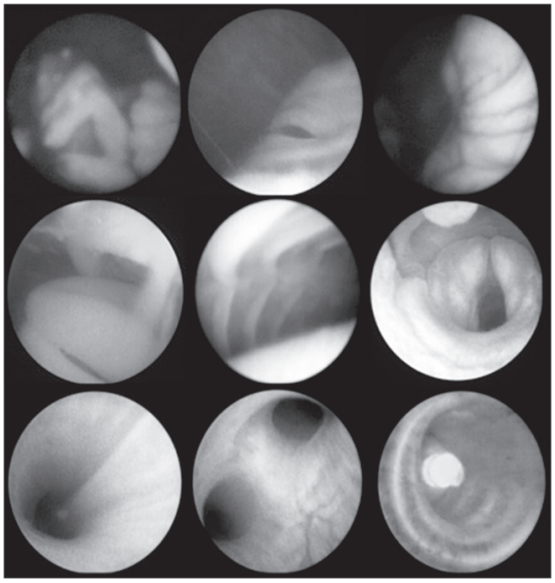


Figure 2. Duration between the beginning of endoscopy and the penetration into the trachea in fetal cynomolgus monkeys (first to sixth case). In cynomolgus monkeys, the duration between onset of endoscopy doesn't decrease with operator experience.



Hand	Eye	Profile
Mouth and tongue	Palate	Vocal cords
Trachea	Bifurcation	Balloon

The monkey model reflects closely human fetal anatomy

Figure 3. Endoscopic views of the fetal monkey.

poor neonatal outcome (27%): 2 intra-uterine fetal deaths and 4 perinatal deaths. Among these cases, only 2 fetal losses were possibly due to the surgery (9%): one intra-uterine fetal death in which fetal reversion was attempted during surgery and one perinatal death for which the only possible detected cause was *Staphylococcus aureus* in the neonatal lungs.

Characteristics of the animals, surgical details and outcomes are displayed in Table 1. Comparison between the two non-human primate models for FETO is displayed in Table 2.

Discussion

Principal findings

Our study demonstrated that non-human primate model is feasible to preform FETO, by showing that FETO was successfully performed by a single port access in all cases. Moreover, the present model, specially the Rhesus monkey, reflected anatomy on fetoscopic views very close to the human fetuses. In addition, FETO was performed mimicking the human surgery by using the same instruments. We believe that this model may be useful for future experimental studies that will investigate (1) the mechanisms related to the fetal growth after FETO [18], (2) the effects of early FETO [19], and (3) the tracheal and pulmonary effects on FETO.

Exclusive percutaneous endoscopic access to the fetal monkey with a single trocar was possible in most cases. However, in spite of the thinness of the subcutaneous layer and uterine wall in monkeys, percutaneous access is not that straightforward due to tissue elasticity. The needle has to be introduced directly, in one smooth motion, under ultrasound guidance. Amnioinfusion of saline is necessary because amniotic liquid is scanty in pregnant monkeys, especially in the last third of pregnancy.

Tracheoscopy was successful in three-quarters of our cases. It was much easier in rhesus monkeys than in cynomolgus ones, because the larynx and trachea are wider. When endoscopic access to the uterine cavity was successful in rhesus monkeys, tracheoscopy failed in only one case because of a problem of visibility due to bleeding. In contrast, most failed tracheoscopy attempts in cynomolgus monkeys were attributed to anatomical issues. It should be noted that the cynomolgus is still an interesting model for fetal surgery because of its non-seasonal breeding.

When TO was attempted it took place without incident. It should be noted that balloon removal was performed in the same surgery session, since the aim of this study was to evaluate the feasibility of TO in the fetal monkey, not the effect of TO on lung growth. We would suggest that other animal models are more suitable for this purpose.

All fetal losses occurred in the cynomolgus group. We assume that this mortality is partly due to a colony issue. Indeed, this group was mostly composed of relatively elder female primigravidas (around 10 years' old). Three fetal losses occurred in mature primigravidas and 2 fetal losses in females who had had a prior fetal loss. Moreover, in a control group of 6 monkeys from the same cynomolgus colony, rates of poor neonatal outcome were even higher: 50% of fetal losses and 83% of early neonatal mortality (before 7 days' life).

Finally, in our series, only 2 fetal losses occurred, which were possibly due to the surgery. It should be noted that in order to avoid premature births, surgery was scheduled for the last third trimester of pregnancy as suggested before by other investigators using the non-human primate model for other fetal surgical procedures [13,20].

Table 1. Characteristics of animals and surgeries.

Cases	Macaque ID	Subspecies	Age (years)	Weight (kg)	Parity	Previous fetal loss	GA at surgery (days)	Surgical procedure	Time to trachea (min)	GA at birth (days)	Fetal outcome	Maternal outcome	Comments
1	1	Rhesus	14.8	7.7	1	0	130	Failure of fetoscopic access	NA	173	Alive	Alive	
2	2	Rhesus	6.0	6.2	2	0	129	Foetoscopy/tracheoscopy	35	149	Alive	Alive	
3	3	Rhesus	3.0	7.1	0	0	134	Foetoscopy/tracheoscopy	29	155	Alive	Alive	
4	4	Rhesus	18.0	6.7	5	0	139	Foetoscopy/tracheoscopy	14	164	Alive	Alive	
5	5	Cynomolgus	12.0	5.9	0	0	127	Foetoscopy	NA	131	Perinatal death	Death	Arm dystocia
6	6	Cynomolgus	11.3	5.3	0	0	127	Foetoscopy/tracheoscopy	25	145	Alive	Alive	
7	7	Cynomolgus	9.3	5.0	0	0	132	Foetoscopy	NA	158	IUFD	Alive	Reversion of fetus during surgery
8	8	Cynomolgus	10.6	6.3	0	0	127	Foetoscopy/tracheoscopy	20	145	Perinatal death	Alive	No maceration of the new-born
9	3	Rhesus	4.0	7.1	1	0	144	Foetoscopy	NA	155	Alive	Alive	
10	6	Cynomolgus	12.0	5.3	1	0	124	Foetoscopy	NA	145	Perinatal death	Alive	Cephalic subcutaneous hematoma
11	1	Rhesus	16.0	7.7	2	0	132	Foetoscopy/tracheoscopy	25	163	Alive	Alive	
12	7	Cynomolgus	10.0	5.0	1	1	135	Foetoscopy/tracheoscopy	24	158	Alive	Alive	
13	9	Rhesus	10.8	9.1	6	2	138	Failure of fetoscopic access	NA	162	Alive	Alive	
14	10	Rhesus	3.9	6.1	0	0	136	Foetoscopy/tracheoscopy	13	153	Alive	Alive	
15	11	Rhesus	13.2	9.0	6	0	138	Foetoscopy/tracheoscopy/plug	11	163	Alive	Alive	
16	12	Rhesus	12.1	7.8	3	0	135	Foetoscopy/tracheoscopy	22	166	Alive	Alive	
17	6	Cynomolgus	12.7	5.3	2	1	139	Foetoscopy	NA	156	Alive	Alive	
18	13	Cynomolgus	8.8	5.7	2	1	134	Foetoscopy/tracheoscopy	13	203	IUFD	Alive	Post-term pregnancy
19	14	Cynomolgus	13.7	6.5	1	1	133	Foetoscopy/tracheoscopy	25	159	Perinatal death	Alive	*Staphylococcus aureus* in neonate lungs
20	15	Rhesus	12.5	7.3	1	0	132	Foetoscopy/tracheoscopy/plug	4	159	Alive	Alive	
21	16	Cynomolgus	13.2	7.7	2	0	136	Foetoscopy/tracheoscopy	28	162	Alive	Alive	
22	9	Rhesus	12.9	9.1	7	2	134	Foetoscopy/tracheoscopy/plug	6	143	Alive	Alive	

NA: not applicable; GA: gestational age; IUFD: intra-uterine fetal death.

Table 2. Comparison between the two non-human primate models for FETO.

	Rhesus monkeys $n = 12$	Cynomolgus monkeys $n = 10$
Age: years (mean ± SD)	10.6 ± 5.1	11.4 ± 1.7
Weight: kg (mean ± SD)	7.6 ± 1.0	5.8 ± 0.8
Previous fetal loss (n, %)	2 (17%)	4 (40%)
GA at surgery: days (mean ± SD)	136 (±4)	131 (±5)
Tracheoscopy (*n*, %)	9 (75%)	6 (60%)
Time to trachea: min (mean ± SD)	17 ± 10	22 ± 5
GA at birth (mean ± SD)	159 ± 8	156 ± 19
IUFD or perinatal death (n)	0 (0%)	6 (60%)
Maternal death (n)	0 (0%)	1 (10%)

SD: standard deviation; GA: gestational age; IUFD: intra-uterine fetal death.

Previous publications

Open fetal surgery with hysterotomy in primates was developed in the 1980s [21–23]. Several operations have been attempted: cystotomy, suprapubic catheterization, relief of urethral obstruction and perineal dissection [22]. Various topics have been studied: scar formation [24], brain development [25] and stem cell and bone transplantation [26–28].

Endoscopic access in fetal monkey model was evaluated in the 1990s [11–13]. However, this did not involve percutaneous endoscopic access, as it is deployed in most current human endoscopic fetal surgical procedures: midline abdominal incision and partial uterine mobilization from the abdominal cavity were performed before endoscopic access.

Experiments on congenital diaphragmatic hernia using the monkey model involved *in utero* repair of the diaphragm [29]. This technique has been abandoned because of failure to improve the newborn outcome and hysterotomy-induced maternal morbidity [30].

Three cases of FETO in baboons (*Papio* spp) have been recently reported [14]. Baboons have the same type of placentation than human but these monkeys have long dog-like muzzles that could be an issue for practicing FETO [10]. Beyond theses cases, no data have been published on exclusively percutaneous endoscopic access for FETO using the fetal monkey model. Only the sheep model has been used for endoscopic FETO [8,9]. The monkey model more closely reflects human fetal anatomy, especially in respect of the fetal face, mouth, vocal cords and trachea.

Conclusions

Experimental FETO is feasible using a monkey model specially using the rhesus *Macaca*. This non-human primate model seems to be very similar to humans, which allows future studies related to the anatomy and consequences of the fetal transitory tracheal occlusion to the lung growth.

Acknowledgements

The authors thank Karl Storz for the laparoscopic unit.

Declaration of interest

This study was funded by the French Ministry of Health MIGAC research fund (Missions of General Interest and Assistance to Contracting) to the Fetal Medicine Department, Strasbourg, France.

References

1. Butler N, Claireaux AE. Congenital diaphragmatic hernia as a cause of perinatal mortality. Lancet 1962;1:659–63.
2. Gallot D, Boda C, Ughetto S, et al. Prenatal detection and outcome of congenital diaphragmatic hernia: a French registry-based study. Ultrasound Obstet Gynecol 2007;29:276–83.
3. Khan PA, Cloutier M, Piedboeuf B. Tracheal occlusion: a review of obstructing fetal lungs to make them grow and mature. Am J Med Genet C Semin Med Genet 2007;145C:125–38.
4. Deprest J, Gratacos E, Nicolaides KH. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results. Ultrasound Obstet Gynecol 2004;24:121–6.
5. Deprest J, Nicolaides K, Done E, et al. Technical aspects of fetal endoscopic tracheal occlusion for congenital diaphragmatic hernia. J Pediatr Surg 2011;46:22–32.
6. Jani JC, Nicolaides KH, Gratacos E, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. Ultrasound Obstet Gynecol 2009;34:304–10.
7. Ruano R, Yoshisaki CT, da Silva MM, et al. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2012;39:20–7.
8. Flageole H, Evrard VA, Vandenberghe K, et al. Tracheoscopic endotracheal occlusion in the ovine model: technique and pulmonary effects. J Pediatr Surg 1997;32:1328–31.
9. Evrard VA, Verbeken EA, Vandenberghe K, et al. Endoscopic In Utero Tracheal Plugging in the Fetal Lamb to Treat Congenital Diaphragmatic Hernia. J Am Assoc Gynecol Laparosc 1996;3:S11.
10. Schlabritz-Loutsevitch NE, Lopez-Alvarenga JC, Comuzzie AG, et al. The prolonged effect of repeated maternal glucocorticoid exposure on the maternal and fetal leptin/insulin-like growth factor axis in Papio species. Reprod Sci 2009;16:308–19.
11. van der Wildt B, Luks FI, Steegers EA, et al. Absence of electrical uterine activity after endoscopic access for fetal surgery in the rhesus monkey. Eur J Obstet Gynecol Reprod Biol 1995;58:213–14.
12. Feitz WF, Steegers EA, Aarnink RG, et al. Endoscopic intrauterine fetal therapy: a monkey model. Urology 1996;47:118–19.
13. Feitz WF, Steegers EA, de Gier RP, et al. Feasibility of minimally invasive intrauterine fetal access in a monkey model. J Urol 1999;161:281–5.
14. Mari G, Deprest J, Schenone M, et al. A novel translational model of percutaneous fetoscopic endoluminal tracheal occlusion – baboons (*Papio* spp.). Fetal Diagn Ther 2014;35:92–100.
15. Abee CR, Mansfield K, Tardif S, Morris T. Nonhuman Primates in Biomedical Research: Biology and Management. London, UK: Academic Press; 2012.
16. Hajivassiliou CA, Nelson SM, Dunkley PD, et al. Evolution of a percutaneous fetoscopic access system for single-port tracheal occlusion. J Pediatr Surg 2003;38:45–50 (discussion 45–50).
17. Luks FI, Deprest JA, Gilchrist BF, et al. Access techniques in endoscopic fetal surgery. Eur J Pediatr Surg 1997;7:131–4.
18. Ruano R, da Silva MM, Campos JA, et al. Fetal pulmonary response after fetoscopic tracheal occlusion for severe isolated congenital diaphragmatic hernia. Obstet Gynecol 2012;119:93–101.
19. Ruano R, Peiro JL, da Silva MM, et al. Early fetoscopic tracheal occlusion for extremely severe pulmonary hypoplasia in isolated congenital diaphragmatic hernia: preliminary results. Ultrasound Obstet Gynecol 2013;42:70–6.
20. Silk JSJ, Roberts J, Kusnitz J. Gestation length in Rhesus macaques (*Macaca mulatta*). Int J Primatol 1993;14:95–104.

21. Michejda M, Bacher J, Johnson D. Surgical approaches in fetal radiography and the study of skeletal age in Macaca mulatta. J Med Primatol 1980;9:50–61.
22. Harrison MR, Anderson J, Rosen MA, et al. Fetal surgery in the primate I. Anesthetic, surgical, and tocolytic management to maximize fetal-neonatal survival. J Pediatr Surg 1982;17: 115–22.
23. Adzick NS, Harrison MR, Glick PL, et al. Fetal surgery in the primate. III. Maternal outcome after fetal surgery. J Pediatr Surg 1986;21:477–80.
24. Lorenz HP, Whitby DJ, Longaker MT, et al. Fetal wound healing. The ontogeny of scar formation in the non-human primate. Ann Surg 1993;217:391–6.
25. Michejda M, Bacher J. Functional and anatomic recovery in the monkey brain following excision of fetal encephalocele. Pediatr Neurosci 1985;12:90–5.
26. Cowan MJ, Chou SH, Tarantal AF. Tolerance induction post in utero stem cell transplantation. Ernst Schering Res Found Workshop 2001;145–71.
27. Mychaliska GB, Rice HE, Tarantal AF, et al. In utero hematopoietic stem cell transplants prolong survival of postnatal kidney transplantation in monkeys. J Pediatr Surg 1997; 32:976–81.
28. Michejda M, Bacher J, Kuwabara T, et al. In utero allogeneic bone transplantation in primates: roentgenographic and histological observations. Transplantation 1981;32:96–100.
29. Esteve C, Toubas F, Gaudiche O, et al. Evaluation of 5 years of experimental in utero surgery for the repair of diaphragmatic hernia. Ann Fr Anesth Reanim 1992;11:193–200.
30. Harrison MR, Adzick NS, Bullard KM, et al. Correction of congenital diaphragmatic hernia in utero VII: a prospective trial. J Pediatr Surg 1997;32:1637–42.

7.2. Analyse protéomique du liquide trachéal chez le singe (résultats préliminaires)

Plate-forme Protéomique
l'innovation en recherche clinique

15 Bd Marechal de Lattre de Tassigny
Bat. Médecine B3
BP37013
21070 Dijon Cedex
Tél : (33) 3 80 39 66 53
Fax : (33) 3 80 39 66 52
geraldine.lucchi@clipproteomic.fr

Certificat N°2001/16873.10
SATT GRAND EST

CLIPP est un prestataire-partenaire de la SATT Grand-Est, dans le cadre de ces activités Welience, certifiée ISO9001

Etude des effets de l'occlusion trachéale : Analyse quantitative label-free de liquide trachéal fœtal de singe

345_02_01

Compte rendu préliminaire

	Rédaction
Nom	Pauline Maes
N° Devis	Devis_345_01_02_180427

Objectif

L'objectif est de réaliser une analyse quantitative en label free des échantillons de liquide trachéal.

1 – Information sur l'échantillon

8 échantillons de 1 à 2mL dont 7 en duplicate ont été reçus à la plateforme. Ces échantillons proviennent de 4 individus dont 2 individus (Greandine et Gargouille) ont été opérés pour une pose de ballon dans la trachée du foetus puis pour enlever ce ballon une semaine après. Les deux autres individus (Charlotte et Marina) servent de témoins. Les échantillons ont été prelevés au temps T1 (avant pose du plug) puis après au temps T2 (après pose du plug)

ID	date intervention	Intervention	Commentaire	Numéro Echantillon Clipp
Charlotte	17/03/2016	Tracheo1		1
Charlotte	07/04/2016	Tracheo2		2
Marina	24/03/2016	Tracheo1		3
Marina	31/03/2016	Tracheo 2		4
Grenadine	12/05/2016	Plug		5
Grenadine	19/05/2016	Unplug		6
Gargouille	25/05/2016	Plug		7
Gargouille	01/06/2016	Unplug	Conditions de prélèvement non optimales	8

A l'examen visuel, il a été constaté que l'échantillon de Grenadine après le plus était très différent des autres car il était blanc trouble et contenait des éléments visqueux (voir fig1) alors que l'échantillon de Gargouille après plug était transparent et similaire aux autres.

Figure 1 Photo de l'échantillon de Grenadine après plug (à gauche) et de l'échantillon de Gargouille après plug (à droite)

2 – Analyses

2.1) Elimination de l'albumine et des IgG

Un 1er dosage BCA des liquides trachéaux après décongélation a été réalisé afin d'évaluer leur concentration protéique totale.

500µg de protéines par échantillons ont été traitées avec 170µL de résine du kit de déplétion de ThermoFisher (Pierce albumin/Igg Removal kit). La déplétion a été réalisée suivant les recommendations du fournisseur. Le filtrat a ensuite été centrifugé à 10000g pendant 1h à 4°C et concentré en utilisant les filtres Amicon 0,5mL (Millipore) avec un cut-off de 3kDa jusqu'à un volume de 35 à 40µL.

200µL de SDS 2%, Tris 10mM et EDTA 1mM ont été ajouté à la résine afin de récupéré la fraction de chaque échantillon retenue par la résine. Cette fraction a également été concentrée avec les filtres Amicon jusqu'à atteindre un volume de 40µL.

Un 2^{e} dosage BCA a été réalisé afin de connaître les concentrations des échantillons après déplétion.

2.2) Electrophorèse 1D

15 µg de protéines par échantillon ont été déposés sur gel SDS-PAGE (10% acrylamide) pour une migration sur toute la longueur du gel. Après électrophorèse et coloration au bleu de Coomassie, nous avons pu observer et comparer le profil protéique des échantillons voir figure 2.

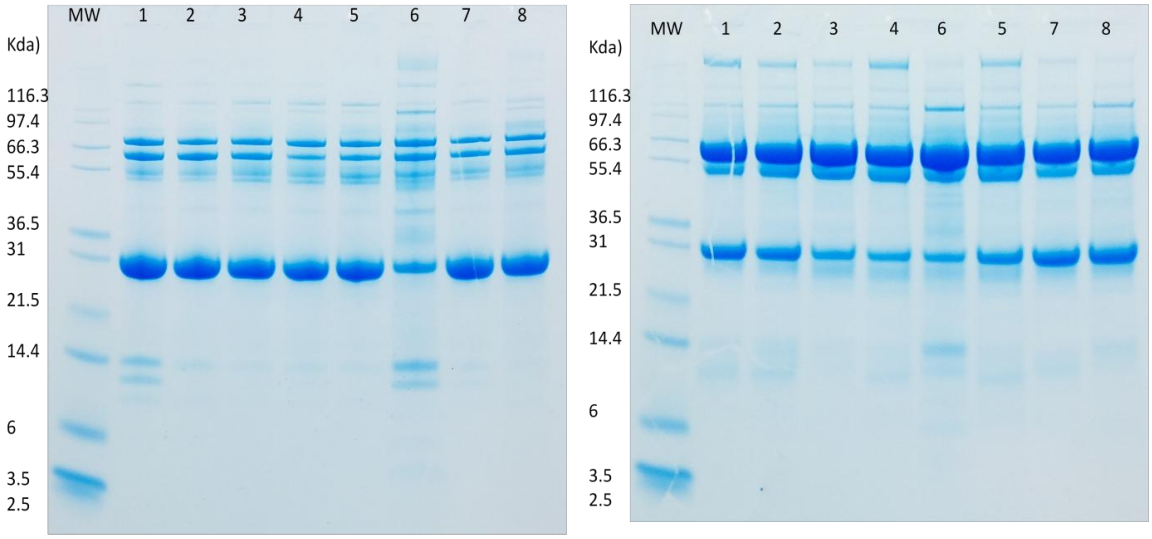


Figure 2 Migration complète des échantillons de liquide trachéal déplétés (à gauche) et les échantillons issus de l'élution de l'albumine (à droite)

On observe bien que la bande d'albumine à 60kDa ainsi que celles des IgG à 50 et 25kDa ont été réduites après déplétion et une bande vers 25-30kDa (d'autres immunoglobulines ?) est devenue la plus intense. De plus l'échantillon 6 présente un profil protéique différent des autres alors que son réplicat biologique l'échantillon 8 est similaire aux autres. Est-ce que l'on peut donc vraiment considérer le 6 et le 8 comme des réplicats biologiques ?

Ensuite 50µg d'échantillons ont été déposés sur un autre gel et la migration éléctrophorétique a été réalisée sur 2 cm de gel afin d'obtenir 5 bandes. Après fixation et coloration au bleu de Coomassie, 5 zones ont été découpées dans chaque piste (les gels sont représentés en figure 3).

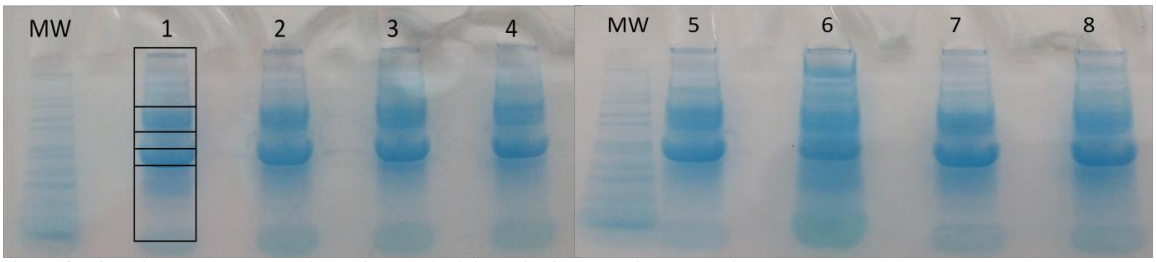


Figure 3 Migration sur 1,5 cm des échantillons déplétés de liquide trachéal avant découpe en 5 zones

Les protéines ont été réduites et alkylées et digérées enzymatiquement dans le gel et à l'issue d'une incubation à 37°C toute la nuit, les peptides de digestion ont été extraits du gel avec de l'acétonitrile (ACN) (voir annexe 1). Le solvant de tous les échantillons a été évaporé (speedvack) et le volume a été ajusté à 20 µL pour les 3 bandes les moins intenses, 100µL pour la bande la plus intense du bas et 60 µL pour la 2^{e} bande la plus intense du haut avec une solution à 2% d'ACN, 0.1% acide formique avant analyse en spectrométrie de masse.

2.4) Analyses MS

0.5 µL de digest sont analysés en MALDI-TOF (UltrafleXtreme, Bruker Daltonics) pour valider l'étape de digestion, l'absence de contaminants tels que les polymères.

L'analyse a été réalisée en utilisant un système nanoUPLC (nanoRSLC, ThermoFisher) couplé à un spectromètre de masse équipé d'une source nanospray Advion TriVersa Nanomate et composé d'une trappe ionique en tandem avec un orbitrap (LTQ-Orbitrap elite, Thermo Scientific).
5µL de chaque échantillon ont été chargés sur une pré-colonne d'enrichissement (Pepmap C18 300µm*5mm, 5µm ; Waters) en utilisant 98% solvant A (2% ACN, 0,1% acide formique) et 2% solvant B (80% ACN, 0,1% acide formique) à un débit de 20µL/min. L'élution des peptides a ensuite été effectuée sur une colonne de séparation (C18 75µm*15mm, 2µm ; ThermoFisher) maintenue à 33°C, à un débit de 300nL/min avec un gradient 1-44% B en 100min pour les échantillons.

Les échantillons ont été injectés dans un ordre aléatoire.

100 fmoles d'un digest de BSA sont utilisés comme test de performance des instruments en nanoLC-ESI-Trap.

Le détail des protocoles se trouve en Annexe 1.
Le résultat du contrôle de BSA est en Annexe 2.

3 – Traitement des données

3.1) Identification des protéines

Identification des protéines :
L'algorithme de recherche Andromeda (Maxquant v1.5.3.30) a été utilisé pour identifier des protéines à partir des données MS/MS. La recherche a été réalisée dans une banque de données protéique restreinte à la taxonomie « human » (Uniprot juin 2018, taxonomie 9606 « Homo sapiens », 173 387 entrées). Une version target-decoy de la banque a alors été générée par Andromeda (mode revert) afin de permettre d'évaluer le taux de faux positif (FDR). Les paramètres

de recherche Andromeda étaient les suivants : 1 sites de clivage manqués par l'enzyme tolérés par peptide, la carbamidomethylation des cystéines spécifiée comme modification fixe, l'oxydation des méthionines et l'acétylation des protéines en N-ter spécifiées comme modifications variables. L'erreur de masse tolérée était en mode MS de 20 ppm (first search), de 10 ppm (main search) et en mode MS/MS de 0,6 Da. Les taux de faux positif (FDR) peptidique et protéique ont été fixés à 1% avec au moins 7 acides aminés par peptide.

Quantification des protéines
A partir des données spectrales (MS) d'un peptide donné, sa quantification a été obtenue après extraction puis intégration de la somme des courants d'ions par le logiciel Maxquant (v1.5.3.30) qui est l'un des rares à permettre de prendre en charge la quantification XIC à partir d'échantillons fractionnés. L'option « Label-Free Quantification » (LFQ) de Maxquant a également utilisé avec ratio count minimum de 1 pour normaliser les intensités peptidiques et protéiques [Cox et al. 2014]. " Match between runs" a également été réalisé après alignement des temps de rétention avec une fenêtre de temps de 3 min. Les peptides modifiés ont été inclus dans la quantification.

3.2) Résultats

L'analyse de l'échantillon 8 (le 2e "unplug") n'a pas été prise en compte en raison de mauvaise condition de prélèvement, de l'aspect macroscopique et des caractéristiques protéiques incohérentes.

Des filtres ont été appliqués sur les valeurs des réplicas par échantillon et n'ont été conservés que les groupes de protéines pour lesquels on avait au min 2 valeurs/3 réplicas et 1 valeur/2 réplicas. Les cas présents/absents ont été conservés. Les contaminants comme la kératine ont également été supprimés.

On obtient 314 protéines quantifiées.

Les analyses biostatistiques et bioinformatiques sont en cours.

Les conditions "âge gestationnel" et "effet du plug" seront testées.

ANNEXE 1 - Protocoles

1 – Gel 1D

- Gel 10% en acrylamide, tampon MES (InVitrogen).
- Migration sur 2 cm (170V) et totale (170V et 190V)
- Fixation 20 min dans EtOH 50%, acide phosphorique 3%
- Lavage des gels 2 x 5 min et 1 x 10min en EmQ.
- Coloration 5 min
- Décoloration 5 min dans un bain d'EmQ
- Découpage en spot de $1mm^3$ dans la zone de migration.

2 – Réduction, alkylation

- Réduire avec 10 mM TCEP (dans 0.1M NH_4HCO_3).
- Incuber 30 min. à 37°C sous agitation à l'obscurité.
- Alkyler avec 55 mM iodoacétamide (dans 0.1M NH_4HCO_3).
- Incuber 20 min à température ambiante à l'obscurité sous agitation.

3 - Digestion

Référence de la trypsine utilisée : Trypsin Gold Mass Spectrometry Grade, PROMEGA, ref. V5280.
- Ajouter 300 ng de trypsine en tampon 0.1MNH_4HCO_3
- Incuber à 37°C pendant la nuit.

4 – Extraction des peptides

- Ajouter 100µL d'ACN 60%, acide formique à 0,1%
- Incuber 1h à RT
- Prélever le surnageant.
- Déshydrater 10 min avec 100 % ACN.
- Pooler le surnageant avec le précédent
- Speedvaquer 1h

5 - Séparation des peptides par nanoLC

- Colonne de séparation : 75 µm id x 15 cm, C18 2 µm 100 A.
- Tampon A : ACN 2 %, acide formique 0.1 %.
- Tampon B : ACN 80 %, acide formique 0.1 %.
- Gradient :

Temps (min)	Tampon B (%)
0	2
75	44
75	98
80	98
80	10

90	10

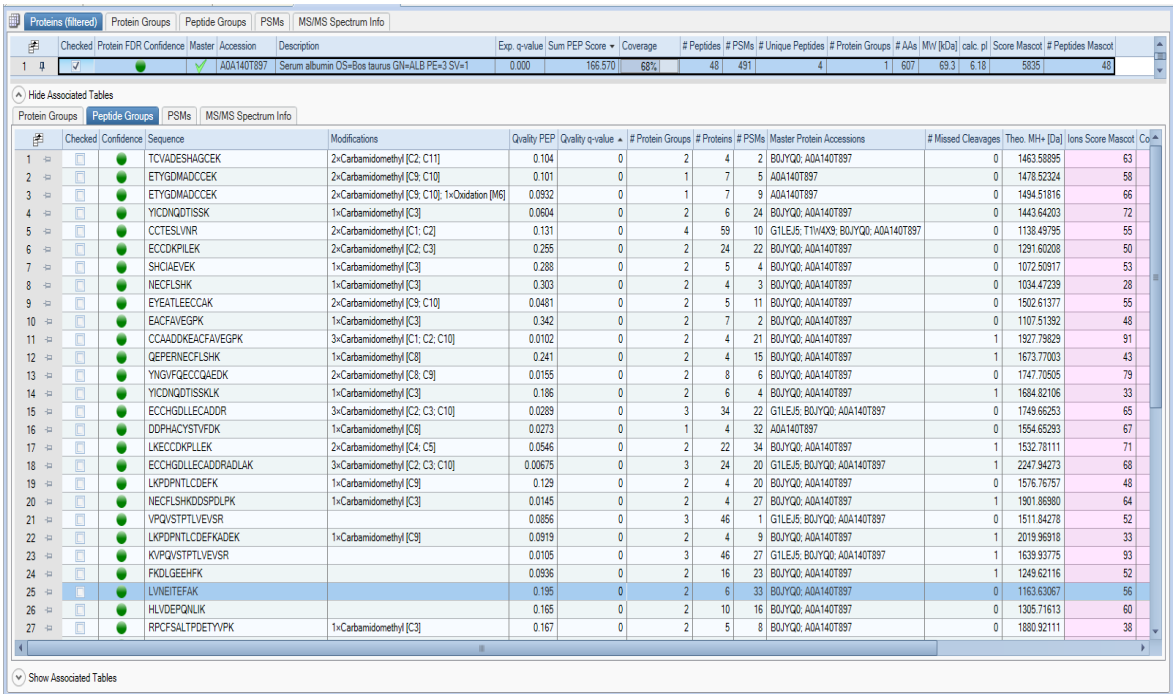
6 - Analyse par spectrométrie de masse ESI-Trap

- Un scan en MS, détection dans l'Orbitrap, à 120000 de résolution et entre 400 – 1700 m/z.
- Suivi de 15 fragmentations en MS/MS sur les 15 ions les plus intenses du spectre MS précédent, détection HCD.
- Les données ont été scorées avec Andromeda, en prenant comme base de données les protéines présentes dans Uniprot pour *Homo sapiens*.
- Conditions de recherche :
    - Enzyme : trypsine.
    - Modification variable : méthionine oxydée
    - Tolérance de masse en MS : 10 ppm.
    - Tolérance de masse en MS/MS : 0.6 Da.
    - FDR à 1% sur le score d'identification

-

ANNEXE 2 – Contrôle de séparation et d'analyse ESI-Trap

Le contrôle danalyse par spectrométrie de masse se fait en interrogeant les données ESI-Trap du digest de BSA :



Proteins (filtered) | Protein Groups | Peptide Groups | PSMs | MS/MS Spectrum Info

	Checked	Protein FDR Confidence	Master	Accession	Description	Exp. q-value	Sum PEP Score	Coverage	# Peptides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Score Mascot	# Peptides Mascot
1	☑		✓	A0A140T897	Serum albumin OS=Bos taurus GN=ALB PE=3 SV=1	0.000	166.570	68%	48	491	4	1	607	69.3	6.18	5835	48

Hide Associated Tables

Protein Groups | Peptide Groups | PSMs | MS/MS Spectrum Info

	Checked	Confidence	Sequence	Modifications	Qvality PEP	Qvality q-value	# Protein Groups	# Proteins	# PSMs	Master Protein Accessions	# Missed Cleavages	Theo. MH+ [Da]	Ions Score Mascot	Co
1	☐		TCVADESHAGCEK	2×Carbamidomethyl [C2; C11]	0.104	0	2	4	2	B0JYQ0; A0A140T897	0	1463.58895	63	
2	☐		ETYGDMADCCEK	2×Carbamidomethyl [C9; C10]	0.101	0	1	7	5	A0A140T897	0	1478.52324	58	
3	☐		ETYGDMADCCEK	2×Carbamidomethyl [C9; C10]; 1×Oxidation [M6]	0.0932	0	1	7	9	A0A140T897	0	1494.51816	66	
4	☐		YICDNQDTISSK	1×Carbamidomethyl [C3]	0.0604	0	2	6	24	B0JYQ0; A0A140T897	0	1443.64203	72	
5	☐		CCTESLVNR	2×Carbamidomethyl [C1; C2]	0.131	0	4	59	10	G1LEJ5; T1W4X9; B0JYQ0; A0A140T897	0	1138.49795	55	
6	☐		ECCDKPILEK	2×Carbamidomethyl [C2; C3]	0.255	0	2	24	22	B0JYQ0; A0A140T897	0	1291.60208	50	
7	☐		SHCIAEVEK	1×Carbamidomethyl [C3]	0.288	0	2	5	4	B0JYQ0; A0A140T897	0	1072.50917	53	
8	☐		NECFLSHK	1×Carbamidomethyl [C3]	0.303	0	2	4	3	B0JYQ0; A0A140T897	0	1034.47239	28	
9	☐		EYEATLEECCAK	2×Carbamidomethyl [C9; C10]	0.0481	0	2	5	11	B0JYQ0; A0A140T897	0	1502.61377	55	
10	☐		EACFAVEGPK	1×Carbamidomethyl [C3]	0.342	0	2	7	2	B0JYQ0; A0A140T897	0	1107.51392	48	
11	☐		CCAADDKEACFAVEGPK	3×Carbamidomethyl [C1; C2; C10]	0.0102	0	2	4	21	B0JYQ0; A0A140T897	1	1927.79829	91	
12	☐		QEPERNECFLSHK	1×Carbamidomethyl [C8]	0.241	0	2	4	15	B0JYQ0; A0A140T897	1	1673.77003	43	
13	☐		YNGVFQECCQAEDK	2×Carbamidomethyl [C8; C9]	0.0155	0	2	8	6	B0JYQ0; A0A140T897	0	1747.70505	79	
14	☐		YICDNQDTISSKLK	1×Carbamidomethyl [C3]	0.186	0	2	6	4	B0JYQ0; A0A140T897	1	1684.82106	33	
15	☐		ECCHGDLLECADDR	3×Carbamidomethyl [C2; C3; C10]	0.0289	0	3	34	22	G1LEJ5; B0JYQ0; A0A140T897	0	1749.66253	65	
16	☐		DDPHACYSTVFDK	1×Carbamidomethyl [C6]	0.0273	0	1	4	32	A0A140T897	0	1554.65293	67	
17	☐		LKECCDKPLLEK	2×Carbamidomethyl [C4; C5]	0.0546	0	2	22	34	B0JYQ0; A0A140T897	1	1532.78111	71	
18	☐		ECCHGDLLECADDRADLAK	3×Carbamidomethyl [C2; C3; C10]	0.00675	0	3	24	20	G1LEJ5; B0JYQ0; A0A140T897	1	2247.94273	68	
19	☐		LKPDPNTLCDEFK	1×Carbamidomethyl [C9]	0.129	0	2	4	20	B0JYQ0; A0A140T897	0	1576.76757	48	
20	☐		NECFLSHKDDSPDLPK	1×Carbamidomethyl [C3]	0.0145	0	2	4	27	B0JYQ0; A0A140T897	1	1901.86980	64	
21	☐		VPQVSTPTLVEVSR		0.0856	0	3	46	1	G1LEJ5; B0JYQ0; A0A140T897	0	1511.84278	52	
22	☐		LKPDPNTLCDEFKADEK	1×Carbamidomethyl [C9]	0.0919	0	2	4	9	B0JYQ0; A0A140T897	1	2019.96918	33	
23	☐		KVPQVSTPTLVEVSR		0.0105	0	3	46	27	G1LEJ5; B0JYQ0; A0A140T897	1	1639.93775	93	
24	☐		FKDLGEEHFK		0.0936	0	2	16	23	B0JYQ0; A0A140T897	1	1249.62116	52	
25	☐		LVNEITEFAK		0.195	0	2	6	33	B0JYQ0; A0A140T897	0	1163.63067	56	
26	☐		HLVDEPQNLIK		0.165	0	2	10	16	B0JYQ0; A0A140T897	0	1305.71613	60	
27	☐		RPCFSALTPDETYVPK	1×Carbamidomethyl [C3]	0.167	0	2	5	8	B0JYQ0; A0A140T897	0	1880.92111	38	

Show Associated Tables

7.3. Développement d'un ballonnet innovant

7.3.1. Objectif

Notre objectif principal était de développer un dispositif médical intelligent pour l'occlusion trachéale fœtale, permettant un retrait du ballonnet qui soit à la fois simple, non-invasif et actionné par contrôle externe.

Un dispositif avec de telles caractéristiques permettrait de pallier ainsi à chacune des difficultés liées au retrait du ballonnet et donc d'améliorer la survie néonatale :

- RETRAIT SIMPLE DU BALLONNET : Les patientes résidant à distance d'un centre de référence et dont le fœtus est atteint de HCD pourraient bénéficier également d'une occlusion trachéale, à partir du moment où une expertise limitée serait nécessaire à la libération des voies aériennes.

- NON-INVASIF : Une procédure de retrait chirurgical serait alors évitée, ainsi que les conséquences en termes de prématurité induite et de mortalité.

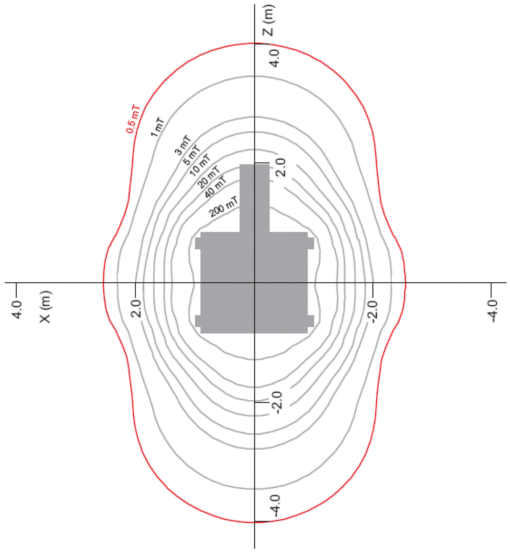
- ACTIONNÉ PAR CONTRÔLE EXTERNE : Le moment de la levée de l'occlusion trachéale pourrait être adapté à chaque situation, en particulier en cas de rupture prématurée des membranes et/ou de début de travail alors que le ballonnet est encore en place.

7.3.2. Solution technique

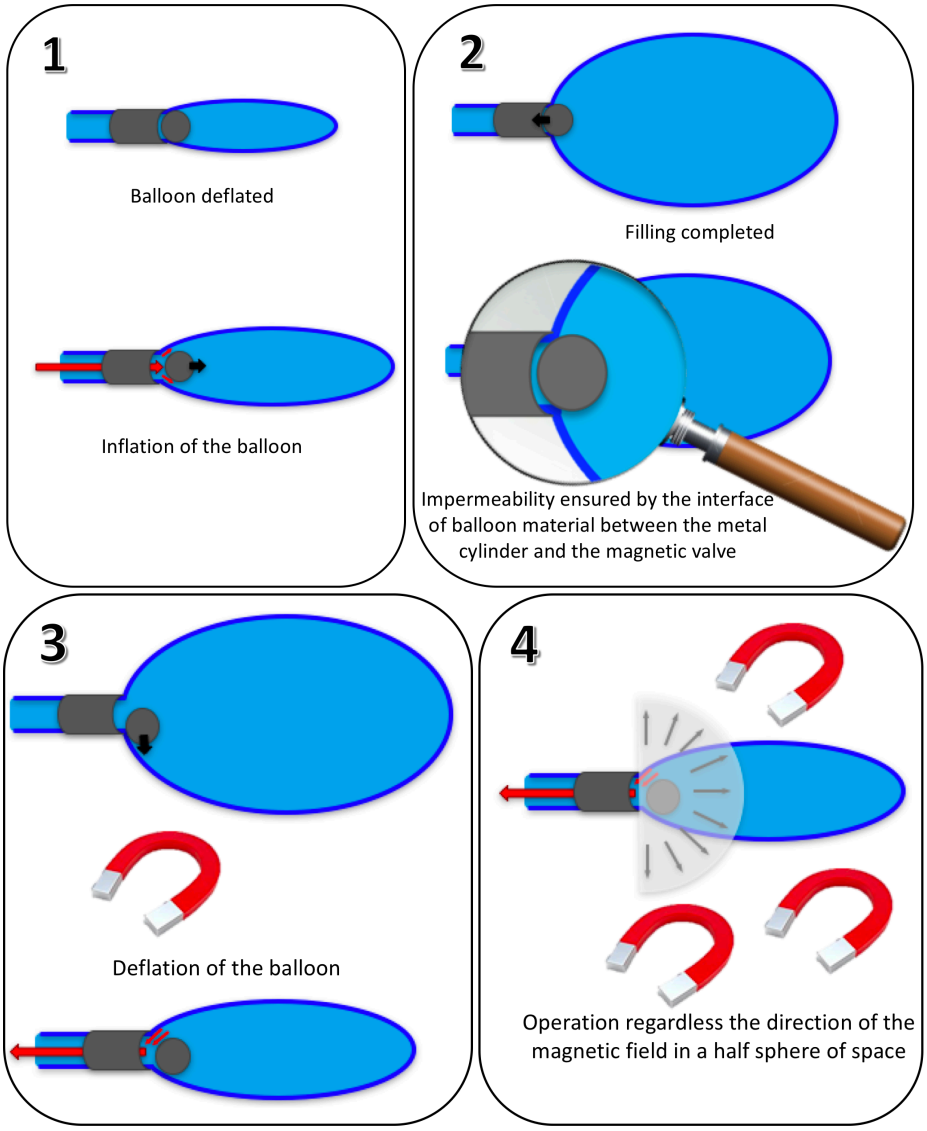
Une valve de remplissage et de vidange est composée d'un tube métallique ferromagnétique et d'une bille aimantée libre de mouvement à l'intérieur du ballonnet. L'injection de sérum physiologique au travers de la valve repousse légèrement la bille magnétique ce qui permet le remplissage du ballon. Une fois que l'injection est terminée, la bille aimantée se plaque à nouveau sur le tube métallique, garantissant alors l'étanchéité. Quad le ballonnet est placé dans un champ magnétique assez puissant, la bille aimantée est attirée en dehors du tube cylindrique, ce qui provoque la vidange du ballonnet. À noter que quelle que soit la direction du champ magnétique dans un hémi-champ de l'espace, l'attraction de la bille en dehors du tube est assurée.

Le champ magnétique utilisé est le camp de fuite d'un appareil d'IRM. Il existe en effet un champ de fuite important autour du cœur de l'IRM, dès lors que la machine est allumée et sans qu'il n'y ait besoin de réaliser une acquisition d'image.

Champ de fuite d'une IRM Siemens 1.5 T Avanto®



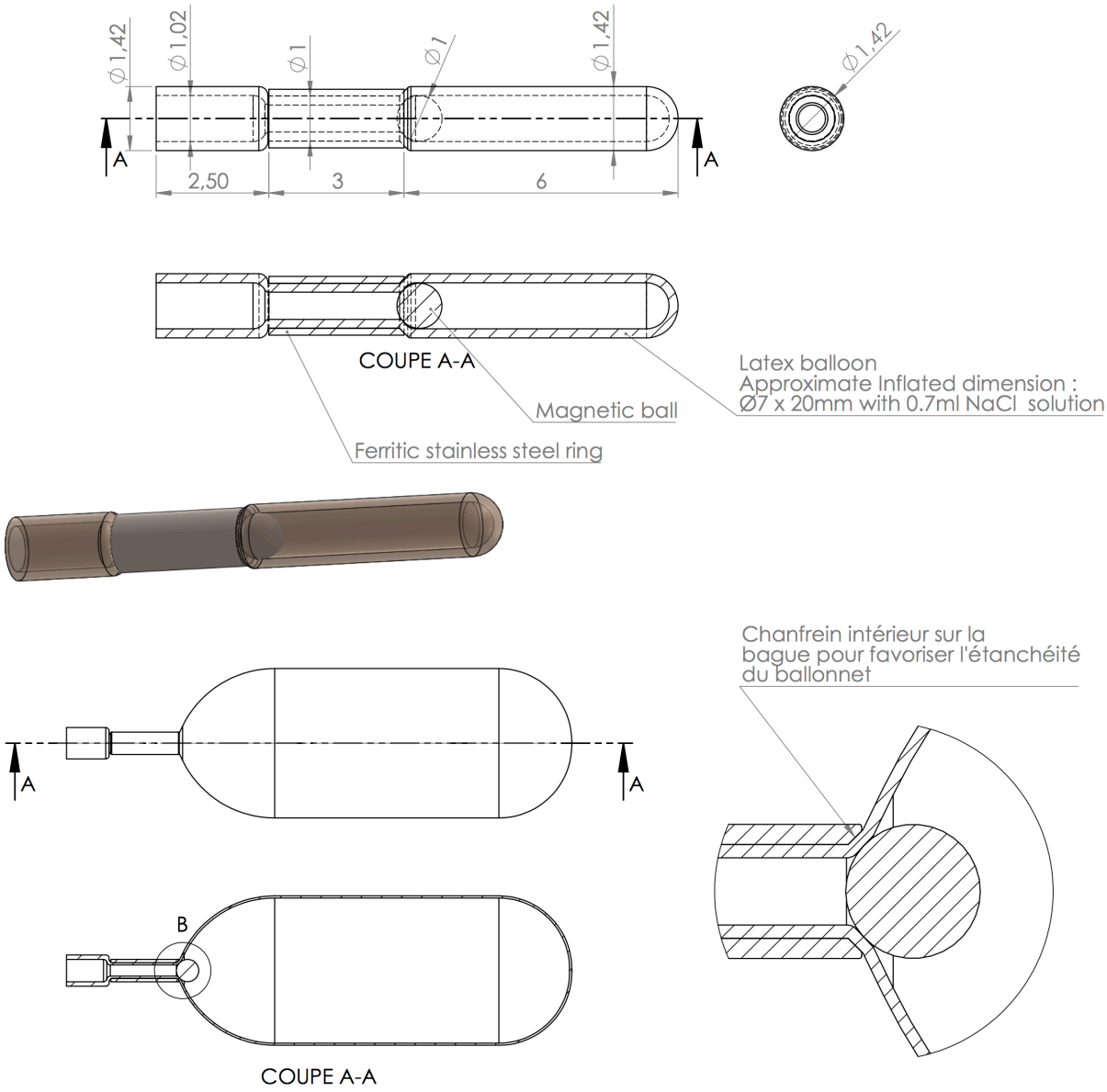
Principe du fonctionnement du ballon



7.3.3. Prototypage du ballonnet

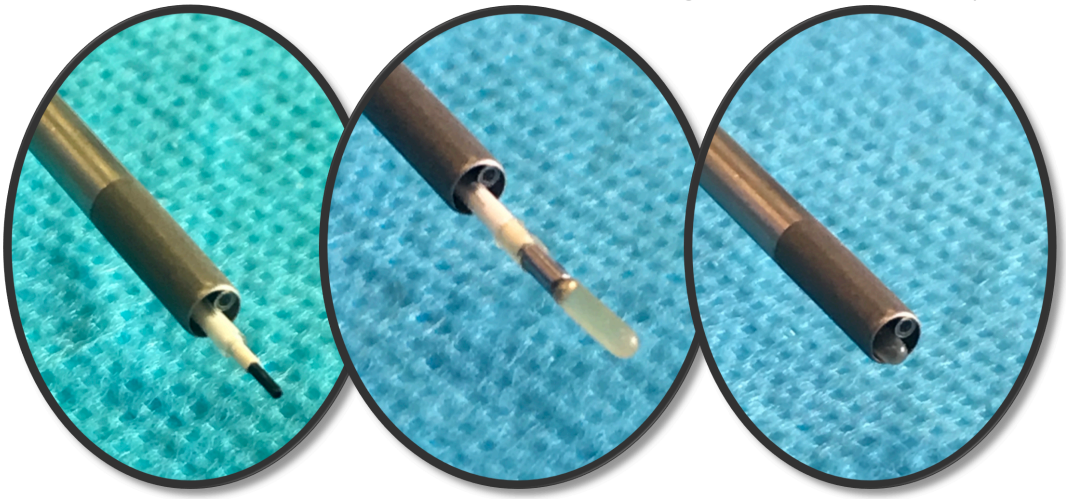
Le prototypage du ballonnet a été réalisé en partenariat avec BS Medical Tech Industry (BSMTI), Niedorroedern.

Le ballon est fait de latex et ses dimensions sont de 1.42mm x 6mm lorsqu'il est vide et 7mm x 20mm lorsqu'il est rempli avec 0.7 mL de sérum physiologique. La bille magnétique est incluse dans le ballon et est libre de mouvement. Elle est composée de Neodyme NdFeB / N35 et son diamètreest de 1mm. Le tube cylindrique cylindrique est composé d'un métal ferromagnétique de type 430 et ses dimensions sont de 1.3mm x 3mm. Le tube métallique et la bille aimanté sont tous les deux recouverts de parylène afin d'assurer la biocompatibilité.

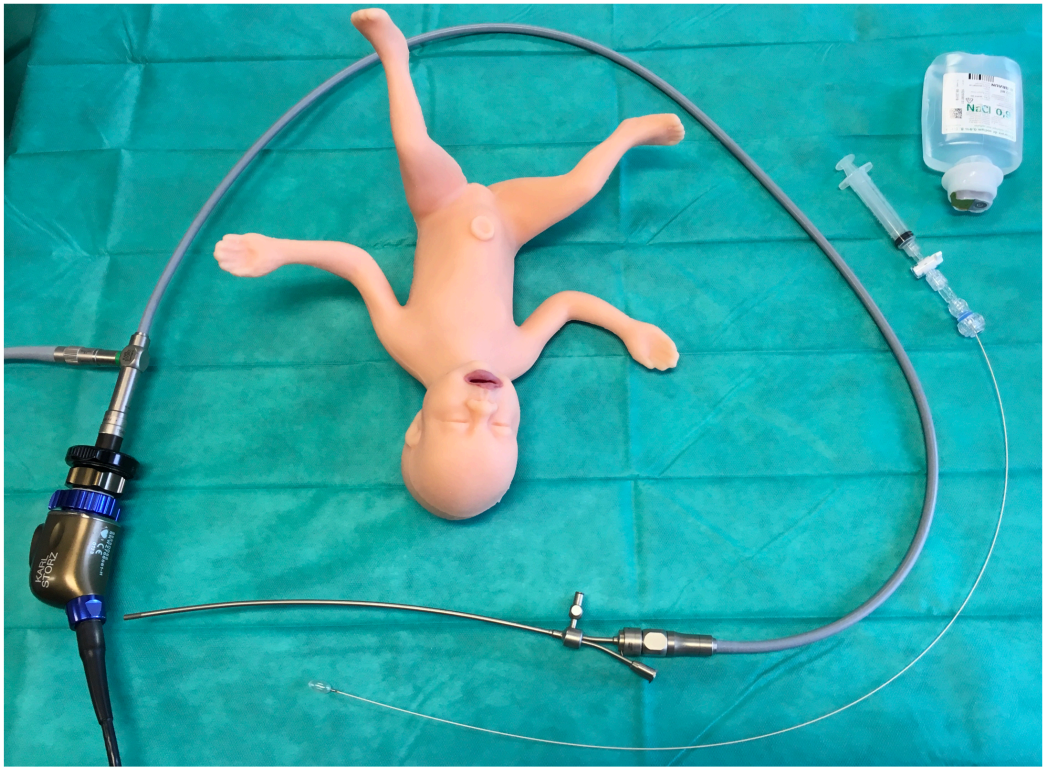


Une preuve de concept avec un premier prototype à l'échelle 1/1 a été conduite devant des machines d'IRM de 1.5 et 2 Teslas. Le positionnement du ballonnet à environ 30-40 cm devant le cylindre de l'IRM (soit environ 40 mT) provoquait sa vidange immédiate. Le prototype a aussi été testé sur simulateur et un aimant de 40 mT avec les mêmes résultats.

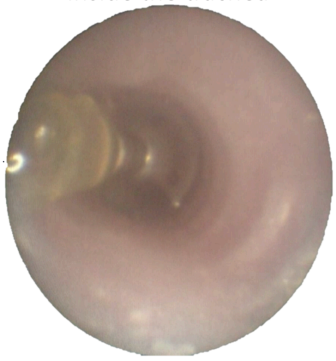
Placement of the balloon on the catheter before moving back inside the fetoscope



High-fidelity simulator for FETO, endoscopic instruments, balloon and delivery catheter

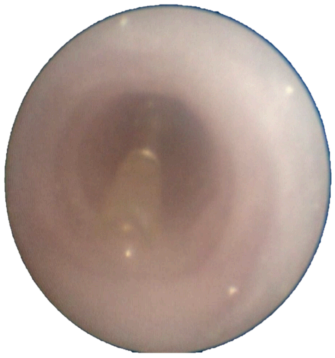


Balloon inflated inside the trachea



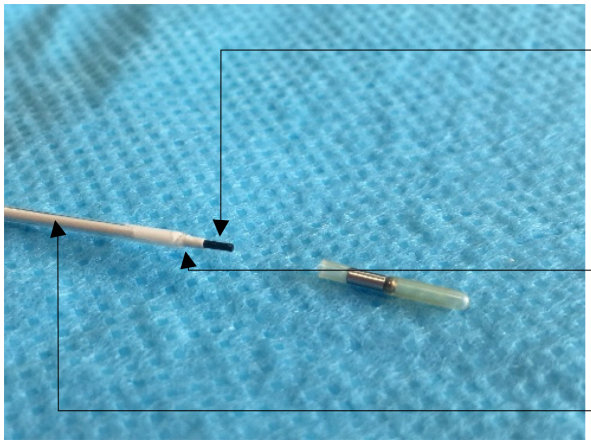
Saline coming out from the balloon

Balloon deflated inside the trachea



7.3.4. Prototypage du système de pose

Le système de pose est composé d'un triple tube. Un tube interne au travers duquel le sérum physiologique est injecté. Un tube intermédiaire bloquant le tube métallique afin que le tube interne ne pénètre pas dans le ballonnet lui-même. Un tube externe ayant le même diamètre que le ballonnet afin d'éviter que le ballonnet ne se désinsère au moment où le système est reculé dans la chemise du foetoscope.



7.3.5. Tests "in vitro"

Des tests d'étanchéité et d'occlusion ont été réalisés. Ils ont montré que les ballonnets étaient bien étanches et conservaient leur caractère d'occlusion sur au moins 6 semaines d'observation.

Des tests de fonctionnement devant une IRM 1,5 T ont été réalisés. Un total de 40 tests ont été réalisés selon 3 différentes hauteurs et 4 angles différents (β=0°, 30°, 60°, and 90°). Le fait présenter le ballonnet devant le tunnel de l'IRM puis de tourner autour provoquait la vidange dans 100% des cas.

7.4. Brevet déposé

R E P U B L I Q U E F R A N C A I S E

INSTITUT NATIONAL DE LA PROPRIÉTÉ INDUSTRIELLE

BREVET D'INVENTION
CERTIFICAT D'UTILITE

Réception électronique de la soumission

Il est certifié par la présente qu'une demande de brevet (ou d'un certificat d'utilité) a été reçue par le biais du dépôt électronique sécurisé de l'INPI. Après réception, un numéro d'enregistrement et une date de réception ont été automatiquement attribués.

Numéro de demande	1653954	
Numéro de soumission	1000345616	
Date de réception	02 mai 2016	
Vos références	BFF 14P0800AH	
Demandeur	Université de Strasbourg	
Pays	FR	
Titre de l'invention	BALLONNET GONFLABLE ET DÉTACHABLE, DESTINÉ À ÊTRE IMPLANTÉ DANS UNE CAVITÉ CORPORELLE, NÉCESSAIRE DE TRAITEMENT ET PROCÉDÉ DE VIDANGE ASSOCIÉS	
Documents envoyés	package-data.xml	requetefr.xml
	application-body.xml	fr-fee-sheet.xml
	requetefr.pdf (5 p.)	validation-log.xml
	comment.pdf (2 p.)	indication-bio-deposit.xml
	fr-office-specific-info.xml	textebrevet.pdf (17 p.)
	dessins.pdf (5 p.)	
Déposé par	EMAIL=paris@lavoix.eu,CN=Philippe BLOT,O=CABINET LAVOIX,C=FR	
Méthode de dépôt	Dépôt électronique	
Date et heure de réception électronique	02 mai 2016, 16:53:59 (CEST)	
Empreinte officielle du dépôt	6D:15:90:CE:25:66:77:46:88:D5:88:91:8D:DD:C4:49:5E:E6:42:D3	

/INPI, section dépôt/

Ballonnet gonflable et détachable, destiné à être implanté dans une cavité corporelle, nécessaire de traitement et procédé de vidange associés

La présente invention concerne un ballonnet gonflable, destiné à être implanté dans une cavité corporelle, comportant :

- une poche formée d'une paroi étanche délimitant un espace intérieur ;
- une vanne de remplissage de l'espace intérieur par un fluide, propre à être obturée après remplissage de l'espace intérieur.

Le ballonnet est propre à être inséré à l'extrémité d'un dispositif de largage et de gonflage et à être détaché du dispositif.

Un tel ballonnet est destiné notamment à être implanté dans la trachée d'un fœtus pour réaliser une occlusion trachéale fœtale lorsque le fœtus est atteint de hernie diaphragmatique congénitale. Le ballonnet peut également être utilisé dans le cadre d'indications potentielles, comme la rupture prématurée des membranes ou toute autre affection associée à une hypoplasie pulmonaire fœtale. La hernie diaphragmatique congénitale est une maladie affectant sporadiquement les fœtus, avec une incidence généralement comprise entre 1/3000 et 1/5000 parmi les nouveau-nés.

Cette hernie se traduit par une invasion d'organes de l'abdomen, tels que l'intestin, l'estomac et/ou le foie dans la cavité thoracique en raison du défaut diaphragmatique. Ceci applique une pression sur les poumons en développement et provoque une hypoplasie pulmonaire susceptible d'engendrer une insuffisance respiratoire, voire parfois la mort du nouveau-né. La mortalité actuelle résultant d'une hernie diaphragmatique congénitale isolée est estimée à 30 % environ par certaines études. L'hypoplasie pulmonaire est plus ou moins sévère en fonction de l'importance de la hernie. Les conséquences une fois l'enfant né sont l'insuffisance respiratoire, mais aussi l'hypertension artérielle pulmonaire.

Pour pallier ce problème, il est connu notamment de l'article « Technical Aspects of Fetal Endoscopic Tracheal Occlusion for Congenital Diaphragmatic Hernia », Journal of Pediatric Surgery, (2011) 46, 22-32, d'implanter un ballon par voie endoscopique dans la trachée du fœtus et de remplir ce ballon de fluide afin de bloquer dans les poumons des sécrétions pulmonaires en amont du ballon entraînant une hyperpression qui stimule le développement pulmonaire.

Lorsqu'une telle technique est appliquée, les études montrent une amélioration significative du développement pulmonaire, augmentant notablement les chances de survie du nouveau-né après la naissance.

Pour être efficace, l'implantation d'un ballon dans la trachée du fœtus doit donc obstruer les voies respiratoires naturelles du fœtus. Il est cependant nécessaire de

dégonfler le ballon, en réalisant une nouvelle endoscopie ou alors en perçant la paroi du ballon, afin de désobstruer les voies respiratoires naturelles.

Cette opération est réalisée in utero vers 34 semaines d'aménorrhée ou avant en cas de rupture de la poche des eaux ou de début de travail. En effet, le retrait du ballon avant la naissance est crucial pour achever une maturation cellulaire adéquate des poumons, qui augmente les chances de survie néonatale. Le retrait in utero facilite en outre la gestion néonatale, et permet d'envisager dans certains cas un accouchement par voie vaginale.

Des difficultés résultent du fait qu'une telle opération ne peut être faite que par une équipe spécialisée, que cette opération n'est pas toujours techniquement réalisable et qu'elle est associée à une morbi-mortalité périnatale élevée.

Par ailleurs, il existe toujours un risque que l'accouchement se produise avant le dégonflage du ballon. Ceci peut avoir des conséquences dramatiques pour le nouveau-né, si l'équipe en charge de l'accouchement ne parvient pas à retirer le ballon lors du travail, ou rapidement après la délivrance.

Les patientes portant un fœtus ayant un ballon dans la trachée sont ainsi astreintes à rester à proximité ou au sein d'un centre hospitalier apte à effectuer une telle intervention rapidement et de manière la plus sûre.

Ceci est fastidieux et coûteux, dans les cas où la patiente n'habite pas au voisinage d'un tel centre hospitalier.

Un but de l'invention est d'obtenir un ballonnet gonflable facile et pratique à implanter dans une cavité corporelle, notamment dans la trachée d'un fœtus, et qui peut néanmoins être simplement dégonflé lorsque cela est souhaité.

À cet effet, l'invention a pour objet un ballonnet du type précité, caractérisé en ce que la poche délimite un orifice de vidange de fluide débouchant dans l'espace intérieur, le ballonnet comportant un organe d'obturation de l'orifice de vidange, l'organe d'obturation étant propre à libérer l'orifice de vidange sous l'effet d'un champ magnétique, pour permettre la vidange au moins partielle du fluide contenu dans l'espace intérieur, l'organe d'obturation étant déplaçable suivant au moins deux axes distincts par rapport à la poche.

Le ballonnet selon l'invention peut comprendre l'une ou plusieurs des caractéristiques suivantes, prise(s) isolément ou suivant toute combinaison techniquement possible :

- l'organe d'obturation est disposé dans l'espace intérieur ;
- l'organe d'obturation est librement déplaçable dans l'espace intérieur défini par la poche sous l'effet d'un champ magnétique ;

- l’organe d’obturation est propre à être maintenu dans une position d’obturation de l’orifice de vidange par aimantation, l’organe d’obturation étant propre à être déplacé à l’écart de l’orifice de vidange sous l’effet d’un champ magnétique propre à vaincre l’aimantation maintenant l’organe d’obturation dans la position d’obturation de l’orifice de vidange ;

- le ballonnet comporte un siège de retenue de l’organe d’obturation, disposé au voisinage de l’orifice de vidange, l’organe d’obturation coopérant par aimantation avec le siège de retenue dans une position d’obturation de l’orifice de vidange ; le siège de retenue étant préférentiellement un anneau, rapporté sur la paroi étanche autour de l’orifice de vidange

- le siège de retenue est couvert d’une couche d’un matériau souple, l’organe d’obturation étant disposé en appui sur la couche de matériau souple dans une position d’obturation de l’orifice de vidange ;

- l’organe d’obturation est une bille ;

- l’organe d’obturation présente une aimantation permanente ;

- l’organe d’obturation est propre à être déplacé jusqu’à au moins deux positions distinctes de libération de l’orifice de vidange sur deux axes sécants passant par l’orifice de vidange ;

- la paroi étanche de la poche est réalisée à partir d’un polymère choisi parmi le silicone, le latex, le polyuréthane, et/ou le polyisoprène ;

- l’orifice de vidange est défini par la vanne de remplissage, l’organe d’obturation étant propre à fermer la vanne de remplissage après remplissage de l’espace intérieur ;

- la vanne de remplissage comporte une bague de guidage d’un tube de gonflage du ballonnet, la bague de guidage et/ou l’organe d’obturation présentant un revêtement biocompatible.

L'invention a également pour objet un nécessaire de traitement d’un patient comportant :

- une ballonnet tel que défini plus haut ;

- un dispositif de gonflage et de largage du ballonnet, comprenant un tuteur de support du ballonnet, le ballonnet étant monté de manière libérable sur le tuteur de support, et un tube de gonflage du ballonnet, propre à être inséré de manière libérable dans la vanne de remplissage.

L'invention a aussi pour objet un procédé de vidange d’un ballonnet tel que défini plus haut, le ballonnet étant implanté dans une cavité corporelle, l’espace intérieur du ballonnet contenant du fluide, l’organe d’obturation obturant l’orifice de vidange. Le procédé comprenant les étapes suivantes :

- soumission du ballonnet à un champ magnétique externe, avantageusement produit par un appareil de résonance magnétique nucléaire, suivant au moins une première direction ;

- déplacement de l'organe d'obturation suivant au moins un axe sous l'effet du champ magnétique externe, pour libérer l'orifice de vidange ;

- vidange au moins partielle du fluide contenu dans l'espace intérieur à travers l'orifice de vidange.

Le procédé selon l'invention peut comprendre l'une ou plusieurs des caractéristiques suivantes, prise(s) isolément ou suivant toute combinaison techniquement possible :

- une étape de soumission du ballonnet à un champ magnétique externe, avantageusement produit par l'appareil de résonance magnétique nucléaire, suivant au moins une deuxième direction, distincte de la première direction.

L'invention a plus généralement pour objet un procédé d'ouverture d'un orifice de vidange d'un fluide dans un implant, l'implant comportant un organe d'obturation de l'orifice de vidange, propre à libérer l'orifice de vidange sous l'effet d'un champ magnétique pour permettre le passage au moins partiel du fluide présent dans l'implant à travers l'orifice de vidange, le procédé comprenant les étapes suivantes :

- soumission de l'implant à un champ magnétique externe issu d'un appareil de résonance magnétique nucléaire suivant au moins une première direction ;

- déplacement de l'organe d'obturation suivant au moins un axe sous l'effet du champ magnétique externe, pour libérer l'orifice de vidange ;

- passage de fluide à travers l'orifice de vidange.

Le procédé d'ouverture peut comprendre une étape de soumission de l'implant au champ magnétique externe produit par l'appareil de résonance magnétique nucléaire, suivant au moins une deuxième direction, distincte de la première direction.

L'invention a également pour objet une méthode de traitement chirurgical comprenant les étapes suivantes :

- fourniture d'un nécessaire tel que défini plus haut ;

- amenée, avantageusement par endoscopie, du ballonnet dans une cavité corporelle, à l'aide du dispositif de largage et de gonflage ;

- gonflage du ballonnet dans la cavité corporelle ;

- largage du ballonnet dans la cavité corporelle et retrait du dispositif de gonflage et de largage hors de la cavité corporelle.

La méthode de traitement chirurgical peut comprendre la caractéristique suivante :

- l'étape d'amenée comporte le convoyage du ballonnet dans la cavité amniotique d'une patiente, puis son introduction dans la trachée d'un fœtus présent dans la cavité amniotique.

L'invention sera mieux comprise à la lecture de la description qui va suivre, donnée uniquement à titre d'exemple, et faite en se référant aux dessins annexés, sur lesquels :

- la figure 1 est une vue schématique d'un premier nécessaire de traitement selon l'invention, avant l'implantation du ballonnet dans une cavité corporelle ;

- la figure 2 est un détail illustrant l'orifice de vidange du ballonnet, et l'organe d'obturation de l'orifice dans une position d'obturation, vu depuis l'intérieur du ballonnet ;

- les figures 3 à 6 illustrent les étapes successives d'introduction du ballonnet dans la cavité corporelle ;

- la figure 7 est une vue en perspective du ballonnet gonflé, lors de son largage ;

- la figure 8 est une vue analogue à la figure 7, lors du déplacement de l'organe d'obturation du ballonnet pour libérer l'orifice de vidange, sous l'effet d'un champ magnétique extérieur à la patiente ;

- la figure 9 est une vue en perspective de trois-quarts face d'un deuxième ballonnet selon l'invention, avant son gonflage ;

- la figure 10 est une vue prise en coupe suivant un plan axial médian du ballonnet de la figure 9, lors de son gonflage ;

- la figure 11 est une vue analogue à la figure 9, après gonflage du ballonnet ;

- la figure 12 est une vue analogue à la figure 10, lors du dégonflage ;

- la figure 13 est une vue analogue à la figure 12 d'un troisième ballonnet selon l'invention ;

- la figure 14 est une vue d'un détail d'une variante de ballonnet selon l'invention.

Un premier nécessaire de traitement 10 selon l'invention est illustré schématiquement sur la figure 1.

Le nécessaire de traitement 10 comporte un ballonnet gonflable 12 selon l'invention, destiné à être implanté dans une cavité corporelle 14, visible sur les figures 4 à 6. Le nécessaire 10 comporte en outre un dispositif 16 de gonflage et de largage du ballonnet gonflable 12 dans la cavité 14, illustré en particulier sur les figures 1 et 3.

Dans l'application particulière représentée sur les figures 3 à 6, la cavité 14 est la trachée d'un fœtus 18, présent dans la cavité amniotique 20 d'une patiente 22. Le fœtus 18 souffre par exemple d'une hernie diaphragmatique congénitale.

En référence aux figures 1 et 2, le ballon 12 comporte une poche 24 gonflable par un fluide, une vanne 26 de remplissage de la poche 24, et selon l'invention, une vanne 28

de vidange, libérable sous l'effet d'un champ magnétique extérieur à la patiente 22. La vanne de vidange 28 est ici distincte de la vanne de remplissage 26.

La poche 24 est formée d'une paroi étanche 30 déformable au toucher, délimitant un espace intérieur 32 de volume variable en fonction de la quantité de fluide qu'il contient.

La paroi étanche 30 est par exemple réalisée à partir d'un matériau polymère tel que du silicone, du latex, ou d'un caoutchouc ou tel que le polyisoprène.

L'épaisseur de la paroi étanche 30 est inférieure à 1 mm et est généralement comprise entre 0,1 mm et 0,5 mm.

La poche 24 présente généralement une forme allongée le long d'un axe A-A', visible sur la figure 2.

En référence à la figure 1, la poche 24 définit un orifice 34 de remplissage de l'espace intérieur 32, obturé sélectivement par la vanne de remplissage 26 et un orifice 36 de vidange, distinct de l'orifice de remplissage 34, obturé de manière sélective par la vanne de vidange 28.

Dans cet exemple, l'orifice de remplissage 34 est situé à une extrémité proximale de la poche 24, prise le long de l'axe A-A'. L'orifice 34 est délimité à sa périphérie par un manchon 38 de montage de la vanne 26 faisant saillie le long de l'axe A-A' par rapport à la paroi 30. Le manchon 38 est d'un seul tenant avec la paroi 30

L'orifice de vidange 36 est ménagé de manière traversante dans une région périphérique distale 40 de la paroi 30, située à l'opposé de l'orifice de remplissage 34 dans cet exemple.

L'étendue transversale de l'orifice de vidange 36 est avantageusement inférieure à 1,5 mm et est comprise par exemple entre 1 mm et 1,5 mm.

L'espace intérieur 32 de la poche 24 est propre à être rempli par un fluide, de préférence par un liquide, à travers la vanne de remplissage 26, pour passer la poche 24 d'une configuration dégonflée, contractée radialement (visible sur la figure 1 ou sur la figure 5) à une configuration gonflée, dilatée radialement (visible sur la figure 7).

Dans la configuration dégonflée, la poche 24 présente avantageusement une étendue transversale maximale et, prise perpendiculairement à l'axe A-A', avantageusement inférieure à 1,5 mm et comprise généralement entre 1 mm et 1,5 mm.

La longueur de la poche 24, prise le long de l'axe A-A', est avantageusement comprise entre 5 mm et 10 mm.

Le volume de l'espace intérieur 32 est alors compris avantageusement entre 3 mm^3 et 10 mm^3.

Dans la configuration gonflée, la poche 24 présente avantageusement une étendue transversale maximale Et, prise perpendiculairement à l'axe A-A', avantageusement inférieure à 10 mm et comprise par exemple entre 5 mm et 9 mm.

La longueur de la poche 24 dans la configuration gonflée, prise le long de l'axe A-A' est supérieure à la longueur de la poche 24 dans la configuration dégonflée. Cette longueur est avantageusement comprise entre 15 mm et 25 mm.

Le volume de l'espace intérieur 32 est alors compris avantageusement entre 250 mm^3 et 1600 mm^3.

Le fluide de gonflage de la poche est par exemple un liquide, notamment un liquide physiologique. Ce liquide contient éventuellement un agent de contraste propre à être visible par radiographie.

En référence à la figure 1, la vanne de remplissage 26 est normalement fermée. Elle définit une lumière centrale 50 d'injection de fluide dans l'espace intérieur 32.

Dans cet exemple, la vanne de remplissage 26 fait saillie axialement par rapport à la paroi 30. Elle est montée autour du manchon 38.

Elle comporte, à l'intérieur du manchon 38, un joint annulaire déformable 52 définissant la lumière centrale 50, et une bague périphérique 54 montée autour du manchon 38 pour enserrer le manchon 38 et le joint 52.

Le joint annulaire 52 est disposé dans l'orifice de remplissage 34. Il est déformable radialement par compression, pour permettre l'introduction d'un élément de remplissage de l'espace intérieur 32. Il est propre à revenir spontanément vers une configuration d'obturation de l'orifice de remplissage 34.

En référence à la figure 2, la vanne de vidange 28 comporte un siège 60 fixé sur la paroi étanche 30, sur la région périphérique 40 autour de l'orifice de vidange 36, et un organe d'obturation 62 de l'orifice de vidange 36, déplaçable suivant au moins deux axes A-A', B-B' distincts, sous l'effet d'un champ magnétique extérieur à la patiente 22.

Dans cet exemple, le siège 60 est rapporté sur la région périphérique 40, à l'extérieur de la paroi étanche 30. Il est par exemple collé sur la région périphérique 40.

Le siège 60 présente ici une forme d'anneau entourant l'orifice de vidange 36.

Le siège 60 est réalisé à l'aide d'un matériau métallique ferromagnétique, propre à être aimanté par un aimant permanent.

L'organe d'obturation 62 est disposé ici dans l'espace intérieur 32. Comme visible sur la figure 2, il présente une étendue transversale maximale e2 supérieure à l'étendue transversale maximale e1 de l'orifice de vidange 36. Dans cet exemple, l'organe d'obturation 62 est une bille.

L'organe d'obturation 62 présente une aimantation permanente. Il est ainsi propre à coopérer magnétiquement avec le siège 60 pour être maintenu dans une position d'obturation de l'orifice de vidange 36, dans laquelle il est appliqué contre la région périphérique 40 en regard du siège 60.

Dans cette position, l'organe d'obturation 62 obture totalement l'orifice 36, et empêche le passage de fluide depuis le volume intérieur 32 vers l'extérieur de la poche 24.

L'étanchéité autour de l'orifice de vidange 36 est renforcée par la présence de la région périphérique 40, sur laquelle s'appuie l'organe d'obturation 62, qui forme une couche intermédiaire déformable.

Sous l'effet d'un champ magnétique extérieur, propre à engendrer une force d'attraction de l'organe d'obturation 62 supérieure à la force de coopération entre l'organe d'obturation 62 et le siège 60, l'organe d'obturation 62 est propre à se déplacer à l'écart de l'orifice de vidange 36 dans l'espace intérieur 32 suivant au moins deux axes A-A', B-B'.

L'intensité du champ magnétique propre à libérer l'organe d'obturation 62 est par exemple supérieure à 0,1 T et est notamment comprise entre 0,5 T et 2 T.

En pratique, lorsqu'il se détache de la position d'obturation, l'organe d'obturation 62 est propre à se déplacer librement suivant une multitude d'axes dans un cône 64 centré sur l'axe A-A' de l'orifice de vidange 36, au niveau de l'orifice 36. Le cône 64 présente un angle d'ouverture vers l'espace intérieur 32 supérieur à 30°, de préférence supérieur à 90°, et avantageusement égal à 180°.

Aucun moyen mécanique de retenue ne raccorde l'organe d'obturation 62 à la poche gonflable 24 et/ou au siège 60.

Une fois détachée de la position d'obturation, l'organe d'obturation 62 est propre à atteindre au moins une position de libération de l'orifice 36, cette position dépendant de l'orientation de la patiente 22, et de celle du champ magnétique extérieur.

En particulier, l'organe d'obturation 62 est propre à occuper une pluralité de positions de libération distinctes, dans l'espace intérieur 32, après avoir quitté la position d'obturation, dont une est illustrée sur la figure 8.

En référence aux figures 1 et 3, le dispositif 16 de gonflage et de largage du ballonnet 12 comporte un tuteur souple 70 portant à son extrémité distale 71 le ballonnet 12, un tube de gonflage 72 disposé dans le tuteur 70, et avantageusement, une tige mandrin 74 disposée dans le tube de gonflage 72 pour le rigidifier.

Le dispositif 16 comporte en outre un embout proximal 76, pour sa manipulation par un praticien, et avantageusement, une gaine amovible 78 de protection du ballonnet 12.

Le tuteur 70 s'étend entre l'embout proximal 76 et l'extrémité distale 71. Il est propre à être déformé pour être introduit dans la patiente 22 avantageusement par voie endoscopique, et atteindre la cavité 14.

Le tube de gonflage 72 s'étend à travers le tuteur 70. Il est raccordé en amont à un réservoir 80 d'injection de fluide.

Le tube de gonflage 72 présente une partie distale 82 qui fait saillie par rapport à l'extrémité distale 71 du tuteur 70 pour être introduite dans la lumière centrale 50 de la vanne de remplissage 26.

La gaine amovible 78 est propre à couvrir le ballonnet 12 lors de son introduction jusqu'à la cavité 14. Elle est mobile longitudinalement autour du tuteur 70 pour découvrir le ballonnet 12, à son point d'implantation dans la cavité 14.

Le fonctionnement du nécessaire de traitement 10 dans le cadre d'une implantation dans une cavité corporelle 14 va maintenant être décrit.

Cette implantation est par exemple réalisée dans la trachée d'un fœtus 18 présent dans la cavité amniotique 20 d'une patiente 22.

Initialement, le ballonnet 12 est monté à l'extrémité distale 71 du tuteur 70 du dispositif de gonflage et de largage 16. La partie distale 82 du tube de gonflage 72 est introduite dans la vanne de remplissage 26, par déformation radiale du joint annulaire 52.

L'organe d'obturation 62 est plaqué en regard du siège 60 contre la région périphérique 40. Il occupe sa position d'obturation de l'orifice 36.

La poche 24 occupe alors sa configuration dégonflée, d'étendue radiale minimale, visible sur les figures 1 et 5.

Le dispositif 16, muni du ballonnet 12 à son extrémité est alors introduit dans la patiente 22, par voie endoscopique. Dans l'exemple représenté sur la figure 3, le praticien l'introduit dans la cavité amniotique 20, puis l'amène à travers les voies respiratoires du fœtus 18 jusqu'à la trachée, en passant à travers les cordes vocales (figure 4).

Une fois l'extrémité distale 71 dans la cavité 14, le praticien extrait le ballonnet 12 hors de la gaine 78, en tirant la gaine 78 vers l'embout proximal 76, comme visible sur la figure 5.

La poche gonflable 24 occupe toujours sa configuration dégonflée.

Le praticien injecte alors du fluide de gonflage dans l'espace intérieur 32 à travers le tube de gonflage 72 introduit dans la vanne de remplissage 26. La poche 24 se dilate

radialement pour atteindre sa configuration gonflée, en appui sur la paroi délimitant la cavité 14, comme visible sur la figure 6.

Puis, le praticien détache le ballonnet 12 du dispositif de gonflage et de largage 16, en extrayant le tube de gonflage 72 hors de la vanne de remplissage 26. La vanne de remplissage 26 se referme par dilatation radiale du joint annulaire 52.

Le praticien retire alors le dispositif 16 de la patiente 22.

Durant le gonflage, et après celui-ci, l'organe d'obturation 62 reste dans sa position d'obturation. Le fluide de gonflage reste donc confiné dans le volume intérieur 32.

La cavité 14 est alors obturée. Dans le cas d'un fœtus 18 souffrant d'hernie diaphragmatique congénitale, le développement pulmonaire du fœtus est amélioré, grâce à la présence du ballonnet 12 gonflé dans la trachée.

Lorsque le ballonnet 12 doit être retiré, la patiente est soumise à un champ magnétique extérieur de forte intensité, par exemple d'intensité supérieure à 0,1 T.

Ce champ magnétique est par exemple produit par un appareil d'imagerie par résonance magnétique. Selon les circonstances, la patiente se positionne dans l'appareil lors d'une acquisition d'image ou de préférence sans acquisition d'images dans l'appareil. Préférentiellement, la patiente n'a pas besoin de se positionner dans l'appareil, le champ de fuite de l'appareil allumé ou à l'entrée du tunnel est avantageusement suffisant pour produire un champ magnétique adéquat pour libérer l'organe d'obturation 62, auquel cas la patiente se tient simplement debout devant la machine.

De préférence, comme illustré par la figure 8, l'orientation relative entre le champ magnétique et la patiente 22 est modifié, par exemple en déplaçant la patiente, pour que le champ magnétique soit appliqué suivant au moins deux axes H1 et H2 distincts, comme illustré par la figure 8.

Le champ magnétique extérieur engendre une force d'attraction sur l'organe d'obturation 62 qui surmonte la force de coopération entre l'organe d'obturation 62 et le siège 60.

Sous l'effet du champ magnétique extérieur, l'organe d'obturation 62 se déplace à l'écart de l'orifice de vidange 36, jusqu'à une position de libération de l'orifice 36, représentée par exemple sur la figure 8.

Au moins une partie du fluide présent dans l'espace intérieur 32 s'écoule alors depuis l'espace intérieur 32 vers l'extérieur, à travers l'orifice de vidange 36, provoquant le dégonflage rapide du ballonnet 12.

Les voies respiratoires du fœtus sont alors à nouveau dégagées. Le ballonnet 12 est alors apte à être expulsé hors du fœtus par la libération du fluide pulmonaire en surpression, ou est retiré quelques jours après la naissance.

La libération de l'organe d'obturation 62 est immédiate et très facile à réaliser. Aucune liaison mécanique n'existant entre d'une part, l'organe d'obturation 62 et d'autre part, la poche gonflable 24 ou le siège 60, cette libération est très fiable et ne dépend pas d'un mécanisme mécanique ou électrique. Au contraire, le dégagement de l'orifice de vidange 36 est exclusivement provoqué par le champ magnétique extérieur appliqué, combiné aux forces de gravité s'appliquant sur l'organe d'obturation 62.

Cette libération est en outre non invasive pour la patiente 22, puisqu'elle peut être réalisée à distance, sans avoir à inciser la patiente 22, voire à pénétrer dans la cavité amniotique 20.

La vanne de vidange 28 ainsi obtenue est peu coûteuse à réaliser, tout en assurant un fonctionnement adéquat, quelles que soient les circonstances.

Les risques pour le fœtus 18 sont donc totalement écartés, puisque le retrait du ballonnet 12 est facilité par son dégonflage immédiat, et son expulsion hors de la trachée, au moment souhaité et sans procédure invasive.

La patiente 22 bénéficie d'un traitement adéquat, assurant le bon développement de son fœtus 18, sans nécessairement être maintenue au voisinage ou au sein d'un centre hospitalier spécialisé, ce qui limite les coûts en maintenant la qualité de soins.

Un deuxième nécessaire de traitement 110 selon l'invention est illustré par les figures 9 à 13.

Le ballonnet 12 du deuxième nécessaire 110 est formé de manière similaire au ballonnet 12 du premier nécessaire 10. En particulier, les dimensions du ballonnet 12 du deuxième nécessaire 110 sont analogues à celles du ballonnet 12 du premier nécessaire 10.

Toutefois, à la différence du premier nécessaire 10, le ballonnet 12 comporte une vanne unique formant à la fois une vanne de remplissage 26 et une vanne de vidange 28.

L'orifice de vidange 36 est constitué par l'orifice de remplissage 34 à l'extrémité de la vanne 26, 28. La poche 24 est donc munie d'un orifice 34, 36 unique permettant le remplissage de l'espace intérieur 32 en fluide et la vidange du fluide contenu dans l'espace intérieur 32.

L'organe d'obturation 62 obture par défaut la vanne de remplissage 26. Ainsi, la bague périphérique 54 délimitant la vanne de remplissage 26 est dépourvue de joint annulaire. Elle définit donc une lumière centrale 50 dégagée en permanence.

Comme pour le ballonnet 12 du premier nécessaire 10, la poche 24 se prolonge par un manchon 38 inséré dans la bague périphérique 54 et délimitant la périphérie de la lumière centrale 50.

Dans cet exemple, le manchon 38 se prolonge au-delà de la bague 54, à l'opposé de la poche 24, par un embout annulaire 112 s'ouvrant vers l'extérieur.

La bague périphérique 54 est ici en matériau métallique ferromagnétique. Elle définit le siège 60, sur lequel s'appuie l'organe d'obturation 62 dans la position d'obturation, sur la région périphérique 40.

L'organe d'obturation 62 présente ici une aimantation permanente. Il s'applique donc au repos sur le siège 60, dans la position d'obturation.

Le fonctionnement du deuxième nécessaire 110 selon l'invention est analogue à celui du premier nécessaire 10.

Toutefois, à la différence du premier nécessaire 10, le tube de gonflage 72 est introduit dans la bague périphérique 54 de la vanne de remplissage 26, sans ouvrir l'orifice de remplissage 34, puisque l'organe d'obturation 62 reste appliqué sur le siège 60.

Comme illustré par la figure 10, la force hydraulique générée par la pression du fluide injecté par le tube de gonflage 72, pousse l'organe d'obturation 62 à l'écart du siège 60 et surmonte au moins partiellement la force magnétique retenant l'organe d'obturation 62 sur le siège 60.

Un interstice se forme alors entre le siège 60 et l'organe d'obturation 62 permettant l'entrée de fluide dans l'espace intérieur 32 et le gonflage de la poche 24.

Lorsque le gonflage s'achève, l'organe d'obturation 62 reprend sa position d'obturation sous l'effet de la force magnétique. L'espace intérieur 32 de fluide est obturé de manière étanche, comme l'illustre la figure 11. Une pression induite, par exemple supérieure à 0,5 bars relatifs, et notamment de l'ordre de 1 bar relatifs subsiste dans l'espace intérieur 32 du ballonnet 12.

Cette pression est nécessaire au démarrage du gonflage (amorçage de l'élasticité du ballonnet 12). Cette pression baisse dans la deuxième phase du gonflage. La pression induite dépend de l'élasticité du matériau, de l'épaisseur de paroi, de la longueur initiale du ballonnet, etc. Elle favorise l'étanchéité de la vanne.

Pour dégonfler le ballonnet 12, la patiente est soumise à un champ magnétique extérieur de forte intensité, comme décrit précédemment.

Comme visible sur la figure 12, ceci engendre la libération de l'organe d'obturation 62 au moins temporairement à l'écart du siège 60 et le passage de fluide autour de l'organe d'obturation 62 vers la lumière centrale 50 pour dégonfler la poche 24.

Le déplacement de l'organe d'obturation 62 à l'écart du siège 60 est avantageusement très limité, par exemple limitée à quelques centièmes, voir quelques dixièmes de millimètres.

L'organe d'obturation 62 est cependant déplaçable suivant au moins deux axes distincts par rapport à la poche 24, comme pour le ballonnet 12 du premier nécessaire 10, ce qui ne nécessite pas de contrôler l'orientation du champ magnétique suivant une direction spécifique pour libérer l'organe d'obturation 62.

Avantageusement, pour chacun des ballonnets 12 décrits précédemment, l'organe d'obturation 62 et/ou la bague périphérique 54 sont munis d'un revêtement biocompatible 114.

Ce revêtement est par exemple un revêtement en titane, en carbone, en polymère fluoré (notamment en polytétrafluoroéthylène), ou/et en parylène. En variante, le revêtement est un film, notamment un film polymère, par exemple en polyisoprène, ou en polyuréthane.

Dans l'exemple représenté sur la figure 13, au moins une surface périphérique extérieure 116 de la bague 54 est munie du revêtement biocompatible. Avantageusement, une surface périphérique intérieure 118 de la bague 54 et la surface extérieure de l'organe d'obturation 62 sont également munies de ce revêtement,

Dans encore une autre variante, visible sur la figure 14, la bague 54 présente un chanfrein 201 à son extrémité formant le siège 60 de l'organe d'obturation 62.

L'organe d'obturation 62 est ainsi apte à entrer plus dans le diamètre intérieur de la bague 54. Ceci augmente la surface de contact et donc l'étanchéité entre l'organe d'obturation 62 et la bague 54, prise au niveau du siège 60.

Dans une variante (non représentée), l'organe d'obturation 62 après avoir quitté la position d'obturation n'est pas nécessairement déplaçable librement dans tout l'espace intérieur 32, mais uniquement dans une région limitée de l'espace intérieur 32. Par exemple, un compartiment de réception de l'organe d'obturation 62 est monté dans l'espace intérieur 32 autour de l'orifice de vidange 36.

Dans une autre variante (non représentée), l'organe d'obturation 62 est disposé dans sa position d'obturation à l'extérieur de l'espace intérieur 32. Un compartiment de réception de l'organe d'obturation 62 est monté sur la poche 24, à l'extérieur de celle-ci, autour de l'orifice de vidange 36. Ce compartiment de réception est de préférence ajouré pour permettre l'écoulement du fluide provenant de l'espace intérieur 32.

Dans d'autres variantes, l'organe d'obturation 62 ne présente pas une forme sphérique mais présente une autre forme, par exemple polyédrique.

REVENDICATIONS

1.- Ballonnet (12) gonflable, destiné à être implanté dans une cavité (14) corporelle, comportant :

- une poche (24) formée d'une paroi étanche (30) délimitant un espace intérieur (32) ;

- une vanne de remplissage (26) de l'espace intérieur (32) par un fluide, propre à être obturée après remplissage de l'espace intérieur (32) ;

caractérisé en ce que la poche (24) délimite un orifice de vidange (36) de fluide débouchant dans l'espace intérieur (32), le ballonnet (12) comportant un organe d'obturation (62) de l'orifice de vidange (36), l'organe d'obturation (62) étant propre à libérer l'orifice de vidange (36) sous l'effet d'un champ magnétique, pour permettre la vidange au moins partielle du fluide contenu dans l'espace intérieur (32), l'organe d'obturation (62) étant déplaçable suivant au moins deux axes distincts par rapport à la poche (24).

2. - Ballonnet (12) selon la revendication 1, dans lequel l'organe d'obturation (62) est disposé dans l'espace intérieur (32).

3. - Ballonnet (12) selon la revendication 2, dans lequel l'organe d'obturation (62) est librement déplaçable dans l'espace intérieur (32) défini par la poche (24) sous l'effet d'un champ magnétique.

4. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel l'organe d'obturation (62) est propre à être maintenu dans une position d'obturation de l'orifice de vidange (36) par aimantation, l'organe d'obturation (62) étant propre à être déplacé à l'écart de l'orifice de vidange (36) sous l'effet d'un champ magnétique propre à vaincre l'aimantation maintenant l'organe d'obturation (62) dans la position d'obturation de l'orifice de vidange (36).

5. - Ballonnet (12) selon l'une quelconque des revendications précédentes, comprenant au moins un siège de retenue (60) de l'organe d'obturation (62), disposé au voisinage de l'orifice de vidange (36), l'organe d'obturation (62) coopérant par aimantation avec le siège de retenue (60) dans une position d'obturation de l'orifice de vidange (36), le siège de retenue (60) étant préférentiellement un anneau, rapporté sur la paroi étanche (30) autour de l'orifice de vidange (36).

6. - Ballonnet (12) selon la revendication 5, dans lequel le siège de retenue (60) est couvert d'une couche d'un matériau souple, l'organe d'obturation (62) étant disposé en appui sur la couche de matériau souple dans une position d'obturation de l'orifice de vidange (36).

7. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel l'organe d'obturation (62) est une bille.

8. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel l'organe d'obturation (62) présente une aimantation permanente.

9. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel l'organe d'obturation (62) est propre à être déplacé jusqu'à au moins deux positions distinctes de libération de l'orifice de vidange (36) sur deux axes sécants passant par l'orifice de vidange.

10. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel la paroi étanche (30) de la poche (24) est réalisée à partir d'un polymère choisi parmi le silicone, le latex, le polyuréthane, et/ou le polyisoprène.

11. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel l'orifice de vidange (36) est défini par la vanne de remplissage (26), l'organe d'obturation (62) étant propre à fermer la vanne de remplissage (26) après remplissage de l'espace intérieur (32).

12. - Ballonnet (12) selon l'une quelconque des revendications précédentes, dans lequel la vanne de remplissage (26) comporte une bague de guidage (54) d'un tube (72) de gonflage du ballonnet (12), la bague de guidage (54) et/ou l'organe d'obturation (62) présentant un revêtement biocompatible (114).

13. - Nécessaire (10) de traitement d'un patient, comportant :

- un ballonnet (12) selon l'une quelconque des revendications précédentes ;

- un dispositif (16) de gonflage et de largage du ballonnet (12), comprenant un tuteur (70) de support du ballonnet (12), le ballonnet (12) étant monté de manière libérable sur le tuteur de support (70), et un tube (72) de gonflage du ballonnet (12), propre à être inséré de manière libérable dans la vanne de remplissage (26).

14. - Procédé de vidange d'un ballonnet (12) selon l'une quelconque des revendications 1 à 12, le ballonnet (12) étant implanté dans une cavité corporelle (14), l'espace intérieur (32) du ballonnet (12) contenant du fluide, l'organe d'obturation (62) obturant l'orifice de vidange (36), le procédé comprenant les étapes suivantes :

- soumission du ballonnet (12) à un champ magnétique externe, avantageusement produit par un appareil de résonance magnétique nucléaire, suivant au moins une première direction (H1) ;

- déplacement de l'organe d'obturation (62) suivant au moins un axe sous l'effet du champ magnétique externe, pour libérer l'orifice de vidange (36) ;

- vidange au moins partielle du fluide contenu dans l'espace intérieur (32) à travers l'orifice de vidange (36).

15. - Procédé selon la revendication 14, comprenant une étape de soumission du ballonnet (12) à un champ magnétique externe, avantageusement produit par l'appareil de résonance magnétique nucléaire, suivant au moins une deuxième direction (H2), distincte de la première direction (H1).

5

ABREGE

Ballonnet gonflable et détachable, destiné à être implanté dans une cavité corporelle, nécessaire de traitement et procédé de vidange associés

Le ballonnet (12) comporte :

- une poche (24) formée d'une paroi étanche (30) délimitant un espace intérieur (32) ;

- une vanne de remplissage de l'espace intérieur (32) par un fluide, propre à être obturée après remplissage de l'espace intérieur (32).

La poche (24) délimite un orifice de vidange (36) de fluide débouchant dans l'espace intérieur (32). Le ballonnet (12) comporte un organe d'obturation (62) de l'orifice de vidange (36), l'organe d'obturation (62) étant propre à libérer l'orifice de vidange (36) sous l'effet d'un champ magnétique, pour permettre la vidange au moins partielle du fluide contenu dans l'espace intérieur (32). L'organe d'obturation (62) est déplaçable suivant au moins deux axes distincts par rapport à la poche (24).

Figure 2

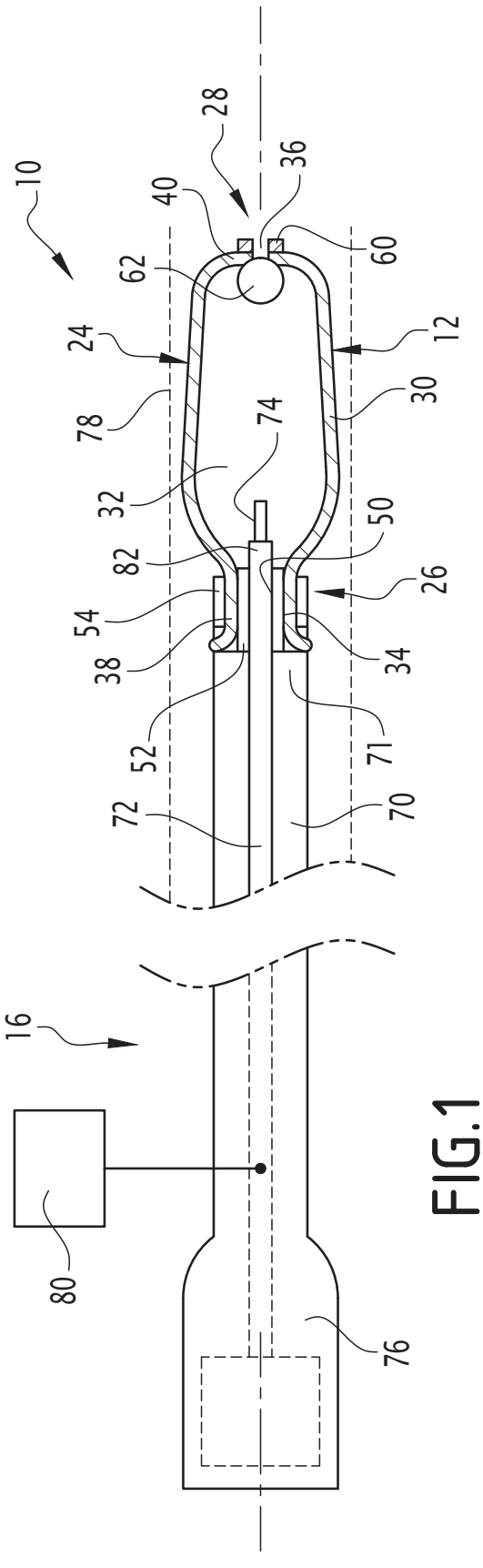

10
28
36
40
62
60
24
12
78
74
30
32
50
82
26
54
34
38
52
71
72
70
16
80
76

FIG.1

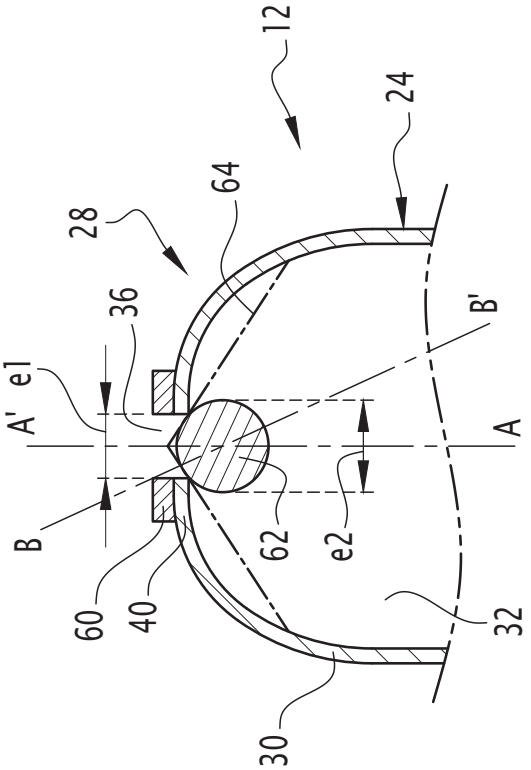

12
24
64
28
36
B'
A' e1
A
B
62
e2
60
40
32
30

FIG.2

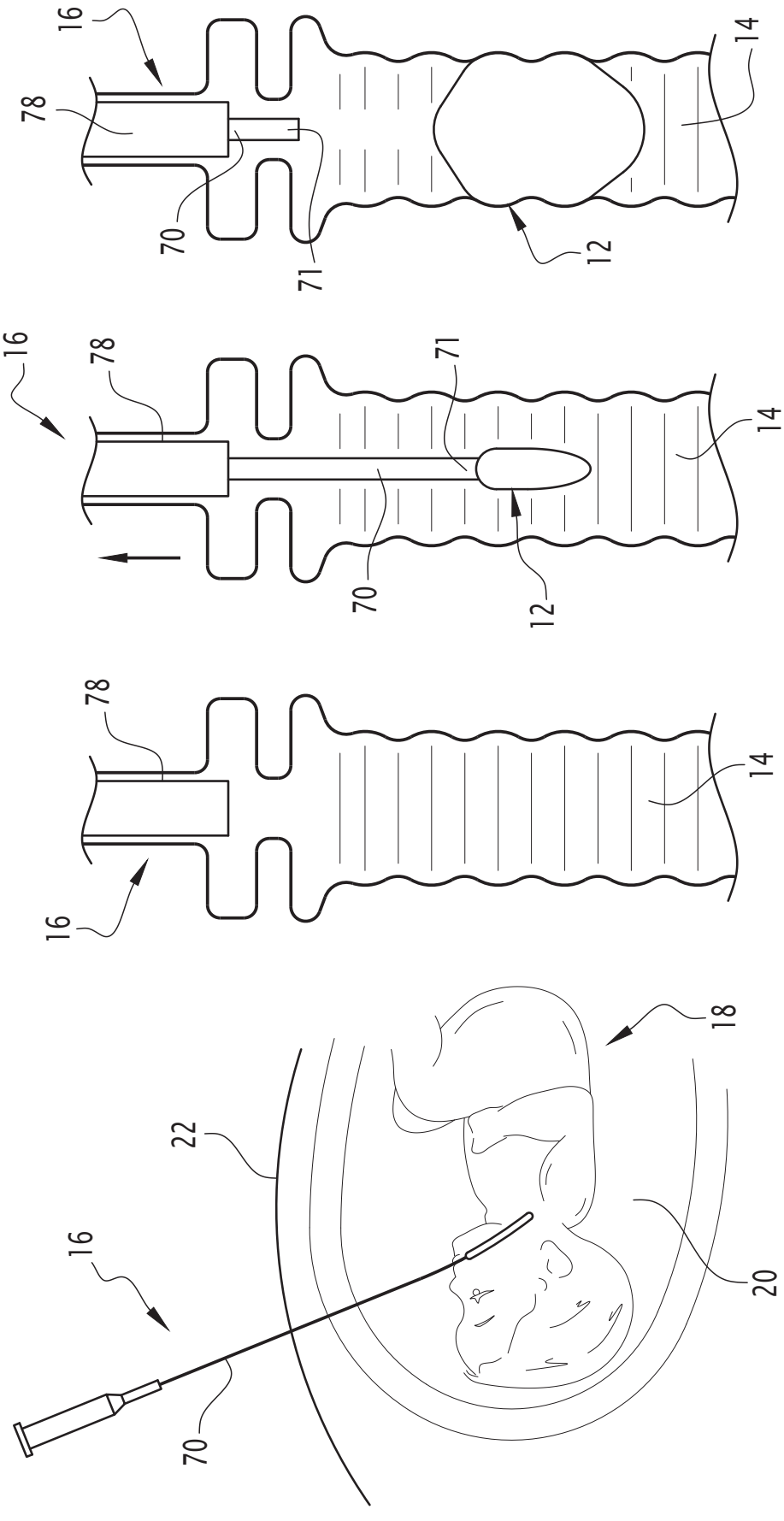


FIG.6

FIG.5

FIG.4

FIG.3

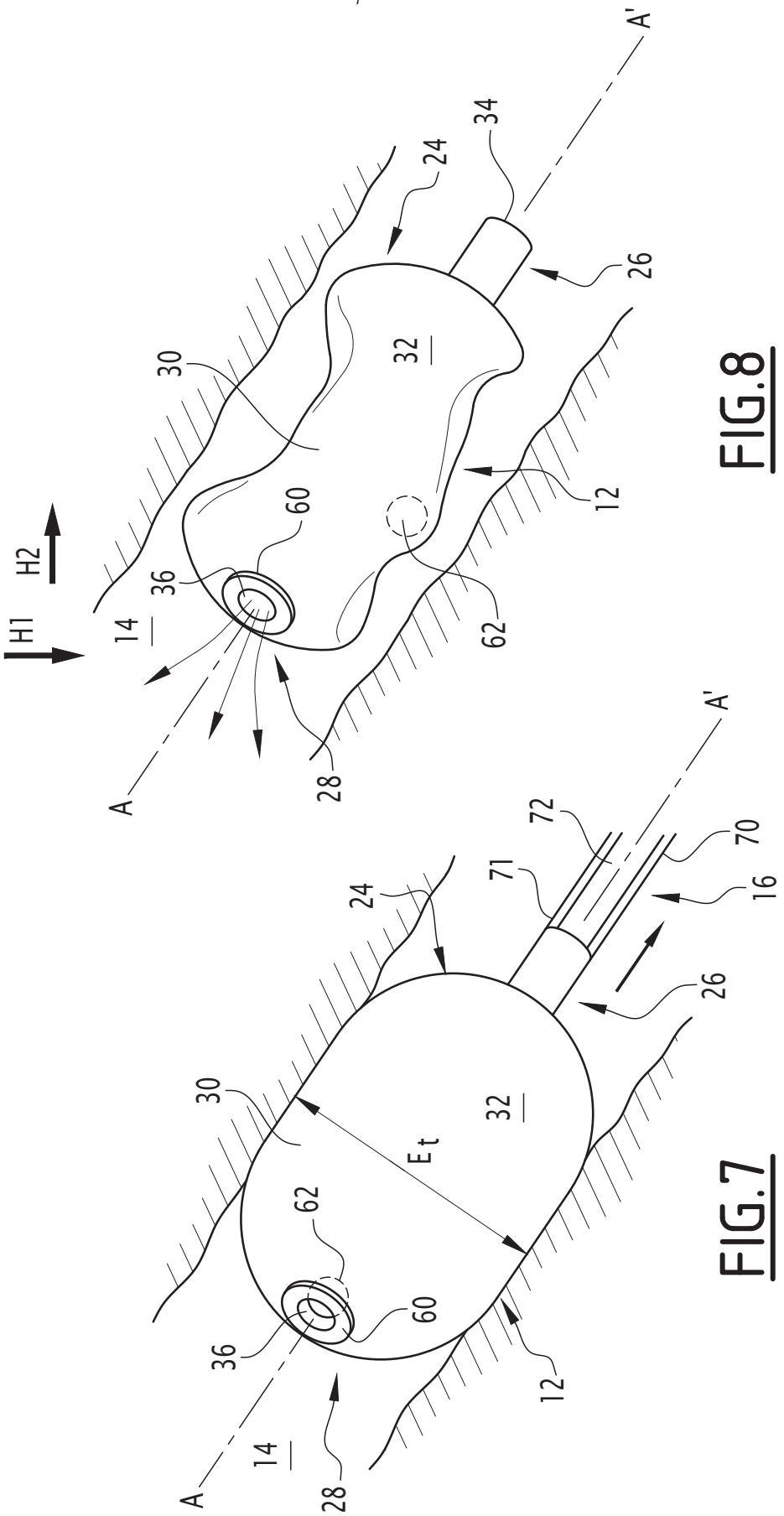

A'
34
24
26
30
32
60
H2
H1
36
14
12
62
A
28

FIG.8

A'
72
70
71
24
16
26
30
32
Et
62
60
36
12
14
A
28

FIG.7

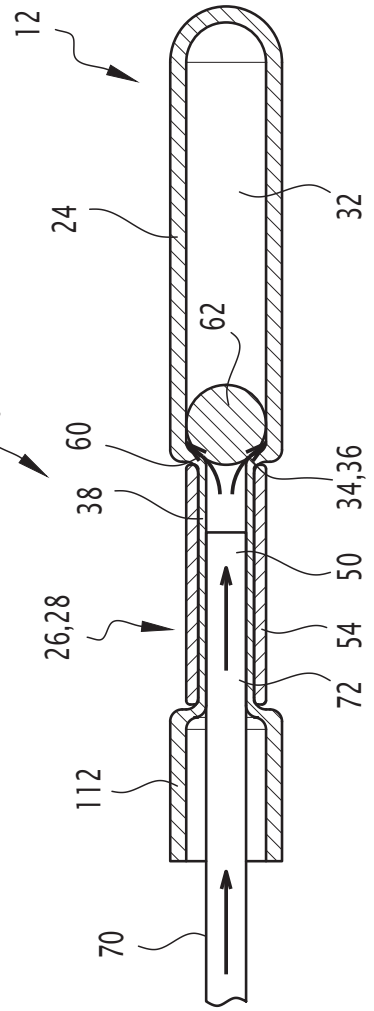


FIG.10

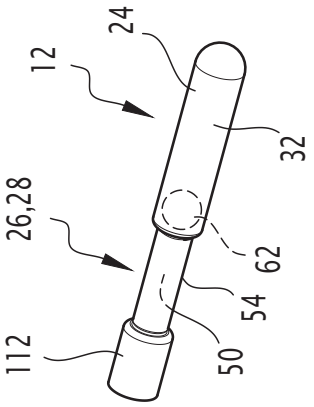


FIG.9

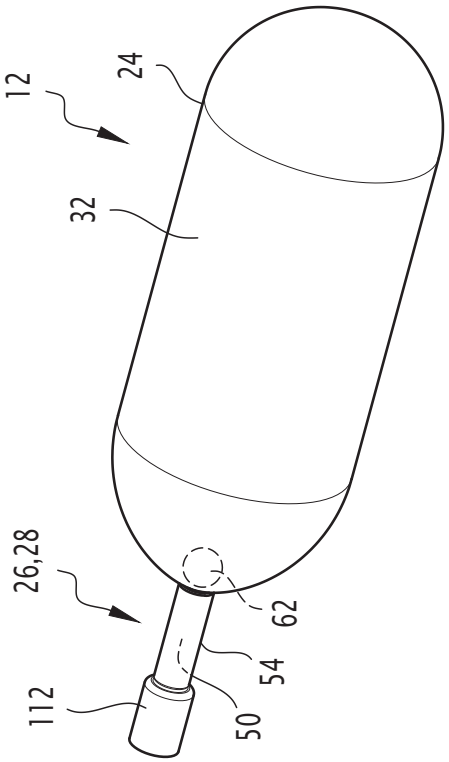


FIG.11

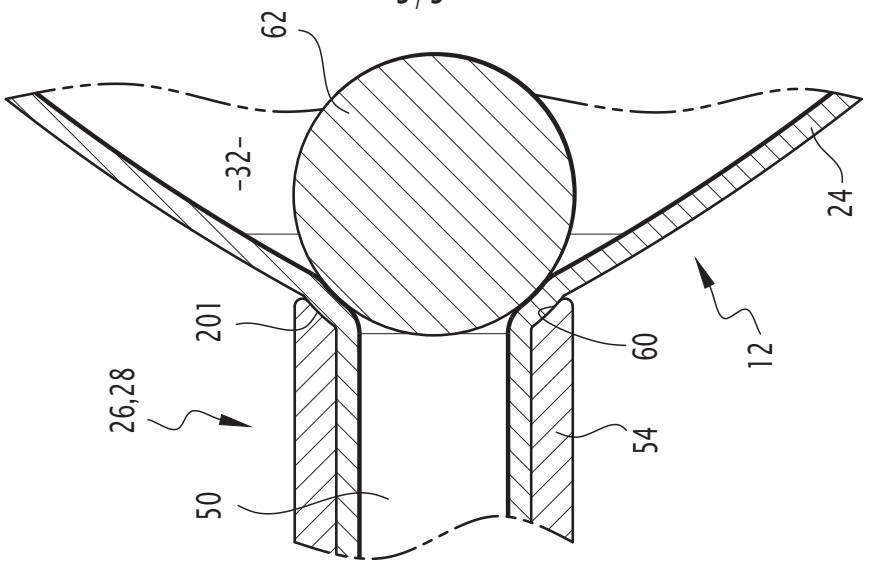


FIG.14

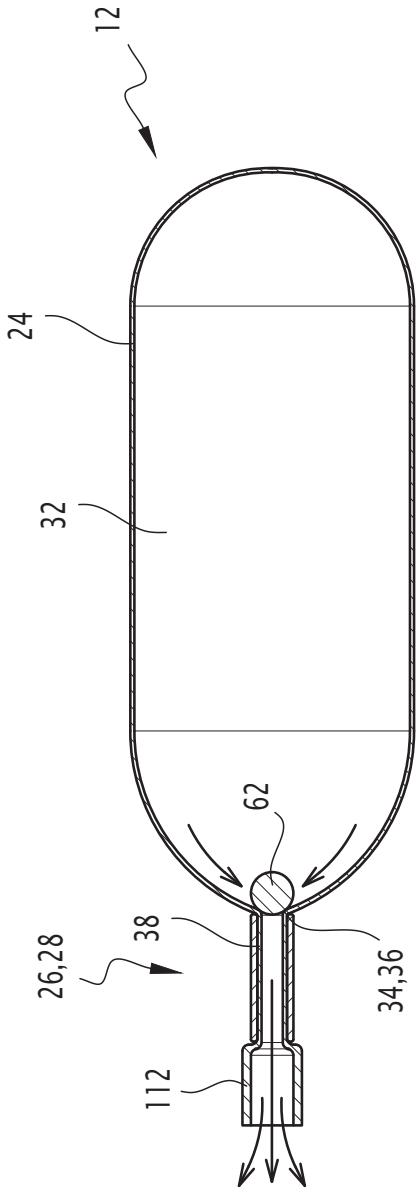


FIG.12

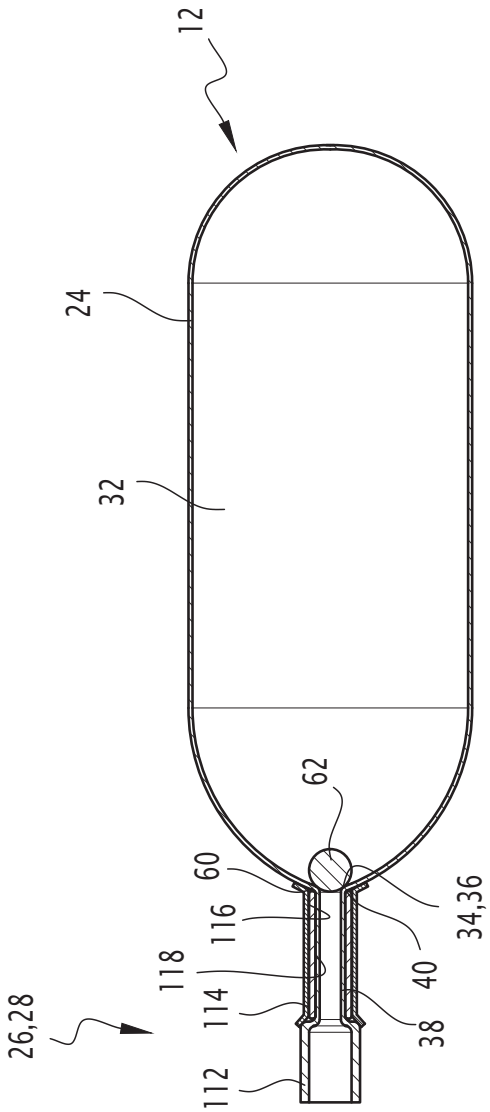


FIG.13

7.5. Tests du ballonnet

7.5.1. Tests sur simulateur

Deflation assessment of the SMART tracheal occlusion system in a low and high fidelity simulator for fetal endotracheal balloon occlusion (FETO).

Rationale: One of the drawbacks of FETO is the need to reverse the occlusion in utero or at the latest at birth. At this moment this mandates the patient to stay close to the FETO center until the airways are freed. The group of the University of Strasbourg has designed an occlusion system that automatically deflates when placed in a strong magnetic field. The procedure would consist of simple ambulation by the pregnant mother around the MR machine, so that she has been walking in different angles throughout this magnetic field. This means that in a non-invasive way the airways could be freed in any hospital that has an operational MR system. This device is currently being tested preclinically. One of the questions is how effective the valve of the balloon can be opened, and the balloon be deflated in different fetal positions.

Hypothesis: SMART-TO balloon deflation will be successful regardless of the fetal position by a standardized single ambulation of an adult carrying a fetus within a simulated in utero environment in a magnetic field of a clinical MRI system.

Methods: This study was conducted in the medical imaging research center of KU Leuven (Belgium). FETO was performed using a high-fidelity fetal simulator specially developed for this type of procedure [Windrim, 2014 #117]. The technique for FETO was the same as previously described with the only difference that the novel SMART-TO balloon and its proper delivery system were used [Deprest, 2011 #84]. For each procedure, a 1.3mm fetoscope housed within a slightly curved 3.3mm sheath (Karl Storz, Tuttlingen, Germany) was advanced into the trachea until the carina was visualized. A detachable balloon SMART-TO balloon (BSMTI, Niederrodern, France) was positioned between the vocal cords and carina with a purpose-designed delivery catheter (BSMTI, Niederrodern, France). Before tracheal PLUG, the balloon and its delivery device were inserted within one of the working channels of the fetoscope, then the integrity of the balloon was tested by filling 0.7 mL saline water (this is the amount recommended by the developers) with a 1 mL Luer Lock syringe, then deflated using the proper stylet. Afterwards, the fetal mannequin was placed inside a plastic balloon filled with 1000ml of water with the overall form of a pregnant uterus.

The fetal mannequin was held by an adult in front of the abdomen in different presentations to cover all possible tracheal orientations in relation to the MR table, hence covering the entire range of possible fetal positions (assuming the baby does not move during the exposure to the magnetic field). For this experiment, the longitudinal axis of the balloon coincides by definition with that of the trachea and the fetal body axis. To describe the position of the tracheal axis (= balloon axis) in space, it can be characterized by a rotation in the horizontal and in the vertical plane. In each plane a 360° rotation is possible, yet the actual direction of the axis of the balloon is irrelevant, all positions within a 180° are sufficient. The mother (and by extension the fetus) however walked around the MR machine (clinical 1.5-tesla unit (Achieva, Philips, Amsterdam, Nederland) as in the IFU, and by doing so, her longitudinal body axis de facto turned around a 360° angle. She actually takes care of all possible orientations in one plane. This means only one variable can describe the tracheal axis throughout the entire experiment. We described this by the angle the trachea takes in a coronal plane, again at an angle between 0 and 90°. The experiment was done at either 0°, 30, 60° and 90°. For each balloon position 8 repetitions were performed, for a total of 32 SMART-TO deflation tests in the standing position at an altitude of 95 cm from the ground.

The experiment mimicked one other variable, that is the altitude of the balloon from the ground. We repeated the experiment with the adult holding the fetus 30 cm higher to mimic a taller pregnant woman (125 cm), a wheel chair (55 cm) and finally a brancard (75 cm). For every scenario a total of 8 repetitions in a random position were performed.

After a single tour around the MR, an ultrasound was performed to assess deflation. When the balloon was not visible during fetoscopy after MR exposure, hence meaning complete deflation, retrieval was made using grasping forceps by fetoscopy.

In the case that we could still visualize the balloon by ultrasound, a fetoscopy was performed to have direct visualization of the findings, and after introducing the mannequin in the balloon filled with water, a second walking tour was performed. If deflation was not achieved, then a third walking tour was performed, and the “patient” was placed inside the MRI as clinically would be attempted under the assumption that it is the field that is not strong enough. In case of failure of deflation after a third attempt, the balloon was punctured using a stylet and retrieved.

Study outcomes: The primary outcome was the rate of deflation after magnetic field exposure by a single standardized ambulation tour, confirmed by direct visualization of the trachea.
Secondary outcomes were the rate of deflation after second or third tour around MRI and the impact of the fetal position and height on deflation.

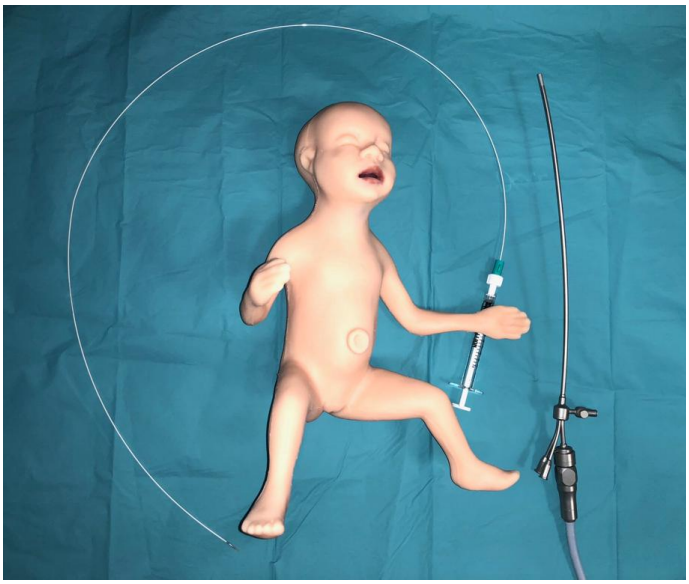
Sample size calculation: Since this is a feasibility study; therefore, no formal sample size calculation was needed. We considered that a total of 8 balloons in each representative position is an acceptable number to prove our hypothesis. The experiment was eventually repeated holding the fetus at higher and lower altitudes, with 8 fetuses each time in a random position. This led to a total of 56 SMART-TO fetoscopies.

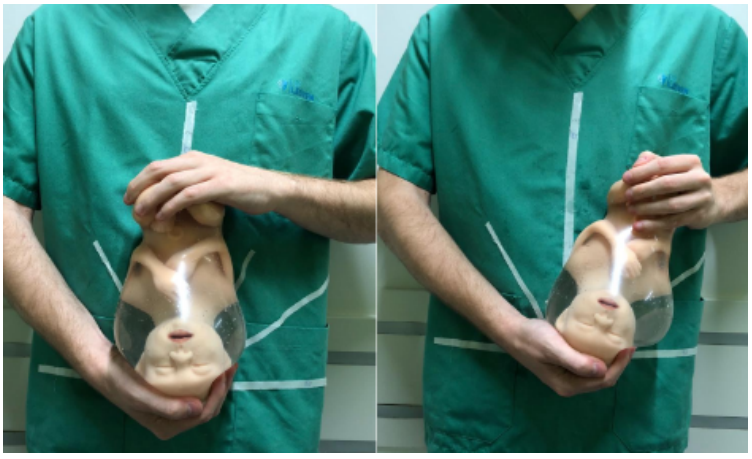
Statistical analysis: Continuous data is presented as mean and standard deviation (SD) or range and was compared by unpaired students t-test. A p-value <0.05 was considered significant. Categorical variables are presented as percentages. Data were analyzed using Prism for mac version 7.0 (Graphpad software, San Diego, CA, USA).

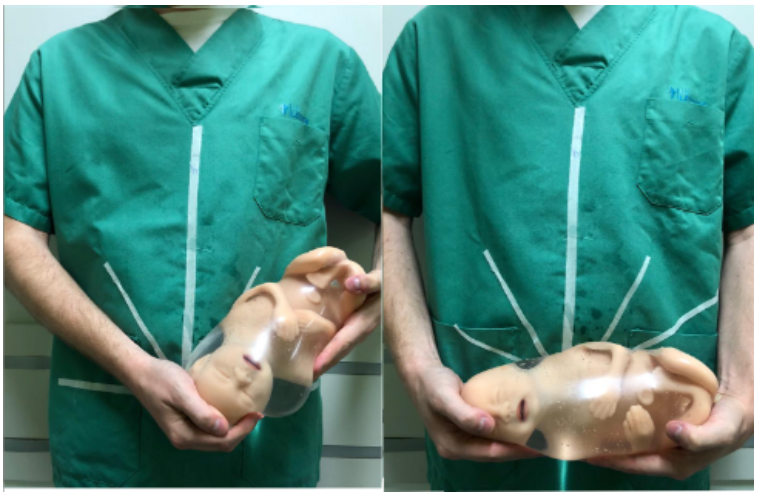
Results: A total of 56 SMART-TO balloons expositions were performed. All balloons were successfully inserted and retrieved without additional findings. The mean time needed to complete a single walking tour (standing position) around the MRI was 23 seconds (range: 20-25 seconds). The overall deflation rate after a single tour around the MRI was 94.7% (53/56). After a single ambulation tour in standing position (as in the instructions of use from the developers) deflation rate was 100% in all possible fetal positions (n=32, tracheal angles of 0°; 30°; 60°; 90); therefore, deflation was achieved regardless the position of the fetus. (Table 1)
When exposing the balloon at a “higher altitude” (30 cm above the normal standing position), deflation rate was achieved in all cases after a single walking tour (n=8). Deflation following exposure on lateral left decubitus on a brancard was also achieved in all cases (n=8). On the contrary, when strolling a wheelchair 3/8 (37.5%) balloons did not deflate after first exposure. Of these, 2/3 were successfully deflated after a second round and 1 failed to deflate after a third tour and exposure inside the MR tunnel. This balloon was then punctured by fetoscopy and retrieved. Deflation rate was significantly lower in the wheelchair compared to the other “maternal positions” (p value <0.001).

Table 1. Summary of observations in different “maternal” positions and angulations.

Maternal position	Balloon position (degrees)	N	Deflation after first exposure N (%)	Deflation after second exposure N (%)	Deflation after third exposure N (%)	Deflation failure N (%)
Standing	0	8	8 (100)	-	-	-
	30	8	8 (100)	-	-	-
	60	8	8 (100)	-	-	-
	90	8	8 (100)	-	-	-
	Total	32	32 (100)	-	-	-
Standing Higher altitude	Random	8	8(100)	-	-	-
Wheel chair	Random	8	5(62.5)	2(25)	0(0)	1(12.5)
Brancard	Random	8	8 (100)	-	-	-







Fetal presentations and angles: high-left: 0°; high-right 30°; low-left: 60° and low right:90°

7.5.2. Tests chez le singe

Evaluation of a new balloon for fetal endoscopic tracheal occlusion in the non-human primate model.

INTRODUCTION

Fetal Endoscopic Tracheal Occlusion (FETO) improves survival in severe cases of Congenital Diaphragmatic Hernia (CDH) according to a large cohort study (1), a non-randomized controlled trial (2), and a randomized controlled trial (3). Two multicenter international randomized controlled trials evaluating the interest of FETO in severe (NCT01240057) and moderate (NCT00763737) CDH are currently ongoing, under the acronym 'TOTAL' (Tracheal Occlusion To Accelerate Lung growth).

One of the major concerns about FETO procedure is the need for balloon removal. The restoration of airway potency is ideally accomplished prior to birth at 34 weeks of gestation, by either another fetal endoscopic procedure or, less frequently, by ultrasound-guided puncture (USGP) (1, 4, 5). The fetoscopy consists in a fetal tracheoscopy during which the balloon is punctured by a sharp stylet. A grasper forceps holds the tail of the balloon and allows its withdrawal. The balloon can also be punctured with a 20-G needle inserted through the maternal abdomen under ultrasound guidance. The balloon is then spontaneously washed out by the fluid coming out from the lungs.

There are several issues related to the prenatal unplug. First, prenatal balloon removal is a difficult procedure, which might not be feasible by fetoscopy nor USGP and then require a removal on placental circulation or even postnatally in 12.6 to 24.7% of the cases (1, 6). Second, balloon removal is performed in an emergency setting in 28.1 to 56% of the cases (1, 6), so the patients should be advised to stay near a FETO center for the whole duration of fetal tracheal occlusion, what might be unacceptable to most of them. Third, in utero balloon removal may precipitate preterm delivery, since 23 to 25% of patients go into labor or rupture their membranes within one week after the procedure (1, 6).

That's why we developed a new balloon, which allows a non-invasive, easily triggered, and externally controlled reversal occlusion. By following these specifications, every single issue related to the prenatal unplug is addressed. The objective of this study was to evaluate the operation of this new balloon in a non-human primate model.

METHODS

New patented balloon for FETO (BS-MTI, Niederroedern, France)

The balloon is made of latex material and its dimensions are 1.42mm x 6mm when deflated and 7mm x 20mm when inflated with 0.7mL of saline. A magnetic ball is included inside the balloon and is free of movements. It is made of Neodymium NdFeB / N35 and its diameter is 1mm. The metal tube cylinder is made of ferritic stainless steel type 430 and its dimensions are 1.3mm x 3mm. At the balloon side, the impermeability is ensured by a chamfer hosting the magnetic ball with a latex interface. On the other side, there is a latex tube whose dimensions are 1.42mm x 2.5mm. The metal tube and the magnetic ball are both coated with parylene. Outlines of the balloon are displayed in figure 1.

The single valve for both inflation and deflation is composed by the external ferromagnetic metal cylinder and the free of movement magnetic ball inside the balloon. Injection of saline through the valve moves away the ball thanks to the hydraulic pressure so the balloon can be filled. When filling is completed, the magnetic ball goes back against the metal cylinder and the impermeability is ensured by the interface of the balloon material. When the balloon is subjected to the magnetic fringe field of an MRI scanner, the ball is attracted out of the cylinder, so the balloon gets deflated. Then, such as when the unplug is performed by ultrasound guided puncture, the balloon is eventually washed out by the fluid coming out from the lungs.

In vitro impermeability, occlusion, and operation tests have already been successfully conducted. A patent application has been filed in 2016 (request #1653954 / submission #1000345616).

Experimental protocol

The monkeys were group-housed in indoor/outdoor facilities in SILABE plateform (Simian Laboratory Europe). They lived in social groups (1 or 2 adult males and 4 to 10 females) in indoor/outdoor facilities of approximately 40 to 85 m^3 for the indoor facility (depending on the animal facility and group) and 40 to 50 m^3 for the outdoor facility. Water and food were distributed ad libitum. Animals were captured regularly in order to perform ultrasound to detect pregnancy and establish the gestational age. The timing of surgery was determined by ultrasound measurement of the biparietal diameter of the fetus in early pregnancy (Voluson E GE®), assuming a gestational length of 165 days (7). Four pregnant rhesus monkeys (Macaca mulata) underwent FETO with the new balloon in the last third trimester of pregnancy, according to a protocol previously published (8). Pregnant females were captured on the day of the surgery. After surgery, they were isolated until the following morning and

were then returned to their social group. The Regional Ethics Committee on Animal Experimentation (CREMEAS) at Strasbourg University, France (number 7270-2016101815136666 v2) approved this study in 2017. Guidelines for the care and use of the monkeys approved by this institution were followed.

For the FETO procedure, the pregnant monkey was first sedated with ketamine (10mg/kg, IM) before general anesthesia with propofol (5mg/kg, IV) and 1 to 2.5% isoflurane after tracheal intubation. Ventilation was controlled by assisted mechanical respiration after tracheal intubation. Maternal breath frequency, electrocardiogram, PO2/PCO2 measurement, halogenated gas and temperature were continually monitored and a heating blanket was applied to maintain body temperature. Maternal blood pressure was monitored with a sphygmomanometer. A single dose of 2% marbocyl was given (Marbofloxacin 2mg/kg, IM) prior to surgery. No tocolytic agent was administered.

Before surgery, fetal monkey curarization was performed by an intra-muscular injection of 0.15 to 0.20 mg cisatracurium in the fetal leg, under ultrasound guidance. After localization of the fetal head and placenta in order to determine the optimal entry site, 5ml 2% xylocaine was injected percutaneously into the myometrium of the pregnant monkey in order to provide additional local anesthesia.

Endoscopic access was performed using the modified Seldinger technique under continuous ultrasound guidance (9, 10). A 17G needle was first introduced into the uterine cavity and an amnioinfusion of 200 to 300mL saline administered. A guide wire was then introduced through the needle and a 10 French-gauge arterial introducer set placed over this guide. Instrument specifications for FETO procedure followed the usual recommendations (4). A 1.3mm fetal tracheoscope was inserted through a 3.3mm sheath 30°-precurved with 3 side ports (11540AA and 11540KE, Karl Storz, Tuttlingen, Germany). The new patented detachable balloon (BS-MTI, Niederroedern, France) was used for TO. Amnioinfusion of saline was performed during the procedure in order to open the fetal mouth and vocal cords. Excessive amount of the infused fluid was drained through the trocar after surgery, under ultrasound guidance.

For the unplug procedure, the pregnant monkey was sedated with ketamine (10mg/kg, IM) and was carried in front of an MRI machine Siemens MAGNETOM Aera 1.5T, in the imaging facility of University Hospital Institute, Strasbourg. The monkey was positioned in front of the MRI scanner, with her abdomen facing the tunnel. Then the monkey was carried all around the MRI scanner keeping its abdomen close to the machine all the way. Ultrasound scan and X-ray were performed afterwards to check the deflation and localization of the balloon.

RESULTS

Four pregnant rhesus monkeys underwent FETO procedure using the new balloon, at 133 (129 to 139) days of gestation. In all cases, at the end of the procedure, the balloon could be visualized inside the fetal trachea by ultrasound scan.

The non-invasive unplug procedure was performed 4.8 (2 to 7) days after, *i.e.* at 140 (135 to 146) days of gestation. In all cases, the balloon was still in a correct position and its shape did not change significantly according to ultrasound scan findings (figure 2). The pregnant monkeys were all carried in front of an MRI scanner 1.5T, and carried all around the machine according to the protocol previously described. The ultrasound scans performed right afterwards could not visualize the balloon anymore in all cases, *i.e.* the balloon got deflated in all cases, what was confirmed at delivery.

Figure 3 illustrates X-ray findings performed after presenting the monkey in front of the MRI scanner. To be noted that in one case, whereas the balloon got deflated, the X-ray showed that it was still inside the fetal trachea. Still, the pregnant monkey delivered the day after and the balloon was eventually washed out since it was found deflated outside the neonate.

The mean right LHR measurements before plug and after unplug were respectively 1.1 and 1.9. The mean left LHR measurements before plug and after unplug were respectively 1.1 and 1.6. The LHR measurements slightly increased but with no statistical difference.

REFERENCES

1. Jani JC, Nicolaides KH, Gratacos E, Valencia CM, Done E, Martinez JM, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. Ultrasound Obstet Gynecol. 2009;34(3):304-10.
2. Peralta CF, Sbragia L, Bennini JR, de Fatima Assuncao Braga A, Sampaio Rousselet M, Machado Rosa IR, et al. Fetoscopic endotracheal occlusion for severe isolated diaphragmatic hernia: initial experience from a single clinic in Brazil. Fetal Diagn Ther. 2011;29(1):71-7.
3. Ruano R, Yoshisaki CT, da Silva MM, Ceccon ME, Grasi MS, Tannuri U, et al. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. Ultrasound Obstet Gynecol. 2012;39(1):20-7.
4. Deprest J, Nicolaides K, Done E, Lewi P, Barki G, Largen E, et al. Technical aspects of fetal endoscopic tracheal occlusion for congenital diaphragmatic hernia. J Pediatr Surg. 2011;46(1):22-32.
5. Deprest J, De Coppi P. Antenatal management of isolated congenital diaphragmatic hernia today and tomorrow: ongoing collaborative research and development. Journal of Pediatric Surgery Lecture. J Pediatr Surg. 2012;47(2):282-90.

6. Jimenez JA, Eixarch E, DeKoninck P, Bennini JR, Devlieger R, Peralta CF, et al. Balloon removal after fetoscopic endoluminal tracheal occlusion for congenital diaphragmatic hernia. Am J Obstet Gynecol. 2017.
7. Tardif S CA, Elmore D, et al. Nonhuman Primates in Biomedical Research: Biology and Management. Academic Press; 2012. p. 201.
8. Sananes N, Ruano R, Weingertner AS, Regnard P, Salmon Y, Kohler A, et al. Experimental fetal endoscopic tracheal occlusion in rhesus and cynomolgus monkeys: nonhuman primate models. J Matern Fetal Neonatal Med. 2015;28(15):1822-7.
9. Hajivassiliou CA, Nelson SM, Dunkley PD, Cameron AD, Frank TG, Cuschieri A, et al. Evolution of a percutaneous fetoscopic access system for single-port tracheal occlusion. J Pediatr Surg. 2003;38(1):45-50; discussion 45-50.
10. Luks FI, Deprest JA, Gilchrist BF, Peers KH, van der Wildt B, Steegers EA, et al. Access techniques in endoscopic fetal surgery. Eur J Pediatr Surg. 1997;7(3):131-4.

Figure 1: Outlines of the innovative balloon (BS-MTI, Niederroedern, France)

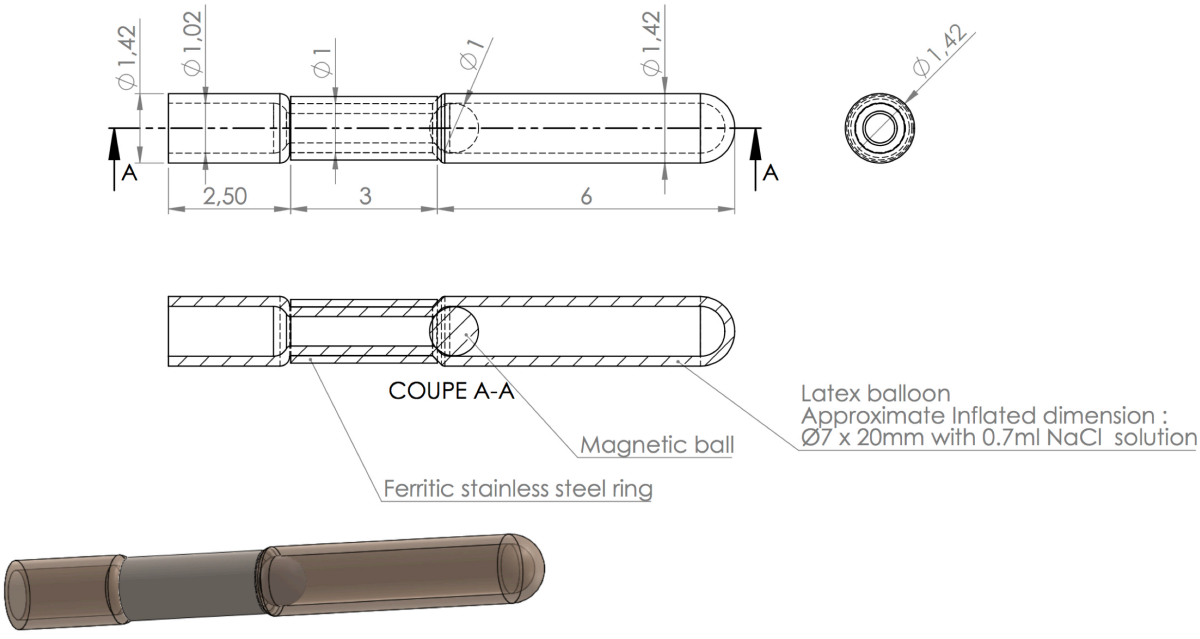


Figure 2: Ultrasound showing the balloon right after the FETO (above) and one week later (below).

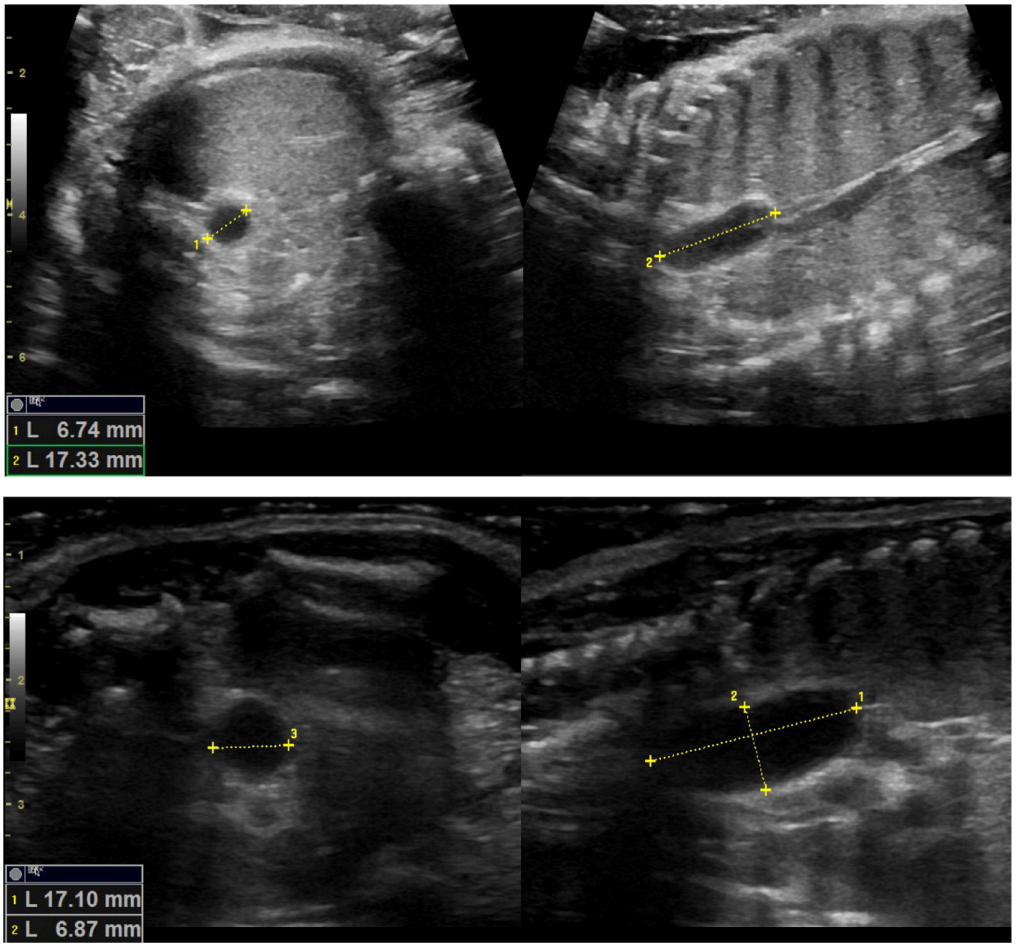
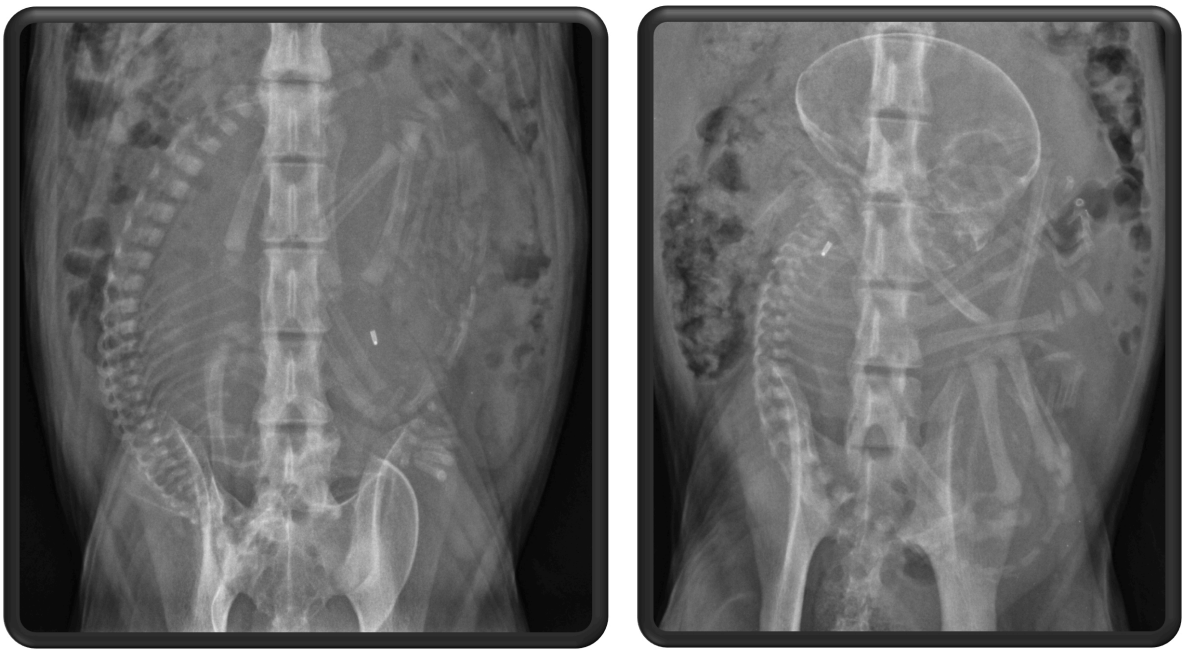


Figure 3: X-rays after presenting monkeys in front of the MRI scanner. The balloon is outside the fetus in the left and still inside the fetal trachea on the right.



7.5.3. Tests chez la brebis

Assessment of the occlusiveness of the SMART-TO balloon in healthy lambs

Rationale: One of the drawbacks of FETO is the need to reverse the occlusion ideally in utero or at the latest at birth. At this moment this mandates patient to stay close to the FETO center until the airways are freed. The group of the University of Strasbourg has designed an occlusion system that automatically deflates when placed in a strong magnetic field. There are no data on the efficacy of the device in terms of lung growth and on its potential tracheal side effects.

Methods: This study was performed in the animal research center of the KU Leuven (Belgium), on healthy Swifter pregnant sheep and fetuses. Non-inferiority study comparing the SMART-TO balloon (BSMTI, Niederrodern, France) to the standard occlusion system (Goldbal 2, Balt, Montmorency, France). The experimental design included two feto-maternal surgeries: Fetal tracheal occlusion at 95 days of gestation (GD) and tracheal endoscopy and reestablishment of airway patency and euthanasia at 116 GD. The choice of timing of TO is correlated to the phase of lung development: at 95 GD the fetal lung of the lambs is in the canalicular phase, which is the same as in human fetuses undergoing TO in the clinical setting.

Fetal foregut endoscopy and tracheal occlusion at GD 95: The ewe was sedated by injecting 0.3mg/kg IM Xylazine, after 30 minutes induction of general anesthesia was initiated with 4 mg/kg IV Propofol and then maintained by continuous IV infusion of Propofol 0.3-0.5 mg/kg/min. Before the start of surgery, 1.5 g IV Cefazolin for prophylactic antibiotics and 0.02 mg/kg IM Buprenorphine for prophylactic analgesia where administered. A 15 cm parasagittal midline laparotomy was performed, then the uterus was exteriorized, and an ultrasound scan was made in order to define the number and orientation of the fetus(es). Fetal analgesia (Fentanyl IM 10ug/kg) was injected through the uterus into one of the fetal limbs under ultrasound guidance and direct palpation. A 2 cm hysterotomy was made and the membranes were fixed with Monocryl 3/0. The fetoscope (10 Fr- 3.3 mm) (Karl Storz, Tuttlingen, Germany) was introduced through the mouth using the palate, tongue, and epiglottis as anatomic landmarks to insert the scope between the vocal cords. The balloon was positioned 2 cm above the right upper lobe orifice, filled with 0.7 cc of saline SMART-TO balloon or 0.6 cc for Goldbal 2 and detached. Following balloon assessment, the fetoscope was retrieved, then the uterus was closed with a two-layer running suture using Monocryl 3/0. The abdominal wall was closed in two layers, first the fascia with PDS 1 loop and then the skin with a subcuticular running suture using Monocryl 2-0. After surgery fentanyl 10 mg IM was given to the ewe for postoperative analgesia.

Tracheoscopy and MR exposure at GD 116: Fetal tracheoscopy was performed as in the first procedure, to confirm the presence of the balloon. The occlusiveness was assessed by gently pushing on the balloon with the fetoscope. In the case of Goldbal 2 balloon, a stylet was introduced, and the balloon was punctured. Afterwards the fetus was repositioned in the uterus and closure of the uterus and abdominal wall were performed. The ewe (and fetus) were euthanized with 0.3ml/kg of T61 (Merck Animal Health, Canada). Following euthanasia, the ewe was moved into the MRI room to allow deflation of the SMART-TO balloon by carrying it between two operators and walking around the MR machine. The tracheal airway patency was then confirmed by ultrasound. The fetus(es) were delivered via cesarean section and the fetal lungs and trachea were collected and stored for histological analysis.

Harvesting and histological analysis:

The fetal weight and total lung weight were measured, allowing the calculation of the lung-to-body-weight ratio (LBWR); the left lung volume was calculated using water displacement method. Afterwards, the samples were fixed in 10% neutral buffered formalin solution for 48 hours and then washed for 1 hour in 70% alcohol solution. Hematoxylin & Eosin(H&E) for histological analysis was performed. The trachea was macroscopically analyzed, specifically for the presence of tracheal dilatation or compression, contour deformations and modification of the pars membranacea. Afterwards, the samples were fixed in 10% neutral buffered formalin solution for 24 hours and then washed for 1 hour in 70% alcohol solution. Hematoxylin & Eosin(H&E) for histological analysis was performed. The histological evaluation will be done on 4 random sections per level: above the plug, at the level of the plug and below the plug using a validated descriptive, semi-quantitative interval scoring system of all anatomical tracheal elements [1]. For each anatomical element, the score could range between absence of any effect and increasingly larger degrees of abnormality. Minimum total score was zero, and maximum was 9 for epithelium, 7 for submucosa, 2 for cartilage and 2 for the pars membranacea, respectively (maximum total microscopic score 20). For the epithelium, presence or absence of the typical folding pattern at lowest magnification were noted. At higher magnification (100X) eventual squamous metaplasia was looked for, i.e. the disappearance of cilia at the apical border of the cells lining the tracheal lumen, and replacement of the normal pseudostratified respiratory epithelium by multilayered squamous epithelium. In the submucosal layers, the absence or presence of acute (infiltration with neutrophils), chronic (infiltration with lymphocytes and plasmocytes) or granulomatous (giant cells and histiocytes) inflammation, calcifications or fibrin deposits was determined. The cartilage and pars membranacea were classified between normal and non- vital.

Two observers, familiar with tracheal histology but blinded to the procedure or localization of the section, will score each section.

Study outcomes:

Primary outcome: LBWR as a proxy of the effectiveness of the occlusion.

Secondary outcomes: histological tracheal score, mean terminal bronchial density (MTBD), amniotic and tracheal fluid electrolytes.

Statistical analysis: Continuous data is presented as mean and standard deviation (SD) or range and was compared by unpaired students t-test. A p-value <0.05 was considered significant. Categorical variables are presented as percentages. Data were analyzed using Prism for mac version 7.0 (Graphpad software, San Diego, CA, USA).

Sample size calculation: A sample size of 3 fetuses per group would allow a 5% significance level, 90% power, non-inferiority limit of 10.5 and standard deviation of outcome of 3.4 for the LBWR. Considered a 20% fetal loss rate by fetal instrumentation, and a 30 % balloon dislodgement and failure rate, 4 fetuses will be required for each treatment group. Therefore 12 fetuses and 8 ewes in total will be used in the study.

Preliminary results:

Occlusiveness test:

A total of 5 ewes and 10 fetuses (1 singleton, 3 twins and 1 triple pregnancy) underwent TO surgery at a mean GA of 95.2 $\pm$ 0.4 weeks. In one case, anesthesia complications occurred during surgery, and the ewe aborted 72 hours later. All the other fetuses were alive at the time of the second surgical procedure (mean GA of 116 $\pm$ 0 weeks), leading to an overall survival rate of 80%. In the SMART-TO (n=4), the inflated balloon was present in the trachea at the time of the second tracheal endoscopy in all cases. No direct trauma to the pharynx, vocal cords or trachea was noted at fetoscopy. In all cases, deflation of the balloon was achieved after a single tour around the MRI. One fetus presented hydrops at the time of the second procedure and was excluded for post-mortem analysis. At obduction, no obvious causes for this complication were detected. A total of 3 cases were therefore included in the analysis. The mean LBWR in the SMART-TO group was 0.080 $\pm$ 0.015%, which was significantly higher than that of healthy historical controls of similar gestational age (0.038 $\pm$ 0.007%, p value 0.04)[2]. At the moment of thracheoscopy in the Goldbal 2 group (n=4), 1 balloon was not present, and 1 balloon was loose. Leaving a total of 2 fetuses for analysis. Mean LBWR was 0.056%. No statistical analysis was made so far for this group due to the reduced number of fetuses.

Assessment of side effect on fetal trachea:

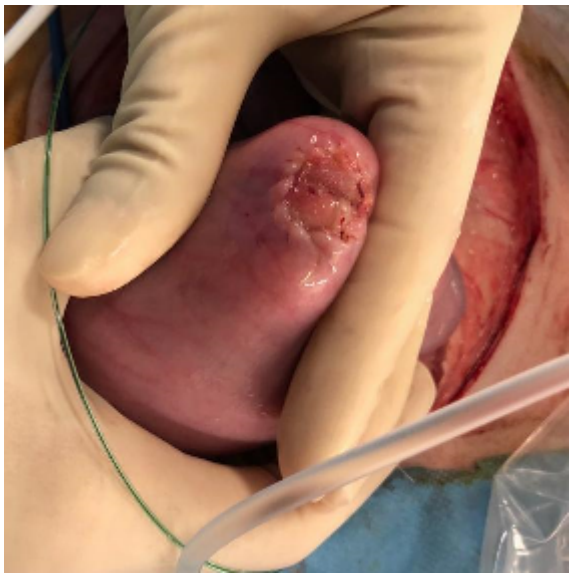
At macroscopic analysis, on the SMART-TO group one fetus (1/3, 33%) had tracheal dilation along all the length. None of the fetuses had presence of tracheal deformations, synechiae or alterations of the pars membranacea. On the Goldbal 2 group, tracheal dilatation was present in one fetus (1/2, 50%) and none had other macroscopic complications (contour deformations, pars membranacea alterations or synechiae).

Microscopically, on the SMART-TO group, changes at the level of the plug were very mild, with a mean total score of 2/20 (range: 1-4). Observed changes involved only the epithelium, in two cases there was generalized unfolding and mild squamous metaplasia and another case presented only local unfolding.

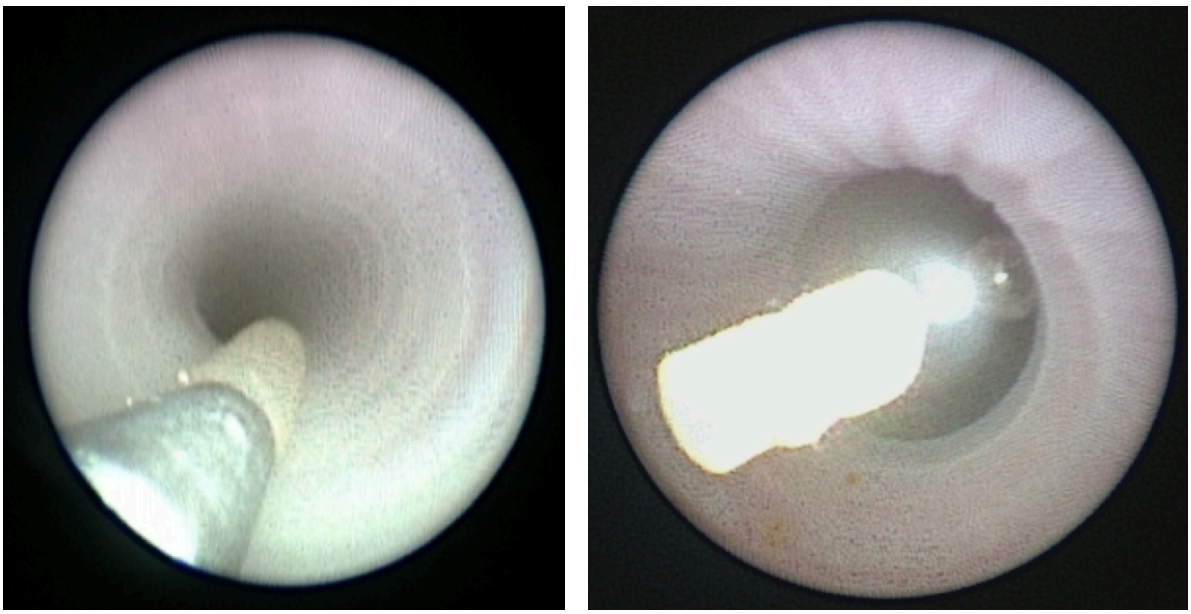
Of note, the tracheal scores above the site of the balloon were higher, with a mean total score of 4/20 (range: 3-5). Again, observed changes involved mainly the epithelium: two cases presented mild changes and in one case there was squamous metaplasia in more than 75% of the contour and moderate unfolding. Only one case (33%) presented localized chronic inflammatory reaction in submucosal tissue. We did not observe acute inflammatory reaction or granulomatous reaction. No alterations were observed below the site of the balloon insertion. Finally, the cartilage and pars membrancea were no damaged.

The microscopically analysis for the Goldbal 2 group will be performed before December.

This experiment will be finished by the end of December, a total of 6 more ewes and 10 fetuses already underwent tracheal occlusion SMART-TO (n=4) and Goldbal 2 (n=6), comparison between the two groups will be performed and reported.



Mini-histerotomy on fetal mouth, site for insertion of the fetoscope



Fetoscopic images showing the SMART-TO balloon insertion (left) and an inflated SMART-TO balloon (right) in the fetal trachea

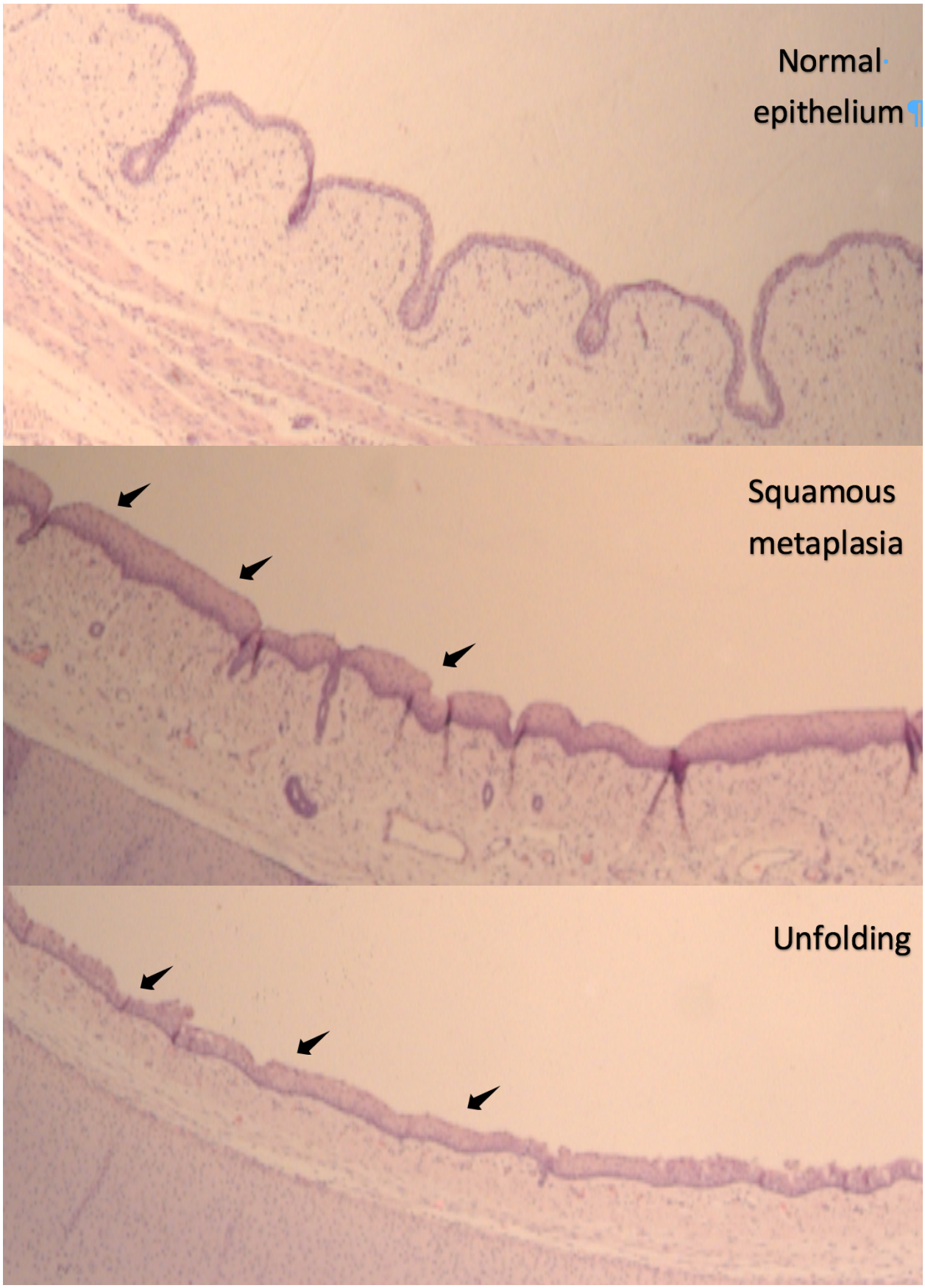
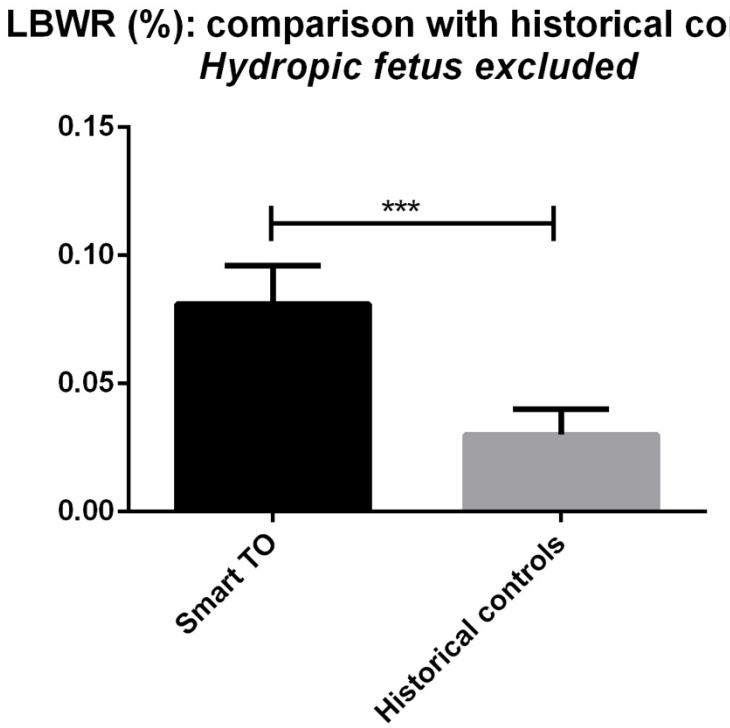

Normal epithelium
Squamous metaplasia
Unfolding

Table 1. Descriptive analysis of the SMART-TO group			
General			
Variable	N	Mean ± SD	Range
Maternal weight (kg)	5	49.2 ± 6	39-55
GA at PLUG	5	95.2 ± 0.4	95-96
GA at UNPLUG	4	116 ± 0	116
Fetus			
Fetal body weight (kg)	3	1.7 ± 0.3	1.4-2.1
Fetal lung volume (mL)	3	98.3 ± 16.1	80-110
Lung weight (g)	3	149.8 ± 28.2	118.1-172.2
LBWR %	3	8.0 ± 1.5	6.3-9.3
Fetal trachea			
Length	3	9.3 ± 0.5	9-10
∅ above balloon	3	2.4 ± 0.2	2.2-2.3
∅ on site of balloon	3	2.2 ± 0.2	2.0-2.5
∅ below balloon	3	2.2 ± 0.3	1.9-2.6
Legend: ∅, diameter			



1. Deprest JA, Evrard VA, Verbeken EK, Perales AJ, Delaere PR, Lerut TE, et al. Tracheal side effects of endoscopic balloon tracheal occlusion in the fetal lamb model. Eur J Obstet Gynecol Reprod Biol. 2000;92(1):119-26.

2. Evrard VA, Flageole H, Deprest JA, Vandenberghe K, Verhaeghe J, Lerut TE. Intrauterine tracheal obstruction, a new treatment for congenital diaphragmatic hernia, decreases amniotic fluid sodium and chloride concentrations in the fetal lamb. Ann Surg. 1997;226(6):753-8.

Growth Patterns of Fetal Lung Volumes in Healthy Fetuses and Fetuses With Isolated Left-Sided Congenital Diaphragmatic Hernia

Rodrigo Ruano, MD, PhD, Ingrid Schwach Werneck Britto, MD, PhD, Nicolas Sananes, MD, Wesley Lee, MD, Haleh Sangi-Haghpeykar, PhD, Russell L. Deter, MD

Objectives—To evaluate fetal lung growth using 3-dimensional sonography in healthy fetuses and those with congenital diaphragmatic hernia (CDH).

Methods—Right and total lung volumes were serially evaluated by 3-dimensional sonography in 66 healthy fetuses and 52 fetuses with left-sided CDH between 20 and 37 weeks' menstrual age. Functions fitted to these parameters were compared for 2 groups: (1) healthy versus those with CDH; and (2) fetuses with CHD who survived versus those who died.

Results—Fetal right and total lung volumes as well as fetal observed-to-expected right and total lung volume ratios were significantly lower in fetuses with CDH than healthy fetuses ($P < .001$) and in those fetuses with CDH who died ($P < .001$). The observed-to-expected right and total lung volume ratios did not vary with menstrual age in healthy fetuses or in those with CDH (independent of outcome).

Conclusions—Lung volume rates were lower in fetuses with left-sided CDH compared to healthy fetuses, as well as in fetuses with CDH who died compared to those who survived. The observed-to-expected right and total lung volume ratios were relatively constant throughout menstrual age in fetuses with left-sided CDH, suggesting that the origin of their lung growth abnormalities occurred before 20 weeks and did not progress. The observed-to-expected ratios may be useful in predicting the outcome in fetuses with CDH independent of menstrual age.

Key Words—congenital diaphragmatic hernia; fetal lung; fetal lung volume; obstetric ultrasound; prenatal diagnosis; pulmonary hypoplasia; 3-dimensional sonography

Received August 3, 2015, from the Department of Obstetrics and Gynecology, Baylor College of Medicine and Texas Children's Hospital Fetal Center, Houston, Texas USA (R.R., I.S.W.B., N.S., W.L., H.S.-H., and R.L.D.); Department of Obstetrics and Gynecology, Medical Science College of Santa Casa of São Paulo, São Paulo, Brazil (I.S.W.B.). Revision requested August 25, 2015. Revised manuscript accepted for publication August 31, 2015.

Address correspondence to Rodrigo Ruano, MD, PhD, Pavilion for Women, Texas Children's Hospital Fetal Center, 6651 Main St, Suite F1020, Houston, TX 77030 USA.

E-mail: ruano@bcm.edu, rodrigoruano@hotmail.com

Abbreviations
CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; 3D, 3-dimensional

doi:10.7863/ultra.15.08005

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 per 2550 live births and can be accurately diagnosed by second-trimester sonography.[1,2] Neonatal mortality and morbidity in cases with isolated CDH depend on the severity of pulmonary hypoplasia and pulmonary arterial hypertension. Fetal tracheal occlusion has been proposed to prevent these complications in severe forms of the disease, increasing neonatal survival.[3–9]

Since pulmonary hypoplasia is defined as a decrease in the size of the lungs, different methods for evaluating fetal lung size have been used to predict outcomes in fetuses with CDH.[10–20] The most frequently used measurement is the lung-to-head ratio, determined by 2-dimensional sonography, in which the contralateral lung area is divided by the head circumference.[8,21–23] However, lung-to-head ratio measurements vary with menstrual age in healthy fetuses and

those with CDH; therefore, investigators suggested the use of observed-to-expected lung-to-head ratio measurements to avoid the influence of menstrual age.[15] In contrast, our recent longitudinal study demonstrated that both lung-to-head ratio measurements and observed-to-expected lung-to-head ratio measurements varied differently with menstrual age when healthy fetuses were compared to those with CDH.[24]

The fetal lung size can also be measured by calculating the total fetal lung volumes using 3-dimensional (3D) sonography or magnetic resonance imaging and can also be expressed as an observed-to-expected ratio in order to avoid the influence of the menstrual age (observed-to-expected fetal lung volume). To date, a few studies evaluated 2 fetal lung volume measurements (second and third trimesters), but to our knowledge, there are no published studies that have evaluated lung volume growth patterns in fetuses with CDH in comparison to healthy fetuses with longitudinal consecutive measurements in the same fetus. Our study investigated the growth patterns of fetal lung volumes in isolated cases of left-sided CDH compared to healthy control fetuses with respect to severity and mortality, considering longitudinal measurements of fetal lung volumes.

Materials and Methods

Sample

Fetal lung volumes were measured in 66 randomly selected healthy fetuses and 52 consecutive fetuses with isolated left-sided CDH every 2 or 4 weeks, depending on maternal availability, between 20 and 37 weeks' menstrual age (January 2004 and December 2010). No fetal surgery was performed in those patients. Inclusion criteria were as follows: (1) singleton pregnancy; (2) live fetus with normal fetal morphologic findings or diagnosis of isolated left-sided CDH (normal fetal karyotype and absence of other structural anomalies); (3) no fetal surgery; (4) more than 3 lung sonographic examinations with intervals varying from 2 to 4 weeks; and (5) menstrual age confirmed by first-trimester measurement of the crown-rump length.[25] Only fetuses with left-sided CDH who had more than 3 sonographic examinations starting in the second trimester were included from 108 fetuses with prenatal diagnosis of CDH previously reported.[8] All healthy fetuses had normal weights at birth. The Institutional Review Board approved this study. Pregnant patients were informed that study results would not be used to modify their perinatal management. The perinatologists, sonographers, and surgeons in charge were not given information on lung evaluations.

Sonographic Evaluation

Each fetus was evaluated every 2 or 4 weeks (depending on patients' availability) by 3D sonography using a Voluson E8 system (GE Healthcare, Milwaukee, WI) with a 4–8-MHz transabdominal transducer. Right and total fetal lung volumes were measured by Virtual Organ Computer-Aided Analysis software (SonoView software; GE Healthcare) as described previously[14,26–29] and compared to the expected values for menstrual age (based on a nomogram described previously)[28,30] by calculating the observed-to-expected right lung volume ratio and observed-to-expected total lung volume ratio.

Perinatal Management of Fetuses With CDH

All fetuses with a prenatal diagnosis of isolated CDH were followed in our fetal medicine unit and were delivered in our children's hospital. All neonates were treated according to the same protocol that was described previously.[8] Briefly, all infants immediately underwent intubation and ventilator support in the delivery room and were transferred to the neonatal intensive care unit with high-frequency oscillatory ventilation when necessary, followed by delayed CDH repair after hemodynamic stabilization. The treatment protocol did not include extracorporeal membrane oxygenation (ECMO). Inhaled nitric oxide was administered in cases of persistent pulmonary arterial hypertension.

Statistical Analysis

The size parameters characterized in this study were the right lung volume, total lung volume, observed-to-expected right lung volume ratio, and observed-to-expected total lung volume ratio. The relationship between the right and total lung volume was determined by the Pearson correlation coefficient. All parameters were $\log_e$ transformed to normalize their distributions. Functions of menstrual age were obtained by using the best-fitting fractional polynomials. For each parameter, the model fit was assessed by the significance of the best-fitting fractional polynomial, residual diagnostic test, 2-log likelihood, Akaike information criterion, and Bayesian information criterion. The observed-to-expected total and right lung volume ratios were fitted by using a linear function, whereas for the total and right lung volume, linear and quadratic functions, respectively, gave optimal fits.

We used generalized linear mixed models (Proc Glimmix; SAS Institute Inc, Cary, NC), with random intercepts and slopes, to generate growth curve models in healthy fetuses and those with CDH. The sets of 3 (2 if linear) coefficients for the fitted curves served to represent the mean curve for

each group or subgroup.[31] Sets of coefficients for different curves were compared by the SAS Contrast procedure and the *F* test. Growth curves were compared in the following groups: (1) healthy fetuses versus fetuses with CDH; and (2) survivals versus deaths (mortality at 6 months of age) among neonates with CDH. Adjustments for multiple non-independent comparisons were made by the Bonferroni method (significance, $P < .0167$). Furthermore, we used the Estimate procedure in SAS to examine whether the patterns of the study parameters (right lung volume, total lung volume, observed-to-expected right lung volume ratio, and observed-to-expected total lung volume ratio) within each group (CDH or healthy fetuses) changed significantly over gestational age (if the slope was significantly different from 0). Intraoperator and interoperator variability was evaluated in 30 randomly selected 3D volumetric images by the intraclass coefficient test and Bland-Altman analysis. $P < .05$ was considered significant in independent comparisons. All analyses were performed with SAS version 9.3 software.

Results

Serial fetal lung measurements were obtained in 66 healthy fetuses (total of 284 ultrasound scans; median number of examinations per fetus, 4; range, 3–7; median menstrual age at first sonographic examination, 24 weeks; range, 21–24 weeks) and in 52 fetuses with isolated left-sided CDH (total of 242 ultrasound scans; median number of examination per fetus, 4; range, 3–7; median menstrual age at first sonographic examination, 24 weeks; range, 20–24 weeks).

The mean maternal age ± SD was 24.2 ± 6.5 years; 41 patients (34.8%) were nulliparous, whereas 77 had 2 or more previous children. Regarding fetal sex, 63 fetuses (53.4%) were male, whereas 55 (46.6%) were female.

In this study, 25 fetuses had severe forms of left-sided CDH; 18 had moderate forms; and 9 had mild forms. Twenty-nine neonates with CDH died, whereas 23 survived. As mentioned before, only fetuses with more than 2 measurements starting before 25 weeks were included in this analysis.

Longitudinal Growth of Lung Volumes as a Function of Menstrual Age in Healthy Fetuses and Those With CDH

Fetuses with CDH had significantly smaller fetal lung volumes (total lung volume, right lung volume, observed-to-expected right lung volume ratio, and observed-to-expected total lung volume ratio) than healthy fetuses ($P < .001$; Figure 1). Considering the growth pattern, both healthy fetuses and those with CDH had significant lung growth for right and total lung volumes as a function of menstrual age (Figure 1, A and B). The observed-to-expected right and total lung volume ratios did not vary with menstrual age in the healthy fetuses (observed-to-expected right lung volume ratio: slope estimation, –0.000055; $P = .99$; and observed-to-expected total lung volume ratio: slope estimation, 0.00015; $P = .80$) and in fetuses with CDH (observed-to-expected right lung volume ratio: slope estimation, –0.00015; $P = .82$; and observed-to-expected total lung volume ratio: slope estimation, –0.0008; $P = .89$; Figure 1, C and D). There was no statistical difference when comparing the slopes of observed-to-expected right and total lung volume ratios between the healthy and CDH groups (observed-to-expected right lung volume ratio: $F = 0.17$; $P = .68$; and observed-to-expected total lung volume ratio: $F = 0.03$; $P = .87$). However, the intercepts of observed-to-expected right and total lung volume ratios were significantly different between the healthy group (observed-to-expected right lung volume ratio: 0.9365; and observed-to-expected total lung volume ratio: 0.5585) and CDH group (observed-to-expected right lung volume ratio: 0.5323; observed-to-expected total lung volume ratio: 0.3260; observed-to-expected right lung volume ratio: $F = 238.03$; $P < .0001$; and observed-to-expected total lung volume ratio: $F = 230.74$; $P < .0001$).

Longitudinal Growth of Lung Measurements in Fetuses With CDH Based on Outcome

Figure 2 shows fetal right and total lung volume growth as a function of menstrual age in CDH cases with or without mortality. Fetuses who died had significantly smaller volumes on average than those who survived ($P < .001$). Concerning growth patterns, both right and total lung volumes increased significantly in fetuses who died (Figure 2, A and B). The observed-to-expected right and total lung volume ratios did not vary with menstrual age in fetuses with CDH who died (observed-to-expected right lung volume ratio: slope estimation, –0.0039; $P = .26$; and observed-to-expected total lung volume ratio: slope estimation, 0.0004; $P = .62$) and survived (observed-to-expected right lung volume ratio: slope estimation, 0.00034; $P = .52$; and observed-to-expected total lung volume ratio: slope estimation, –0.0011; $P = .36$; Figure 2, C and D). There was no statistical difference in slope comparison between survivors and nonsurvivors (observed-to-expected right lung volume ratio: $F = 1.60$; $P = .21$; and observed-to-expected total lung volume ratio: $F = 1.17$; $P = .28$). However, the intercepts of observed-to-expected right and total lung volume ratios were significantly differ-

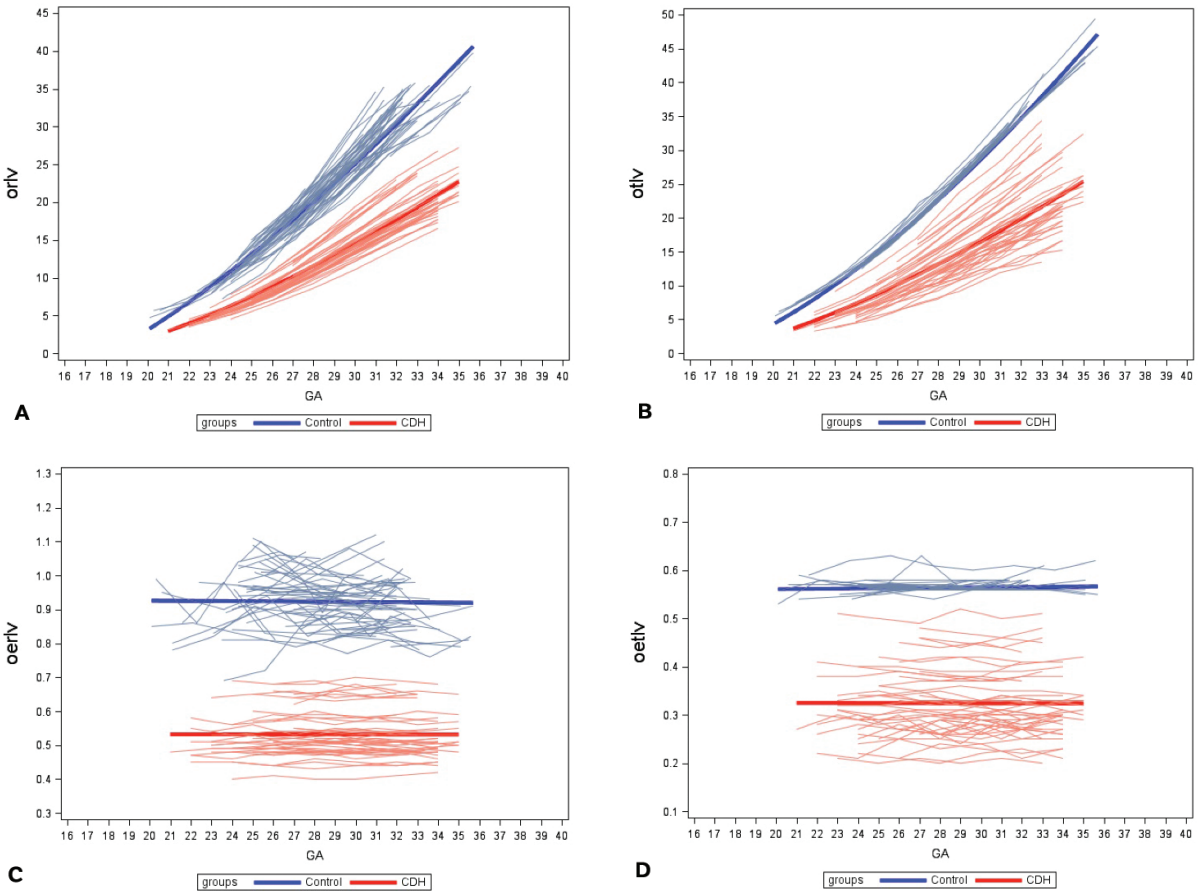
ent between survivors (observed-to-expected right lung volume ratio: 0.5860; and observed-to-expected total lung volume ratio: 0.4029) and nonsurvivors (observed-to-expected right lung volume ratio: 0.5004; observed-to-expected total lung volume ratio: 0.2812; observed-to-expected right lung volume ratio: $F = 22.97$; $P < .0001$; and observed-to-expected total lung volume ratio: $F = 38.90$; $P < .0001$).

A good correlation was observed between right and total lung volumes (Pearson correlation coefficient, =0.972; $P < .001$). Good intraoperator reproducibility was observed for right lung volumes (intraclass correlation coefficient, 0.99 [95% confidence interval, 0.98–0.99]; and bias, 0.10 [limits of agreement, –0.87 to +1.07]) and for total lung volumes (intraclass correlation coefficient, 0.99 [95% confidence interval, 0.98–0.99]; and bias, –0.08 [limits of agreement, –1.82 to +1.65]). Good interoperator reproducibility was observed for right lung volumes (intraclass correlation coefficient, 0.96 [95% confidence interval, 0.95–0.99]; and bias, –0.02 [limits of agreement, –1.94 to +1.90]) and for total lung volumes (intraclass correlation coefficient, 0.95 [95% confidence interval, 0.94–0.98]; and bias, –0.21 [limits of agreement, –1.80 to +1.39]).

Discussion

This longitudinal study provides unique information about growth patterns of fetal lung volumes measured by 3D sonography in healthy fetuses and those with isolated left-sided CDH who did not undergo fetal intervention.

Figure 1. **A**, Growth curves for the observed right lung volume (orlv) in healthy fetuses and those with CDH. **B**, Growth curves for the observed total lung volume (otlv) in healthy fetuses and those with CDH. **C**, Growth curves for the observed-to-expected right lung volume ratio (oerlv) in healthy fetuses and those with CDH. **D**, Growth curves for the observed-to-expected total lung volume ratio (oetlv) in healthy fetuses and those with CDH. GA indicates gestational age.



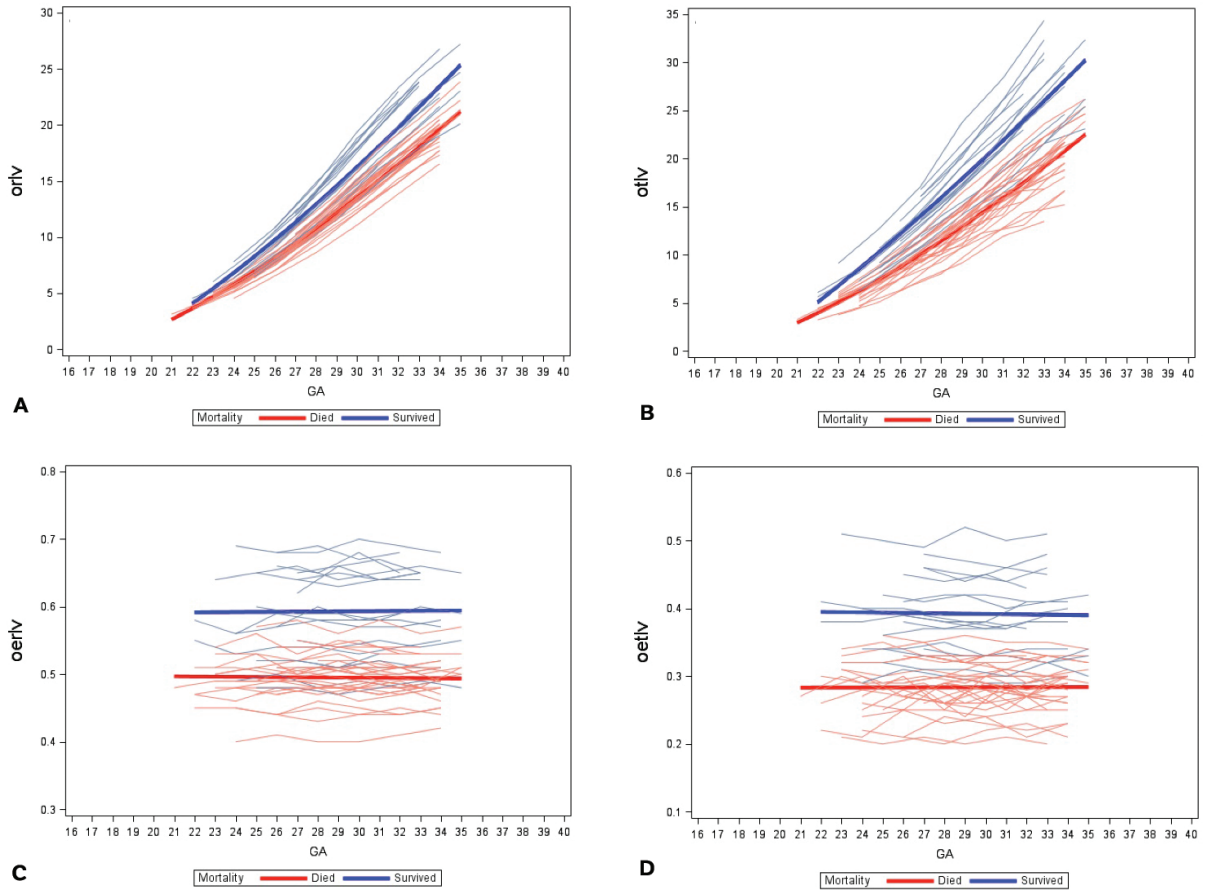
This study demonstrates that fetal right and total lung volumes grow throughout menstrual age in healthy fetuses and those with CDH.

Our study of the observed-to-expected, which is a measure of the difference in average growth rates before the time of measurement in observed and reference populations (expressed as a decimal fraction of the reference growth rate; Appendix), indicated no change with menstrual age during the 20- to 35-week interval for either right or total lung volume. The observed average growth rate of healthy fetuses is much closer to that in the reference sample for right lung volume than it is for total lung volume, even in the same fetuses.

Our findings that the observed-to-expected ratios did not vary as a function of menstrual age confirm the hypothesis that the observed-to-expected right and total lung volume ratios can be used clinically to evaluate fetal lung size and growth independent of fetal age. This study also confirms previous observations reported in the literature that fetuses with CDH have consistently lower values for observed-to-expected right and total lung volume ratios compared to their healthy counterparts, and fetuses with more severe forms of CDH resulting in death had even more significantly reduced observed-to-expected right and total lung volume ratio values. This study did not aim to evaluate cutoffs for the observed-to-expected right and total lung volume ratios to predict survival, since they have been reported previously.[13,26]

In addition to the clinical implications, our results also provide further information regarding the physiopatho-

Figure 2. **A**, Growth curves for the observed right lung volume (orlv) in fetuses with left-sided CDH according to outcome. **B**, Growth curves the observed total lung volume (otlv) in fetuses with left-sided CDH according to outcome. **C**, Growth curves for the observed-to-expected right lung volume ratio (oerlv) in fetuses with left-sided CDH according to outcome. **D**, Growth curves for the observed-to-expected total lung volume ratio (oetlv) in fetuses with left-sided CDH according to outcome. GA indicates gestational age..



logic characteristics of the disease. The absence of changes in the observed-to-expected right and total lung volume ratios over the 20- to 35-week menstrual age interval suggests that the severity of pulmonary hypoplasia does not progress and that disease severity may be determined very early in pregnancy. A similar observation was reported recently; in 2015, Coleman et al[32] reported a study in which lung volumes were measured in the same fetus twice (initially at 20–30 weeks and then after 30 weeks), and they found that the observed-to-expected total lung volume ratio did not change in these 2 measurements. They clearly stated: "observed-to-expected total lung volume demonstrated no specific change pattern."[32] However, in another recent study, Hagelstein et al[33] showed that the observed-to-expected total lung volume ratio measured on magnetic resonance imaging decreased by an average of 9% in 61% of fetuses, increased by an average of 7% in 26% of the cases, and stayed stable in 13% of cases but without any correlation with survival. These results, different from those reported by Coleman et al[32] and this study, seem to be due to 2 main raisons: (1) different nomograms were used to generate the expected values; and (2) most of the last measurements in fetuses with CDH in the study by Hagelstein et al[33] were after 35 weeks, whereas the nomogram by Rypens et al[34] that was used to generate the observed-to-expected total lung volume ratio had only a few cases after 35 weeks. In addition, both studies only evaluated 2 points on the fetal lung growth trajectory, whereas our study evaluated the fetal lung growth patterns at 4 time points (median, 4; range, 3–7).

To our knowledge, no previously published studies have characterized lung volume growth patterns with serial measurements or have compared observed-to-expected fetal lung volume ratios in healthy fetuses and those with CDH (who did not undergo fetal intervention) using longitudinal measurements. This study also evaluated these ratios in fetuses with CDH who had different outcomes (survival and mortality) for the first time. Further longitudinal investigations beginning in the first trimester (or even before 20 weeks' gestation) will be necessary to evaluate when this process starts and to determine whether earlier intervention is possible.

In our study, we observed a strong correlation between right and total lung volumes, which was expected, since the right lung volume is part of the total lung volume. The reproducibility of fetal lung volume measurements by 3D sonography has also been extensively reported.[14,23,26,28,30,35,36] Although it was not the primary objective of this study, we observed good reproducibility of fetal lung volumes using our method. Measuring the ipsilateral lung volumes is especially challenging with this technique, which requires operator expertise and training.[17,23,35] Fetal magnetic resonance imaging may have less operator variability and may be more reproducible than 3D sonography, despite the fact that previous studies reported by expert operators demonstrated a good level of agreement between these methods.[14,37] Magnetic resonance imaging has more clinical applications in tertiary centers where fetuses with CDH are treated.[14,38–40]

This study provides novel information on the growth patterns of fetal lung volumes measured by 3D sonography. Our results provide novel insight into the variation and growth of the observed-to-expected right and total lung volume ratios in both healthy fetuses and those with CDH. However, this study did not evaluate 2 aspects: (1) fetuses with right-sided CDH were excluded because we believe that our hypothesis should be tested separately in fetuses with right-sided CDH; and (2) the liver position (herniation) was not considered, since our goal was to investigate changes in lung volume in fetuses with isolated left-sided CDH in comparison to healthy fetuses independent of herniated organs. We will investigate the variation in the amount of liver herniation into the fetal chest throughout gestation as well as its impact on fetal lung volume growth. However, further longitudinal studies are necessary to establish whether the amount of liver herniation varies with menstrual age.

One criticism of this study may be the fact that our neonates were not treated with the ECMO after birth. The benefits of ECMO in neonates with CDH still need scientific confirmation. However, this study did not aim to evaluate whether fetal lung growth could predict outcomes but, rather, aimed to evaluate fetal lung growth patterns in healthy fetuses and those with CDH. This study provided evidence that those ratios (observed-to-expected right lung volume ratio and observed-to-expected total lung volume ratio) did not change throughout pregnancy, independent of outcome. Therefore, the results of this study would not be influenced by whether ECMO was used.

In conclusion, the observed-to-expected right lung volume ratio and observed-to-expected total lung volume ratio do not show significant variations between 20 and 35 weeks, so they can be used to evaluate the severity of left-sided CDH independent of fetal age.

Appendix: Ratio of Observed and Expected Measurements as a Measure of the Ratio of Observed and Expected Growth Rates

At some menstrual age after the last menstrual period date (menstrual age = 0), the lung can be identified microscopically. At this age, the lung volume can be considered

equal to 0 (it will not be 0 but very close to it). This menstrual age (MA_0) is called the 0 point or start point.

An estimate of an individual menstrual age (MA_{0i}) can be obtained if one assumes approximate linear growth in the first and second trimesters. A linear function can be fit to the second trimester cube root values of the right lung volume or total lung volume measurements and the resulting line extrapolated back to where it crosses the menstrual age axis. In the reference group, the MA_{0i} values can be determined for each fetus and averaged to give an MA_{0R} value.

At any subsequent time point, say, 30 weeks (MA_{30}) in a given individual, we have a measured observed right lung volume value ($o\text{-}RLV_{30i}$) and an expected right lung volume value ($e\text{-}RLV_{30}$), the latter being the expected value at 30 weeks derived from a reference sample. The $o\text{-}RLV_{30i}$ is the result of a growth process occurring between MA_{0i} and MA_{30}. The $e\text{-}RLV_{30}$ can be considered to be the value obtained from a fetus growing along the 50th percentile line of the reference sample from MA_{0R} to MA_{30}.

The average right lung volume growth rates ($av\ RLV\ gr$) between MA_{0i} or MA_{0R} and MA_{30} are:

$$av\ o\text{-}RLV\ gr_i = (o\text{-}RLV_{30i} - 0)/(MA_{30} - MA_{0i}) = o\text{-}RLV_{30i}/(MA_{30} - MA_{0i});$$

$$av\ e\text{-}RLV\ gr = (e\text{-}RLV_{30} - 0)/(MA_{30} - MA_{0R}) = e\text{-}RLV_{30}/(MA_{30} - MA_{0R}).$$

The ratio of average growth rate is:

$$av\ o\text{-}RLV\ gr_i/av\ e\text{-}RLV\ gr = [o\text{-}RLV_{30i}/(MA_{30} - MA_{0i})]/[e\text{-}RLV_{30}/(MA_{30} - MA_{0R})].$$

If $MA_{0i} = MA_{0R}$:

$$av\ o\text{-}RLV\ gr_i/av\ e\text{-}RLV\ gr = o\text{-}RLV_{30i}/e\text{-}RLV_{30}.$$

Differences between MA_{0i} and MA_{0R} might be expected to have a greater effect early in pregnancy when the $MA\text{-}MA_0$ interval is shorter. However, individual plots of the observed-to-expected values for right lung volume and total lung volume (Figures 1C, 1D, 2C, and 2D) do not show clear evidence of such an effect.

References

1. Langham MR Jr, Kays DW, Ledbetter DJ, Frentzen B, Sanford LL, Richards DS. Congenital diaphragmatic hernia. epidemiology and outcome. *Clin Perinatol* 1996; 23:671–688.
2. Cannie MM, Jani JC, De Keyzer F, Allegaert K, Dymarkowski S, Deprest J. Evidence and patterns in lung response after fetal tracheal occlusion: clinical controlled study. *Radiology* 2009; 252:526–533.
3. Cannie M, Jani J, Meersschaert J, et al. Prenatal prediction of survival in isolated diaphragmatic hernia using observed to expected total fetal lung volume determined by magnetic resonance imaging based on either gestational age or fetal body volume. *Ultrasound Obstet Gynecol* 2008; 32:633–639.
4. Gucciardo L, Deprest J, Doné E, et al. Prediction of outcome in isolated congenital diaphragmatic hernia and its consequences for fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008; 22:123–138.
5. Hsieh YY, Chang FC, Tsai HD, Hsu TY, Yang TC. Accuracy of sonography in predicting the outcome of fetal congenital diaphragmatic hernia. *Zhonghua Yi Xue Za Zhi (Taipei)* 2000; 63:751–757.
6. Ishikawa S, Kamata S, Usui N, Sawai T, Nose K, Okada A. Ultrasonographic prediction of clinical pulmonary hypoplasia: measurement of the chest/trunk-length ratio in fetuses. *Pediatr Surg Int* 2003; 19:172–175.
7. Jani JC, Benachi A, Nicolaides KH, et al. Prenatal prediction of neonatal morbidity in survivors with congenital diaphragmatic hernia: a multicenter study. *Ultrasound Obstet Gynecol* 2009; 33:64–69.
8. Ruano R, Takashi E, da Silva MM, Campos JA, Tannuri U, Zugaib M. Prediction and probability of neonatal outcome in isolated congenital diaphragmatic hernia using multiple ultrasound parameters. *Ultrasound Obstet Gynecol* 2012; 39:42–49.
9. Ruano R, Yoshisaki CT, da Silva MM, et al. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2012; 39:20–27.
10. Askenazi SS, Perlman M. Pulmonary hypoplasia: lung weight and radial alveolar count as criteria of diagnosis. *Arch Dis Child* 1979; 54:614–618.
11. Wigglesworth JS, Desai R. Use of DNA estimation for growth assessment in normal and hypoplastic fetal lungs. *Arch Dis Child* 1981; 56:601–605.
12. Wigglesworth JS, Desai R, Guerrini P. Fetal lung hypoplasia: biochemical and structural variations and their possible significance. *Arch Dis Child* 1981; 56:606–615.
13. Mahieu-Caputo D, Sonigo P, Dommergues M, et al. Fetal lung volume measurement by magnetic resonance imaging in congenital diaphragmatic hernia. *BJOG* 2001; 108:863–868.
14. Ruano R, Joubin L, Sonigo P, et al. Fetal lung volume estimated by 3-dimensional ultrasonography and magnetic resonance imaging in cases with isolated congenital diaphragmatic hernia. *J Ultrasound Med* 2004; 23:353–358.
15. Jani J, Nicolaides KH, Keller RL, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007; 30:67–71.
16. Jani J, Peralta CF, Benachi A, Deprest J, Nicolaides KH. Assessment of lung area in fetuses with congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007; 30:72–76.
17. Jani JC, Cannie M, Peralta CF, Deprest JA, Nicolaides KH, Dymarkowski S. Lung volumes in fetuses with congenital diaphragmatic hernia: comparison of 3D US and MR imaging assessments. *Radiology* 2007; 244:575–582.

18. Jani JC, Cordier AG, Martinovic J, et al. Antenatal ultrasound prediction of pulmonary hypoplasia in congenital diaphragmatic hernia: correlation with pathology. *Ultrasound Obstet Gynecol* 2011; 38:344–349.
19. Peralta CF, Jani JC, Van Schoubroeck D, Nicolaides KH, Deprest JA. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. *Am J Obstet Gynecol* 2008; 198:60.e1–60.e5.
20. Britto IS, Tedesco GD, Herbst SR, et al. New anatomical landmarks to study the relationship between fetal lung area and thoracic circumference by three-dimensional ultrasonography. *J Matern Fetal Neonatal Med* 2012; 25:1927–1932.
21. Metkus AP, Filly RA, Stringer MD, Harrison MR, Adzick NS. Sonographic predictors of survival in fetal diaphragmatic hernia. *J Pediatr Surg* 1996; 31:148–152.
22. Lipshutz GS, Albanese CT, Feldstein VA, et al. Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. *J Pediatr Surg* 1997; 32:1634–1636.
23. Jani JC, Peralta CF, Ruano R, et al. Comparison of fetal lung area to head circumference ratio with lung volume in the prediction of postnatal outcome in diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007; 30:850–854.
24. Ruano R, Britto IS, Sangi-Haghpeykar H, et al. Longitudinal assessment of lung area measurements by two-dimensional ultrasound in fetuses with isolated left-sided congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2015; 45:566–571.
25. Hadlock FP, Shah YP, Kanon DJ, Lindsey JV. Fetal crown-rump length: reevaluation of relation to menstrual age (5–18 weeks) with high-resolution real-time US. *Radiology* 1992; 182:501–505.
26. Ruano R, Benachi A, Joubin L, et al. Three-dimensional ultrasonographic assessment of fetal lung volume as prognostic factor in isolated congenital diaphragmatic hernia. *BJOG* 2004; 111:423–429.
27. Ruano R, Benachi A, Martinovic J, et al. Can three-dimensional ultrasound be used for the assessment of the fetal lung volume in cases of congenital diaphragmatic hernia? *Fetal Diagn Ther* 2004; 19:87–91.
28. Ruano R, Martinovic J, Dommergues M, Aubry MC, Dumez Y, Benachi A. Accuracy of fetal lung volume assessed by three-dimensional sonography. *Ultrasound Obstet Gynecol* 2005; 26:725–730.
29. Ruano R, Martinovic J, Aubry MC, Dumez Y, Benachi A. Predicting pulmonary hypoplasia using the sonographic fetal lung volume to body weight ratio: how precise and accurate is it? *Ultrasound Obstet Gynecol* 2006; 28:958–962.
30. Ruano R, Joubin L, Aubry MC, et al. A nomogram of fetal lung volumes estimated by 3-dimensional ultrasonography using the rotational technique (virtual organ computer-aided analysis). *J Ultrasound Med* 2006; 25:701–709.
31. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982; 38:963–974.
32. Coleman A, Phithakwatchara N, Shaaban A, et al. Fetal lung growth represented by longitudinal changes in MRI-derived fetal lung volume parameters predicts survival in isolated left-sided congenital diaphragmatic hernia. *Prenat Diagn* 2015; 35:160–166.
33. Hagelstein C, Weidner M, Kilian AK, et al. Repetitive MR measurements of lung volume in fetuses with congenital diaphragmatic hernia: individual development of pulmonary hypoplasia during pregnancy and calculation of weekly lung growth rates. *Eur Radiol* 2014; 24:312–319.
34. Rypens F, Metens T, Rocourt N, et al. Fetal lung volume: estimation at MR imaging—initial results. *Radiology* 2001; 219:236–241.
35. Ruano R, Aubry MC, Barthe B, Dumez Y, Zugaib M, Benachi A. Ipsilateral lung volumes assessed by three-dimensional ultrasonography in fetuses with isolated congenital diaphragmatic hernia. *Fetal Diagn Ther* 2008; 24:389–394.
36. Ruano R, Aubry MC, Barthe B, Dumez Y, Zugaib M, Benachi A. Three-dimensional sonographic measurement of contralateral lung volume in fetuses with isolated congenital diaphragmatic hernia. *J Clin Ultrasound* 2008; 36:273–278.
37. Gerards FA, Twisk JW, Bakker M, Barkhof F, van Vugt JM. Fetal lung volume: three-dimensional ultrasonography compared with magnetic resonance imaging. *Ultrasound Obstet Gynecol* 2007; 29:533–536.
38. Matsuoka S, Takeuchi K, Yamanaka Y, Kaji Y, Sugimura K, Maruo T. Comparison of magnetic resonance imaging and ultrasonography in the prenatal diagnosis of congenital thoracic abnormalities. *Fetal Diagn Ther* 2003; 18:447–453.
39. Jani J, Cannie M, Doné E, et al. Relationship between lung area at ultrasound examination and lung volume assessment with magnetic resonance imaging in isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007; 30:855–860.
40. Kehl S, Kalk AL, Eckert S, et al. Assessment of lung volume by 3-dimensional sonography and magnetic resonance imaging in fetuses with congenital diaphragmatic hernias. *J Ultrasound Med* 2011; 30:1539–1545.

Standardization of Sonographic Lung-to-Head Ratio Measurements in Isolated Congenital Diaphragmatic Hernia

Impact on the Reproducibility and Efficacy to Predict Outcomes

Ingrid Schwach Werneck Britto, MD, PhD, Nicolas Sananes, MD, Oluyinka O. Olutoye, MD, PhD, Darrell L. Cass, MD, Haleh Sangi-Haghpeykar, PhD, Timothy C. Lee, MD, Christopher I. Cassady, MD, Amy Mehollin-Ray, MD, Stephen Welty, MD, Caraciolo Fernandes, MD, Michael A. Belfort, MD, PhD, Wesley Lee, MD, Rodrigo Ruano, MD, PhD

Received November 25, 2014, from the Texas Children's Fetal Center, Houston, Texas USA (I.S.W.B., N.S., O.O.O., D.L.C., T.C.L., C.I.C., A.M.-R., S.W., C.F., M.A.B., W.L., R.R.); and Department of Obstetrics and Gynecology (I.S.W.B., N.S., H.S.-H., M.A.B., W.L., R.R.), Michael E. DeBakey Department of Surgery (O.O.O., D.L.C., T.C.L.), Department of Radiology (C.I.C., A.M.-R.), and Department of Pediatrics, Section of Neonatology (S.W., C.F.), Baylor College of Medicine, Houston, Texas USA. Revision requested December 10, 2014. Revised manuscript accepted for publication December 16, 2014.

Dr Britto is a recipient of a postdoctoral scholarship grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo (process number 2013/12493-1), and Dr Sananes is a recipient of a scholarship grant from the Fulbright Franco-American Commission for Education Exchange (process number 68141003).

Address correspondence to Rodrigo Ruano, MD, PhD, Pavilion for Women, Texas Children's Fetal Center, 6651 Main St, Suite F1020, Houston, TX 77030 USA.

E-mail: ruano@bcm.edu, rodrigoruano@hotmail.com

Abbreviations

AUC, area under the curve; CDH, congenital diaphragmatic hernia; CI, confidence interval; ECMO, extracorporeal membrane oxygenation; MRI, magnetic resonance imaging; 3D, 3-dimensional; 2D, 2-dimensional

doi:10.7863/ultra.15.14.11064

Objectives—The purpose of this study was to evaluate the impact of standardization of the lung-to-head ratio measurements in isolated congenital diaphragmatic hernia on prediction of neonatal outcomes and reproducibility.

Methods—We conducted a retrospective cohort study of 77 cases of isolated congenital diaphragmatic hernia managed in a single center between 2004 and 2012. We compared lung-to-head ratio measurements that were performed prospectively in our institution without standardization to standardized measurements performed according to a defined protocol.

Results—The standardized lung-to-head ratio measurements were statistically more accurate than the nonstandardized measurements for predicting neonatal mortality (area under the receiver operating characteristic curve, 0.85 versus 0.732; $P = .003$). After standardization, there were no statistical differences in accuracy between measurements regardless of whether we considered observed-to-expected values ($P > .05$). Standardization of the lung-to-head ratio did not improve prediction of the need for extracorporeal membrane oxygenation ($P > .05$). Both intraoperator and interoperator reproducibility were good for the standardized lung-to-head ratio (intraclass correlation coefficient, 0.98 [95% confidence interval, 0.97–0.99]; bias, 0.02 [limits of agreement, –0.11 to +0.15], respectively).

Conclusions—Standardization of lung-to-head ratio measurements improves prediction of neonatal outcomes. Further studies are needed to confirm these results and to assess the utility of standardization of other prognostic parameters.

Key Words—congenital diaphragmatic hernia; fetal lung; lung-to-head ratio; obstetric ultrasound; pulmonary hypoplasia; standardization

Congenital diaphragmatic hernia (CDH) occurs in approximately 1 per 2500 live births and is associated with high morbidity and mortality depending on the severity of pulmonary hypoplasia and pulmonary arterial hypertension.[1–5] Accurate prenatal assessment in fetuses with isolated CDH is of interest because it can guide appropriate counseling and prenatal care (fetal endoscopic tracheal occlusion, expectant management, or termination of pregnancy).[6–9] The most widely used prognostic factor is

the sonographic measurement of the lung-to-head ratio, which was first described for left-sided CDH before 25 weeks' gestation.[10] Its efficacy in predicting outcomes varies substantially in different studies.[10–13] Recently, Jani et al[14] suggested that conflicting results might occur from using different methods of measuring the lung-to-head ratio. These investigators proposed the need for standardization of lung-to-head ratio measurements based on adequate imaging, operator training, and correction for gestational age. However, so far, there is a paucity of data that have examined the impact of lung-to-head ratio standardization on clinical outcomes.

Our hypothesis was that standardization of lung-to-head ratio measurements would improve the accuracy of predicting outcomes of fetuses with isolated CDH and improve intraoperator and interoperator reproducibility. The primary and secondary objectives of this study were to evaluate the impact of standardization of lung-to-head ratio measurements on prediction of neonatal outcomes and reproducibility of these standardized measurements, respectively.

Materials and Methods

Study Design

We conducted a retrospective cohort study of all patients who underwent at least 1 prenatal sonographic scan for fetal isolated CDH and who delivered at Texas Children's Hospital and were at least 6 months of life between January 2004 and July 2012. In this cohort of patients, there was no infant 6 months or older who underwent fetal endoscopic tracheal occlusion. Two operators (I.S.W.B. and R.R.), who were blinded to the neonatal outcome and each operator's measurements, reviewed all video clips from comprehensive fetal sonography and measured the contralateral lung areas and the lung-to-head ratios. The study was approved (H-29006) by the Institutional Review Board of Baylor College of Medicine.

Sonographic Measurements

We compared the lung-to-head ratio measurements that were performed prospectively at our institution (nonstandardized lung-to-head ratio) during clinical evaluation of the patients to the lung-to-head ratio measurements that we performed by reviewing the video clips and after adjusting the images according to the proposal of Jani et al[14] (standardized lung-to-head ratio measurements) for the same fetus at the same gestational age. Only 1 sonographic examination per patient (the first examination) was considered for analysis in this study.

The nonstandardized lung-to-head ratio measurements were performed by maternal-fetal medicine specialists, radiologists, and sonographers. The lung area was estimated by multiplication of the longest diameter of the lung by its longest perpendicular diameter. Then, the area of the lung was divided by the head circumference (in millimeters).[10] The observed-to-expected lung-to-head ratio values were calculated by using nomograms reported by Peralta et al.[15]

The standardized lung-to-head ratio measurements were performed according to the following protocol described by Jani et al[14]: (1) lung measurements should be obtained in an axial view at the level of the 4-chamber view of the fetal heart, with the lung contralateral to the diaphragmatic defect close to the probe, avoiding shadows produced by the ribs; (2) the image should be frozen before final magnification to ensure that all landmarks are clearly visible, and the image should then be magnified so that the axial view of the fetal thorax occupies the whole screen; and (3) the calipers should be placed according to the method being used to measure the lung area. For each fetus, the lung area was estimated by 3 different techniques and divided by the head circumference to calculate the lung-to-head ratio: by multiplying the 2 longest perpendicular diameters, by multiplying the anteroposterior diameter by the perpendicular diameter located at the midpoint, and by manually tracing the area around the lung. These 3 methods are illustrated in Figure 1. All images were obtained from video clips of the axial view of the fetal chest with the contralateral lung close to the ultrasound probe. The images were then frozen and magnified before measurements. The observed-to-expected lung-to-head ratio was calculated by using nomograms reported by Jani et al[14] and Britto et al.[16]

Perinatal Management

In this study, there was no case of termination of pregnancy based on state law. Therefore, the lung-to-head ratio was not used to guide prenatal management in these cases. All neonates were treated according to a standard perinatal protocol.[17] All neonates underwent immediate endotracheal intubation with orogastric decompression and avoidance of bag-mask ventilation by a staff neonatologist. Gentle mechanical ventilation using a permissive hypercapnia protocol was used. Escalation of ventilatory support to a maximal peak inspiratory pressure and positive end-expiratory pressure of 30 and 6 cm H_2O, respectively, and use of high-frequency oscillatory ventilation to a maximum mean airway pressure of 15 cm H_2O were based on preestablished criteria. The need for extracorporeal membrane

oxygenation (ECMO) was determined by the presence of persistent hypoxia (preductal oxygen saturation <80%), persistent acidosis (pH <7.2), or inadequate tissue perfusion. The timing of surgical repair was managed according to the infant's physiologic stability.

Statistical Analysis

Results were reported as mean ± standard deviation unless otherwise specified. Each method for obtaining the lung-to-head ratio measurement was assessed as a potential predictor of 6-month neonatal mortality and need for ECMO (primary outcome). The performances of the different predictors were evaluated by receiver operating characteristic curve analysis, with estimation of areas under the curves (AUCs) with 95% confidence interval (CI) and sensitivity and specificity for the best cutoffs. Statistical comparisons between the AUCs of the different predictors were performed according to the methods of DeLong et al[18] and Hanley et al.[19,20] The intraoperator reproducibility and interoperator reproducibility were analyzed by the intraclass correlation test and Bland-Altman analysis, respectively. $P < .05$ was considered statistically significant. The SPSS version 19.0 statistical software package (IBM Corporation, Armonk, NY) was used for all data analyses, except for comparisons of AUCs, for which MedCalc version 11.6 software (MedCalc, Mariakerke, Belgium) was used.

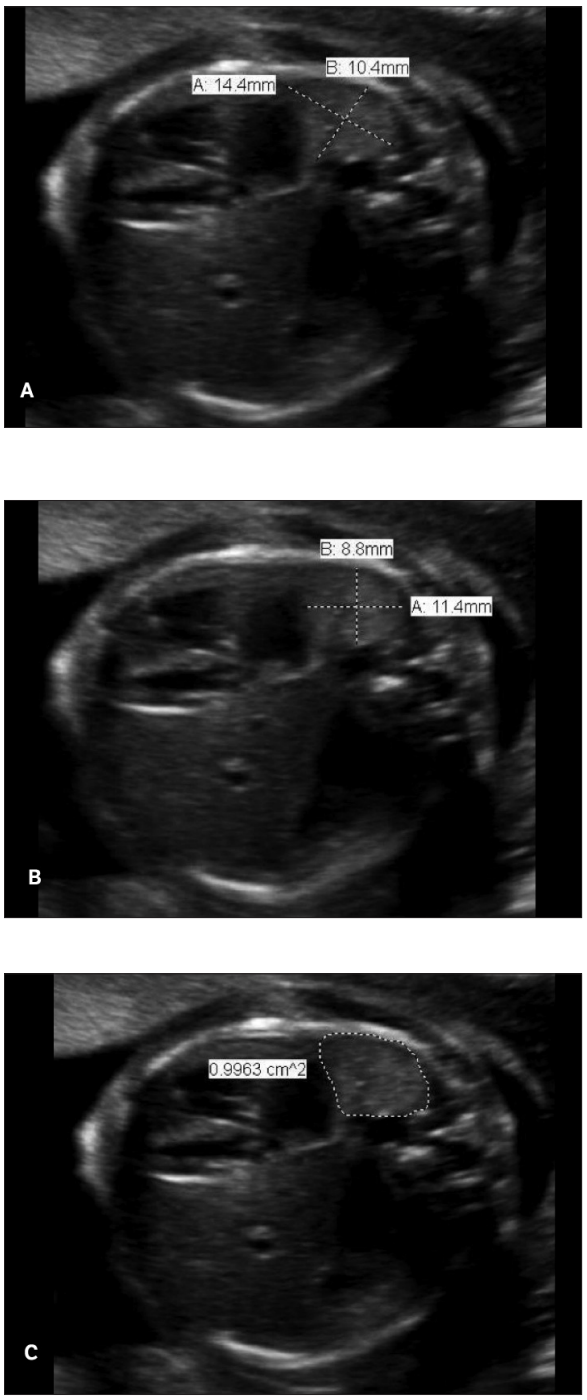
Results

A total of 80 patients were identified, but 3 cases were excluded because of inability to assess the images and video clips adequately. Therefore, 77 fetuses with isolated CDH were included in the study. Patients' demographics and characteristics were previously reported[21]: the mean maternal age ± SD was 27.5 ± 5.8 years; and mean gestational ages at diagnosis and delivery were 21.9 ± 5.8 and 38.2 ± 1.9 weeks, respectively. The 6-month mortality rate was 20.7% (16 of 77). Extracorporeal membrane oxygenation was used in 35.5% of neonates (27 of 76); 1 fetus died in utero.

Table 1 demonstrates that all lung-to-head ratio measurements were statistically associated with mortality and the need for ECMO. Figures 2 and 3 show receiver operating characteristic curves for standardized and nonstandardized measurements to predict mortality and the need for ECMO, respectively.

Receiver operating characteristic analyses are shown in Table 2. In considering prediction of mortality (Figure 2), the standardized longest and observed-to-expected longest lung-to-head ratio measurements were statistically more

Figure 1. **A**, Two-dimensional sonogram of the longest right lung area in a fetus with right CDH. **B**, Two-dimensional sonogram of the antero-posterior right lung area in a fetus with right CDH. **C**, Two-dimensional sonogram of the tracing right lung area in a fetus with right CDH.



accurate than the nonstandardized measurements (P = .003; P = .024). After standardization, there was no statistical difference among the measurements regardless of whether the lung-to-head ratio was adjusted for gestational age by using the observed-to-expected lung-to-head ratio (standardized ratio versus standardized observed-to-expected ratio according to Jani et al[14] and Britto et al[16]; P = .711) or the method of measuring the contralateral lung area (standardized longest diameter versus standardized anteroposterior diameter versus standardized tracing area; P > .05).

Regarding prediction of the need for ECMO, standardization of the lung-to-head ratio measurements improved the accuracy from an AUC of 0.67 to an AUC of 0.74. However, those differences were not statistically significant (P > .05; Table 2 and Figure 3).

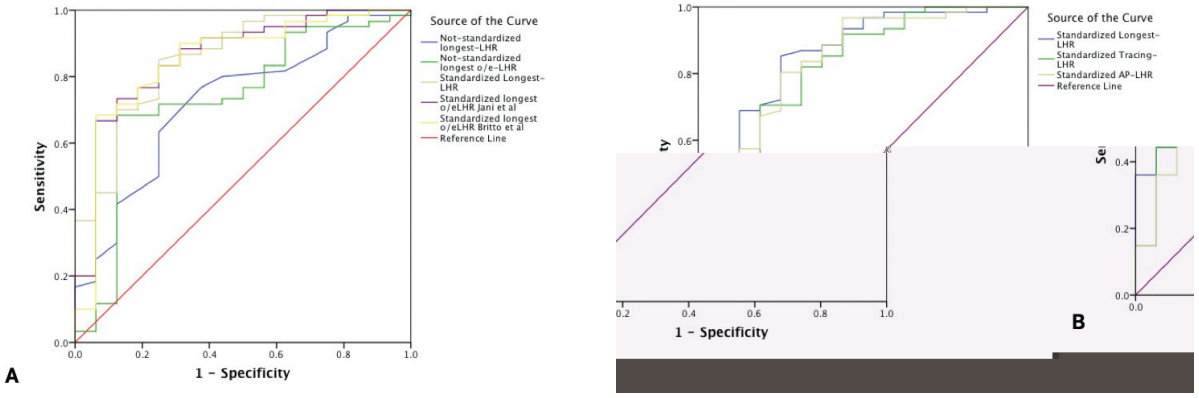
Good intraoperator reproducibility was observed for the standardized lung-to-head ratio measurements (anteroposterior diameter: intraclass correlation coefficient, 0.97 [95% CI, 0.95–0.98]; longest diameter: intraclass correlation coefficient, 0.98 [95% CI, 0.97–0.99]; and tracing area: intraclass correlation coefficient, 0.99 [95% CI,

Table 1. Sample Characteristics and Results Related to Mortality and Need for ECMO in 77 Fetuses With Isolated CDH

	Mortality			Need for ECMO		
Characteristic	Alive (n = 60)	Death (n = 16)	*P*	No (n = 49)	Yes (n = 27)	*P*
Maternal age, y	27.3 ± 5.6	28.1 ± 6.8	.62	27.2 ± 6.7	27.2 ± 5.7	.97
GA at diagnosis, wk	22.4 ± 5.4	22.5 ± 5.9	.75	22.4 ± 6.1	23.0 ± 6.4	.79
Side of CDH, n						
Left	53	15		44	23	
Right	8	1	.68	5	4	.48
Nonstandardized longest LHR	1.82 ± 0.68	1.31 ± 0.53	<.01	1.85 ± 0.69	1.49 ± 0.60	.24
Standardized longest LHR	1.91 ± 0.70	1.15 ± 0.40	<.01	1.92 ± 0.70	1.45 ± 0.65	<.01
Standardized anteroposterior LHR	1.40 ± 0.49	0.90 ± 0.36	<.01	1.41 ± 0.49	1.11 ± 0.49	.01
Standardized tracing area LHR	1.34 ± 0.53	0.86 ± 0.37	<.01	1.35 ± 0.53	10.8 ± 0.48	.03
Nonstandardized observed-to-expected LHR (Peralta et al[15])	0.51 ± 0.19	0.38 ± 0.19	<.01	0.51 ± 0.19	0.44 ± 0.19	.12
Standardized observed-to-expected LHR (Jani et al[14])	0.51 ± 0.15	0.32 ± 0.12	<.01	0.52 ± 0.15	0.40 ± 0.15	<.01
Standardized observed-to-expected LHR (Britto et al[16])	0.45 ± 0.13	0.28 ± 0.11	<.01	0.47 ± 0.14	0.35 ± 0.13	<.01
GA at birth, wk	37.0 ± 1.9	38.1 ± 1.2	.32	37.4 ± 1.5	37.9 ± 2.8	.64
Newborn weight, g	3208.0 ± 740.9	3154.2 ± 493.5	.75	3154.1 ± 713.3	3067.1 ± 497.8	.75
Age at repair, d	3 (2–8)	8 (2–18)	.17	3 (1–8)	10 (2–25)	.01

Data are presented as mean ± SD and median (range) where applicable. GA indicates gestational age, and LHR, lung-to-head ratio.

Figure 2. **A**, Receiver operating characteristic curves for prediction of 6-month neonatal mortality for standardized and nonstandardized lung-to-head ratio (LHR) measurement methods. **B**, Receiver operating characteristic curves for prediction of 6-month neonatal mortality for the 3 standardized lung-to-head ratio measurement methods. Diagonal segments are produced by ties; AP indicates anteroposterior; and o/e, observed-to-expected.



0.98–0.99]). Good interoperator reproducibility was also observed for the standardized measurements (anteroposterior diameter: bias, –0.01 [absolute limits of agreement, –0.16 to +0.15]; longest diameter: bias, 0.02 [absolute limits of agreement, –0.11 to +0.15]; and tracing area: bias, –0.01 [absolute limits of agreement, –0.12 to +0.10]).

Discussion

Our study demonstrates that standardization of lung-to-head ratio measurements as proposed by Jani et al[14] significantly improves prediction of mortality at 6 months of life, independently from the method of measuring the contralateral lung area (anteroposterior diameter versus longest diameter versus tracing area). The standardized observed-to-expected lung-to-head ratio measurements were slightly more accurate in predicting the need for ECMO than the standardized lung-to-head ratio measurements (without adjusting the expected value for specific gestational age), albeit the difference did not reach statistical significance. To our knowledge, a study evaluating the utility of a standardization protocol for measuring the lung-to-head ratio has not been reported previously.

Prediction of the prognosis in CDH assists in counseling parents, helps in the selection of cases that may benefit from fetal surgery, and determines the choice of a specialized medical center for delivery and neonatal care.[22] Neonatal mortality is associated with severe pulmonary hypoplasia; therefore, it is important to measure fetal lung size by 2-dimensional sonography, 3-dimensional sonography, or magnetic resonance imaging (MRI).

Three-dimensional sonographic and MRI measurements of total fetal lung volumes might predict outcomes more accurately than the lung-to-head ratio and other 2D sonographic ratios.[23–25] Both 3D sonography and MRI have their own advantages: 3D sonography is less expensive and allows longitudinal monitoring, whereas MRI may be less operator dependent and may allow a better evaluation of liver-related parameters. However, 2D sonography is still of interest, as it is more frequently available.

The most widely used 2D sonographic prognostic factor is the lung-to-head ratio, but its efficacy in predicting outcome varies in different studies.[10–13] Therefore, stan-

Figure 3. Receiver operating characteristic curves for prediction of the need for ECMO for standardized and nonstandardized lung-to-head ratio (LHR) measurement methods. Diagonal segments are produced by ties; o/e indicates observed-to-expected.

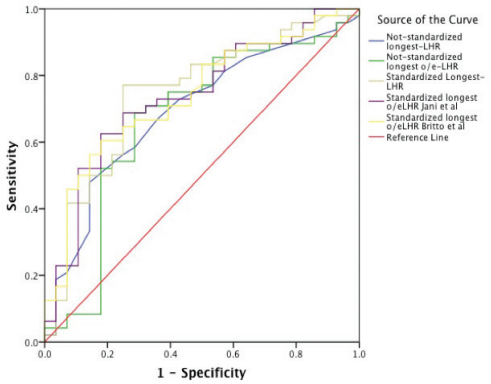


Table 2. Sensitivity, Specificity, and AUC for Each Lung-to-Head Ratio Measurement Method

Measurement Method	Sensitivity/Specificity, %	AUC (95% CI)
6-mo mortality		
Nonstandardized longest LHR	62.5/76.7	0.72 (0.59–0.86)
Nonstandardized observed-to-expected longest LHR (Peralta et al[15])	87.5/67.2	0.73 (0.59–0.88)
Standardized Longest LHR	75.0/85.2	0.85 (0.75–0.96)
Standardized observed-to-expected longest LHR (Jani et al[14])	87.5/72.1	0.86 (0.75–0.97)
Standardized observed-to-expected longest LHR (Britto et al[16])	93.7/68.9	0.85 (0.73–0.96)
Standardized anteroposterior LHR	75.0/80.3	0.82 (0.69–0.95)
Standardized tracing area LHR	81.2/70.5	0.81 (0.68–0.94)
Need for ECMO		
Nonstandardized longest LHR	85.2/67.9	0.69 (0.57–0.81)
Nonstandardized observed-to-expected longest LHR (Peralta et al[15])	70.4/67.3	0.67 (0.54–0.81)
Standardized longest LHR	74.1/75.5	0.75 (0.63–0.86)
Standardized observed-to-expected longest LHR (Jani et al[14])	81.5/61.2	0.74 (0.62–0.85)
Standardized observed-to-expected longest LHR (Britto et al[16])	81.5/59.2	0.74 (0.63–0.85)
Standardized anteroposterior LHR	77.8/61.2	0.72 (0.60–0.84)
Standardized tracing area LHR	70.4/73.5	0.71 (0.59–0.83)

LHR indicates lung-to-head ratio.

dardization of the lung-to-head ratio measurement technique is of interest.[14] Our study demonstrates that standardization significantly improves prediction of mortality. Interestingly, we found that standardization mattered even more than the choice of the lung-to-head ratio measurement technique itself. Indeed, there was no difference in the mortality predictions made by each method (anteroposterior diameter versus longest diameter versus tracing area).

Some studies in the literature have suggested that the lung-to-head ratio varies throughout gestational age; therefore, the use of an observed-to-expected ratio has been proposed.[15,26] However, our study demonstrated that there was no statistical difference in predicting outcomes among the standardized measurements regardless of whether we considered the observed-to-expected measurements. In addition, longitudinal studies demonstrated that even the observed-to-expected lung-to-head ratio seems to vary throughout gestational age, suggesting that there is no need to adjust the measurements for gestational age.[27,28] Additionally, it seems that evaluating the fetal total lung volumes (observed-to-expected total fetal lung volume ratio) has better accuracy for predicting outcomes (mortality and need for ECMO) than 2D sonographic measurements.[23,29]

In this study, standardization of the lung-to-head ratio did not improve prediction of the need for ECMO. Evidently, measurement of lung size does not accurately predict the use of ECMO. This idea might be explained by the fact that, regardless of the method by which lung size is evaluated, we are not considering future lung function or pulmonary arterial hypertension. Evaluation of vasculature and pulmonary arterial hypertension with 2D and 3D Doppler studies may be a better option for predicting pulmonary arterial hypertension, as some authors have proposed.[30–32]

Among the different methods for measuring the lung area using 2D sonography, tracing has been considered the most reproducible.[33] However, in our experience, all 3 methods for measuring the lung-to-head ratio (longest diameter versus anteroposterior diameter versus tracing area) had similar predictive values and reproducibility after standardization in our cohort of fetuses, suggesting that they can be used with similar results. In our opinion, we think that the tracing area seems to be easier to perform, since it can provide the area value directly, and it may be more reproducible in a larger population.

The strength of this study was that we tested the hypothesis that standardized lung-to-head ratio measurements might improve the reproducibility and accuracy of predicting outcome in fetuses with isolated CDH. However, this study's limitations include the retrospective design. Despite the retrospective nature of the study, we were able to obtain adequate images from video clips, since acquisition of a video clip of the fetal chest in an axial view is part of the standard sonographic examination in our center. To avoid bias, the operator measured the lung sizes on images from video clips without knowing the outcomes of any of the fetuses.

In conclusion, we have shown that standardization of lung-to-head ratio measurements improves prediction of neonatal outcomes. Further prospective studies are necessary to confirm these results in other patient cohorts and to assess the utility of standardization of other prognostic parameters.

References

1. Cannie MM, Jani JC, De Keyzer F, Allegaert K, Dymarkowski S, Deprest J. Evidence and patterns in lung response after fetal tracheal occlusion: clinical controlled study. *Radiology* 2009; 252:526–533.
2. Gucciardo L, Deprest J, Done E, et al. Prediction of outcome in isolated congenital diaphragmatic hernia and its consequences for fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2008; 22:123–138.
3. Langham MR Jr, Kays DW, Ledbetter DJ, Frentzen B, Sanford LL, Richards DS. Congenital diaphragmatic hernia: epidemiology and outcome. *Clin Perinatol* 1996; 23:671–688.
4. Jani JC, Benachi A, Nicolaides KH, et al. Prenatal prediction of neonatal morbidity in survivors with congenital diaphragmatic hernia: a multicenter study. *Ultrasound Obstet Gynecol* 2009; 33:64–69.
5. Ruano R, Aubry MC, Barthe B, Mitanchez D, Dumez Y, Benachi A. Predicting perinatal outcome in isolated congenital diaphragmatic hernia using fetal pulmonary artery diameters. *J Pediatr Surg* 2008; 43:606–611.
6. Jani JC, Nicolaides KH, Gratacos E, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. *Ultrasound Obstet Gynecol* 2009; 34:304–310.
7. Peralta CF, Jani JC, Van Schoubroeck D, Nicolaides KH, Deprest JA. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. *Am J Obstet Gynecol* 2008; 198:60.e1–60.e5.
8. Ruano R, da Silva MM, Campos JA, et al. Fetal pulmonary response after fetoscopic tracheal occlusion for severe isolated congenital diaphragmatic hernia. *Obstet Gynecol* 2012; 119:93–101.
9. Ruano R, Duarte SA, Pimenta EJ, et al. Comparison between fetal endoscopic tracheal occlusion using a 1.0-mm fetoscope and prenatal expectant management in severe congenital diaphragmatic hernia. *Fetal Diagn Ther* 2011; 29:64–70.
10. Metkus AP, Filly RA, Stringer MD, Harrison MR, Adzick NS. Sonographic predictors of survival in fetal diaphragmatic hernia. *J Pediatr Surg* 1996; 31:148–152.
11. Arkovitz MS, Russo M, Devine P, Budhorick N, Stolar CJ. Fetal lung-head ratio is not related to outcome for antenatal diagnosed congenital diaphragmatic hernia. *J Pediatr Surg* 2007; 42:107-111.

12. Laudy JA, Van Gucht M, Van Dooren MF, Wladimiroff JW, Tibboel D. Congenital diaphragmatic hernia: an evaluation of the prognostic value of the lung-to-head ratio and other prenatal parameters. *Prenat Diagn* 2003; 23:634–639.
13. Lipshutz GS, Albanese CT, Feldstein VA, et al. Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. *J Pediatr Surg* 1997; 32:1634–1636.
14. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. *Ultrasound Obstet Gynecol* 2012; 39:2–6.
15. Peralta CF, Cavoretto P, Csapo B, Vandecruys H, Nicolaides KH. Assessment of lung area in normal fetuses at 12–32 weeks. *Ultrasound Obstet Gynecol* 2005; 26:718–724.
16. Britto IS, Araujo Júnior E, Sangi-Haghpeykar H, et al. Reference ranges for 2-dimensional sonographic lung measurements in healthy fetuses: a longitudinal study. *J Ultrasound Med* 2014; 33:1917–1923.
17. Lazar DA, Cass DL, Rodriguez MA, et al. Impact of prenatal evaluation and protocol-based perinatal management on congenital diaphragmatic hernia outcomes. *J Pediatr Surg* 2011; 46:808–813.
18. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44:837–845.
19. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983; 148:839–843.
20. McNeil BJ, Hanley JA, Funkenstein HH, Wallman J. Paired receiver operating characteristic curves and the effect of history on radiographic interpretation: CT of the head as a case study. *Radiology* 1983; 149:75–77.
21. Ruano R, Lazar DA, Cass DL, et al. Fetal lung volume and quantification of liver herniation by magnetic resonance imaging in isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2014; 43:662–669.
22. Ruano R, Yoshisaki CT, da Silva MM, et al. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2012; 39:20–27.
23. Ruano R, Takashi E, da Silva MM, Haeri S, Tannuri U, Zugaib M. Quantitative lung index, contralateral lung area, or lung-to-head ratio to predict the neonatal outcome in isolated congenital diaphragmatic hernia? *J Ultrasound Med* 2013; 32:413–417.
24. Victoria T, Bebbington MW, Danzer E, et al. Use of magnetic resonance imaging in prenatal prognosis of the fetus with isolated left congenital diaphragmatic hernia. *Prenat Diagn* 2012; 32:715–723.
25. Kehl S, Kalk AL, Eckert S, et al. Assessment of lung volume by 3-dimensional sonography and magnetic resonance imaging in fetuses with congenital diaphragmatic hernias. *J Ultrasound Med* 2011; 30:1539–1545.
26. Jani J, Nicolaides KH, Keller RL, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2007; 30:67–71.
27. Ruano R, Takashi E, da Silva MM, Campos JA, Tannuri U, Zugaib M. Prediction and probability of neonatal outcome in isolated congenital diaphragmatic hernia using multiple ultrasound parameters. *Ultrasound Obstet Gynecol* 2012; 39:42–49.
28. Ruano R, Britto IS, Sangi-Haghpeykar H, et al. Longitudinal assessment of lung area measurements by two-dimensional ultrasound in fetuses with isolated left-sided congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2015; 45:566–571.
29. Ruano R, Joubin L, Sonigo P, et al. Fetal lung volume estimated by 3-dimensional ultrasonography and magnetic resonance imaging in cases with isolated congenital diaphragmatic hernia. *J Ultrasound Med* 2004; 23:353–358.
30. Ruano R, Aubry MC, Barthe B, Mitanchez D, Dumez Y, Benachi A. Quantitative analysis of fetal pulmonary vasculature by 3-dimensional power Doppler ultrasonography in isolated congenital diaphragmatic hernia. *Am J Obstet Gynecol* 2006; 195:1720–1728.
31. Cruz-Martinez R, Hernandez-Andrade E, Moreno-Alvarez O, Done E, Deprest J, Gratacos E. Prognostic value of pulmonary Doppler to predict response to tracheal occlusion in fetuses with congenital diaphragmatic hernia. *Fetal Diagn Ther* 2011; 29:18–24.
32. Mahieu-Caputo D, Aubry MC, El Sayed M, Joubin L, Thalabard JC, Dommergues M. Evaluation of fetal pulmonary vasculature by power Doppler imaging in congenital diaphragmatic hernia. *J Ultrasound Med* 2004; 23:1011–1017.
33. Jani JC, Cordier AG, Martinovic J, et al. Antenatal ultrasound prediction of pulmonary hypoplasia in congenital diaphragmatic hernia: correlation with pathology. *Ultrasound Obstet Gynecol* 2011; 38:344–349.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE

http://informahealthcare.com/jmf
ISSN: 1476-7058 (print), 1476-4954 (electronic)

J Matern Fetal Neonatal Med, Early Online: 1–5
 DOI: 10.3109/14767058.2015.1114080

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Prematurity and fetal lung response after tracheal occlusion in fetuses with severe congenital diaphragmatic hernia

Nicolas Sananes[1,2], Carlota Rodo[3], Jose Luis Peiro[3], Ingrid Schwach Werneck Britto[1], Haleh Sangi-Haghpeykar[1], Romain Favre[2], Arnaud Joal[2], Adrien Gaudineau[2], Marcos Marques da Silva[4], Uenis Tannuri[4], Marcelo Zugaib[5], Elena Carreras[2], and Rodrigo Ruano[1,5]

[1]Department of Obstetrics and Gynecology, Texas Children's Fetal Center, Baylor College of Medicine, Houston, TX, USA, [2]Service De Gynécologie Obstétrique, CMCO – HUS, Hôpitaux Universitaires De Strasbourg, Strasbourg, France, [3]Fetal Surgery Program, Hospital Universitari Vall D'hebron, Barcelona, Spain, [4]Department of Pediatric Surgery and [5]Department of Obstetrics and Gynecology, Faculdade De Medicina, Universidade De Sao Paulo, Sao Paulo, Brazil

Abstract

Objective: To evaluate the independent association of fetal pulmonary response and prematurity to postnatal outcomes after fetal tracheal occlusion for congenital diaphragmatic hernia.
Methods: Fetal pulmonary response, prematurity (<37 weeks at delivery) and extreme prematurity (<32 weeks at delivery) were evaluated and compared between survivors and non-survivors at 6 months of life. Multivariable analysis was conducted with generalized linear mixed models for variables significantly associated with survival in univariate analysis.
Results: Eighty-four infants were included, of whom 40 survived (47.6%) and 44 died (52.4%). Univariate analysis demonstrated that survival was associated with greater lung response (p=0.006), and the absence of extreme preterm delivery (p=0.044). In multivariable analysis, greater pulmonary response after FETO was an independent predictor of survival (aOR 1.87, 95% CI 1.08–3.33, p=0.023), whereas the presence of extreme prematurity was not statistically associated with mortality after controlling for fetal pulmonary response (aOR 0.52, 95% CI 0.12–2.30, p=0.367).
Conclusion: Fetal pulmonary response after FETO is the most important factor associated with survival, independently from the gestational age at delivery.

Keywords

Congenital diaphragmatic hernia, fetal lung, fetal surgery, fetoscopy, lung-to-head ratio, prematurity

History

Received 4 April 2015
Revised 20 October 2015
Accepted 25 October 2015
Published online 1 December 2015

Introduction

Congenital diaphragmatic hernia (CDH) occurs in ~1 in 2500 live births and is associated with high rates of neonatal mortality, especially in cases with severe pulmonary hypoplasia and abnormal pulmonary vasculature [1–4]. Fetal endoscopic tracheal occlusion (FETO) improves survival due to lung growth and decrease of pulmonary arterial hypertension [5–8].

Fetal lung size and position of the liver relative to the diaphragm are both predictors of neonatal outcome in CDH cases being considered for prenatal assessment for FETO [9,10]. Predicting neonatal survival after FETO is of interest as well for prenatal counseling of the parents and for planning adequate perinatal care. Several studies have suggested that FETO promotes fetal pulmonary growth that seems to be associated with increased survival [7,11,12]. On the other hand, FETO is associated with preterm premature rupture of membranes and prematurity [5,6,8–10]. Prematurity has been associated with poor prognosis in infants with CDH without FETO [13,14]. Recent studies have suggested that prematurity is also associated with increased mortality in fetuses with CDH undergoing FETO [9,15,16]. However, no study has so far evaluated the influence of the fetal pulmonary response and prematurity on postnatal outcomes after FETO.

Our hypothesis is that the survival of fetuses with CDH that undergo FETO is more influenced by the fetal pulmonary response to the procedure than the gestational age at delivery. Therefore, the objective of this study was to evaluate the impact of these two factors simultaneously and independently in a multicenter cohort of fetuses that underwent FETO.

Materials and methods

Study design

This was a retrospective cohort study of all patients who underwent FETO for CDH, between 2002 and 2014, and who delivered in three regional centers (Sao Paulo, Brazil; Barcelona, Spain and Strasbourg, France). Eighty-four fetuses

Address for correspondence: Rodrigo Ruano, Pavilion for Women – Texas Children's Fetal Center, 6651 Main Street, Suite F1020, Houston, TX 77030, USA. E-mail: ruano@bcm.edu; rodrigoruano@hotmail.com

with isolated and severe congenital diaphragmatic hernia that underwent fetal intervention were evaluated {48 fetuses from Sao Paulo, Brazil (43 fetuses were reported in previous studies [6–8]), 30 patients from Barcelona, Spain (20 were reported before [17,18]) and 6 from Strasbourg, France (no cases reported before)}. Inclusion criteria were: absence of chromosomal or major structural anomalies, lung-to-head ratio <1.0 (or observed-to-expected lung-to-head ratio <0.25) and at least one-third of liver herniation into fetal chest based on prenatal imaging [5–9]. The local institutional review boards approved the study and all patients had informed consent.

Fetal endoscopic tracheal occlusion

FETO was performed similarly in all institutions using a previously described technique between 22 and 30 weeks [3,5,6,8]. Briefly, fetal intervention was performed under maternal regional or local anesthesia and fetal systemic anesthesia, using a 1.0-mm (11510A; Karl Storz, Tuttlinglen, Germany), 1.2-mm (11530AA; Karl Storz) or 1.3-mm (11540AA; Karl Storz) fetoscope. Direct visualization of the fetal trachea was achieved in all cases prior to occlusion by a detachable balloon filled with saline (GOLDBAL 2 or 4; Balt, Montmorency, France) [4,6,8,15,16]. Fetal intervention was performed by R. Ruano in Brazil, J.L. Peiro and E. Carreras in Spain and J. Deprest in Belgium (cases from France).

Fetal lung assessment

Fetal lung parameters were evaluated immediately before fetal surgery and every 2 weeks following FETO, using a Voluson 730 or E8 ultrasound machine (GE Medical Systems, Zipf, Austria). Lung-to-head ratio and observed-to-expected lung-to-head ratio were measured for the contralateral lung for all fetuses in the present series, at the level of the four-chamber heart view, using two-dimensional ultrasonography [19,20]. The observed-to-expected lung-to-head ratio was calculated by measuring the ratio between the lung area and the head circumference compared with the expected value for gestational age [21].

Perinatal management

The balloon was removed prenatally or by *ex utero* intrapartum therapy delivery (EXIT procedure). Perinatal management was standardized in all centers. All neonates underwent immediate endotracheal intubation in the delivery room and admitted to the neonatal intensive care unit for ventilator support and high-frequency oscillatory ventilation when necessary. Inhaled nitric oxide was administered in cases of persistent pulmonary arterial hypertension. Extracorporeal membrane oxygenation was available if necessary based on clinical criteria (presence of persistent hypoxia, persistent acidosis and/or inadequate tissue perfusion). The time of surgery repair was managed according to the infant physiologic stability [22,23].

Statistical analysis

The primary study outcome of interest was survival of the neonate at 6 months of life. The following variables were analyzed: primary centers where the patients were followed, gestational age at fetal intervention, duration of the procedure, prophylactic tocolysis during the procedure, side of the defect, lung size immediate before the procedure [initial (observed-to-expected) lung-to-head ratio] and before delivery [latest (observed-to-expected) lung-to-head ratio, that was calculated in the same week of the delivery], lung growth after the procedure (percentage of LHR growth = difference of the latest and initial values divided by initial value and the difference of observed-to-expected lung-to-head ratio = difference between the latest and initial values), gestational at delivery, duration of tracheal occlusion (days between fetal ''plugging'' and ''unplugging'' fetal trachea), interval in days between the removal of the balloon and the delivery and the use of extracorporeal membrane oxygenation. Results were reported as number and percentage for categorical variables and mean ± standard deviation for continuous variables. Fisher's exact test and χ^2 test were used for univariate comparisons. Estimation of odds ratios (OR) and their 95% confidence interval were also computed to predict survival.

Multivariable analysis was then conducted with generalized linear mixed models for variables significantly associated with survival in univariate analysis. The different methods to assess lung size weren't included in the same multivariable model because of their colinearity and therefore several models were built. Correlation between measurements of lung size and gestational age at delivery was assessed using Pearson's coefficient, before multivariable analysis. Adjustment for within-center clustering was performed by including the center as random effect in the GLIMMIX procedure in SAS. The GLIMMIX procedure fits generalized linear mixed models and estimates the parameters by maximum likelihood. A p value <0.05 was considered as statistically significant.

Results

A total of 84 cases of CDH treated by FETO were included, among which 40 survived beyond 6 months of life (47.6%) and 44 died (52.4%). Survival rates were respectively 50.0% (24/48), 40.0% (12/30) and 66.7% (4/6) in Sao Paulo, Barcelona and Strasbourg, without statistically significant difference (p=0.432). The mean duration of the surgery was 19.5 ± 9.5 min. The mean duration of tracheal occlusion in all sites was 52.9 ± 21.1 days.

A total of 52 (61.9%) patients delivered before 37 weeks, 23 (27.4%) patients delivered before 34 weeks and 16 (19.1%) delivered before 32 weeks. Survival rates were 48.1, 30.4 and 25.0%, respectively.

Univariate comparisons between survivors and non-survivors are reported in Table 1. Survivors had statistically greater lung response, based on both percentage of increase of lung-to-head ratio after FETO (157 versus 94%, p=0.006) and difference between observed-to-expected lung-to-head ratio before and after FETO (0.20 versus 0.14, p=0.007). Survival was significantly lower in infants born before 32 weeks than after (p=0.044). None of the other parameters analyzed were significantly associated with survival: side of the defect, lung size before FETO, gestational age at FETO, duration of FETO procedure, type of prophylactic tocolytic agent used, duration

Table 1. Univariate comparison between survivors and non-survivors.

Parameters	Survivors (n=40)	Non-survivors (n=44)	OR (95% CI)	p value
Center				
Sao Paulo	24 (50.0%)	24 (50.0%)	1 (reference)	0.432
Barcelona	12 (40.0%)	18 (60.0%)	0.7 (0.3–1.7)	
Strasbourg	4 (66.7%)	2 (33.3%)	2.0 (0.3–11)	
Side				
Left	31 (50.0%)	31 (50.0%)	1 (reference)	0.737
Right	8 (40.0%)	12 (60.0%)	0.7 (0.2–1.9)	
Bilateral	1 (50.0%)	1 (50.0%)	1 (0.1–17)	
Initial lung-to-head ratio, mean ± SD	0.82 ± 0.15	0.81 ± 0.23	1.1 (0.1–10)	0.903
Initial observed-to-expected lung-to-head ratio, mean ± SD	0.12 ± 0.02	0.12 ± 0.04	1.0 (0.2–4.3)	0.962
Gestational age at FETO (weeks), mean ± SD	26.7 ± 1.8	27.1 ± 1.7	0.9 (0.7–1.1)	0.299
Duration of procedure (minutes), mean ± SD	19.9 ± 7.8	19.1 ± 11.3	1.0 (0.9–1.1)	0.761
Prophylactic tocolysis, N (%)	37 (46.3%)	43 (53.7%)	0.3 (0.0–2.9)	0.261
Latest lung-to-head ratio after FETO, mean ± SD	2.0 ± 0.58	1.6 ± 1.12	1.7 (1.0–2.9)	0.059
Latest observed-to-expected lung-to-head ratio after FETO, mean ± SD	0.32 ± 0.08	0.26 ± 0.14	1.7 (1.1–2.6)	0.018
Percentage of increase of lung-to-head ratio between before and after FETO, mean ± SD	157 ± 104	94 ± 91	1.1 (1.0–1.2)	0.006
Difference between observed-to-expected lung-to-head ratio before and after FETO, mean ± SD	0.20 ± 0.08	0.14 ± 0.12	1.9 (1.2–3.2)	0.007
Prenatal removal of the balloon	14 (41.2%)	20 (58.8%)	0.6 (0.3–1.6)	0.330
Gestational age at delivery (weeks), mean ± SD	35.2 ± 2.4	34.5 ± 3.3	1.1 (0.9–1.3)	0.268
Duration of tracheal occlusion (days), mean ± SD	52.9 ± 21.1	46.2 ± 22.1	1.0 (0.9–1.1)	0.165
Interval between removal of the balloon and delivery (days), mean ± SD	20.6 ± 19.0	18.4 ± 16.3	1.0 (0.9–1.1)	0.737
Interval between FETO and delivery (in days), mean ± SD	60.3 ± 18.7	54.1 ± 23.2	1.0 (0.9–1.1)	0.195
Delivery before 37 weeks, N (%)	25 (48.1%)	27 (51.9%)	1.0 (0.4–2.5)	0.915
Delivery before 34 weeks, N (%)	7 (30.4%)	16 (69.6%)	0.4 (0.1–1.0)	0.053
Delivery before 32 weeks, N (%)	4 (25.0%)	12 (75.0%)	0.3 (0.1–1.0)	0.044
Use of extracorporeal membrane oxygenation	0 (0%)	2 (100.0%)	0.0 (0.0–99)	0.185

SD, standard deviation. OR associated with observed-to-expected lung-to-head ratio and increase of lung-to-head ratio was calculated for each 10% increase.

Table 2. Multivariable analysis of odds for survival.

Model	Parameters	aOR	95% CI	p value
Model 1	Delivery before 32 weeks	0.50	0.12–2.12	0.339
	Percentage of increased lung-to-head ratio after FETO	1.08	1.01–1.15	0.029
Model 2	Delivery before 32 weeks	0.52	0.12–2.30	0.380
	Difference between observed-to-expected lung-to-head ratio before and after FETO	1.87	1.08–3.33	0.023

In each model, correlation within each center is adjusted by including it as a random effect. aOR associated with observed-to-expected lung-to-head ratio and increase of lung-to-head ratio was calculated for each 10% increase.

of tracheal occlusion, prenatal versus EXIT removal of the balloon, time between removal of the balloon and delivery, time between FETO and delivery, the use of extracorporeal membrane oxygenation.

The gestational age at delivery did not significantly correlate with the fetal pulmonary response assessed by percentage of increase of lung-to-head ratio between before and after FETO (r=0.127, p=0.235) and difference between observed-to-expected lung-to-head ratio before and after FETO (r=0.11, p=0.17).

Table 2 provides the results obtained from multivariable analysis including the centers as a random variable in each model. Two models were built, each including extreme prematurity (delivery before 32 weeks) and one of the two fetal pulmonary response variables. In the first multivariable model (Model 1), the percentage of increased lung-to-head ratio after FETO was independently associated with survival (aOR 1.08, 95% CI 1.01 < 1.15, p=0.029) whereas the present of extreme prematurity was not significantly associated with survival (aOR 0.50, 95% CI 0.12–2.12, p=0.339). In a second multivariable model (Model 2), difference of the observed-to-expected lung-to-head ratio from FETO to delivery was significantly associated with survival (aOR 1.87, 95% CI 1.08–3.33, p=0.023) while extreme prematurity was not significantly associated with outcome (aOR 0.52, 95% CI 0.12–2.30, p=0.380).

Discussion

Our data suggest that fetal pulmonary response after FETO is the most important factor associated with survival in fetuses with severe isolated CDH undergoing fetal intervention in our series. Prematurity (especially before 32 weeks gestation) also impacts survival; however, in our cohort after performing a multivariable analysis, this factor became secondary to the fetal pulmonary response.

Different predictive factors of survival in fetuses with CDH undergoing FETO have been previously reported, including lung size before the procedure (the lung-to-head ratio, the observed-to-expected lung-to-head ratio and the observed-to-

expected total lung volume before FETO), gestational age at fetal intervention, fetal pulmonary response after the intervention, duration of the tracheal occlusion, time between balloon removal and delivery, gestational age at delivery and prematurity [7,9,12,15,16,24,25]. However, so far, there are very limited data evaluating the association between adequate pulmonary response after FETO and neonatal survival, especially among those infants that born at extreme premature gestational ages have been limited.

In our study, only fetal lung response and prematurity were associated with outcome. Fetal lung response was found to be associated with survival independently of prematurity. Our findings related to the impact of gestational age at delivery following FETO differ slightly from prior studies. Ali et al. [15] recently suggested that birth before 35 weeks was strongly associated with mortality in patients who underwent FETO for CDH. However, there were no survivors that were born prior to 33 weeks, whereas in our series 4 out of 16 (25%) fetuses that were born before 32 weeks survived. The largest series reported in the literature by Jani et al. [24] demonstrated that gestational age at delivery was a predictor of survival with an odds ratio of 1.024 (95% CI 1.007–1.042, p=0.007). In addition, Done et al. [16] found that the difference of mean gestational age at delivery between survivors and non-survivors was 10 days. In our study, prematurity (birth before 37 weeks) did not impact survival, however extreme prematurity (birth before 32 weeks) was statistically associated with mortality in univariate analysis. Nevertheless, the multivariable analysis revealed that even extreme prematurity became a less important predictor of mortality when considering the adequacy of the fetal pulmonary response. Prior studies have suggested that fetuses who deliver prematurely are less likely to survive because their lungs do not have enough time to grow sufficiently [16]. In our series, we did not observe a significant correlation between lung growth and gestational age at delivery. This result can be explained by the fact that the fetal pulmonary response is achieved significantly within 2 weeks after the procedure with a maximal growth 4 weeks after FETO performed between 26–30 weeks [7]. All of our cases but four were born at least 2 weeks after FETO.

The present study had some limitations, including the retrospective analysis of databases from different centers. In addition, patients were managed in different centers. However, the indication for FETO and the perinatal management were similar in all three centers. In addition, we considered the ''centers'' as a random variable in our analysis and thus took into consideration the similarity (correlation) among patients and procedures within each center, revealing that different 'centers' were not associated with outcome. Another potential limitation of the present study was limited use of ECMO in our patients. In our series, ECMO was used only in eight patients and it was not statistically associated with outcome. In reality, ECMO was used more often in patients that died than in those that survived. It is still controversial if ECMO improves the long-term survival (after 6 months as it was evaluated in the present study) in patients that undergo FETO [26–28]. Further studies are necessary to evaluate this aspect. Finally another limitation of the present study may be the small sample size to demonstrate prematurity to be an independent risk factor of mortality in case of CDH treated by FETO. Clearly, in univariate analysis our study had adequate power to show prematurity, specifically delivery before 32 weeks, to be a risk factor for mortality with an OR of 0.3, p=0.04 (70% reduction in odds for survival in presence of prematurity). However, in multivariable analysis, where other variables were included and adjusted for in the model, influence of prematurity became lessened at aOR = 0.5, $p > 0.05$ (50% reduction in odds for survival). Although, in general the presence of other variables in the model reduces power, our results show that lung response, but not prematurity, is an independent risk factor for mortality.

In conclusion, we found that fetal pulmonary response after FETO is the most important factor associated with survival, independently from the gestational age at delivery. Further prospective studies with larger series are necessary to confirm the present findings.

Acknowledgements

N.S. is a recipient of a scholarship grant from FULBRIGHT Franco-American Commission for Education Exchange (process no. 68141003) and I.S.W.B. is a recipient of post-doctoral scholarship grant from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, process no. 2013/12493-1). Fetal interventions for cases from Strasbourg, France, were performed in Leuven, Belgium.

Declaration of interest

The authors report no conflicts of interest.

References

1. Samangaya RA, Choudhri S, Murphy F, et al. Outcomes of congenital diaphragmatic hernia: a 12-year experience. Prenat Diagn 2012;32:523–9.
2. Wright JC, Budd JL, Field DJ, Draper ES. Epidemiology and outcome of congenital diaphragmatic hernia: a 9-year experience. Paediatr Perinat Epidemiol 2011;25:144–9.
3. Ruano R, Takashi E, da Silva MM, et al. Prediction and probability of neonatal outcome in isolated congenital diaphragmatic hernia using multiple ultrasound parameters. Ultrasound Obstet Gynecol 2012;39:42–9.
4. Gucciardo L, Deprest J, Done E, et al. Prediction of outcome in isolated congenital diaphragmatic hernia and its consequences for fetal therapy. Best Pract Res Clin Obstet Gynaecol 2008;22:123–38.
5. Deprest J, Gratacos E, Nicolaides KH, Group FT. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results. Ultrasound Obstet Gynecol 2004;24:121–6.
6. Ruano R, Yoshisaki CT, da Silva MM, et al. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2012;39:20–7.
7. Ruano R, da Silva MM, Campos JA, et al. Fetal pulmonary response after fetoscopic tracheal occlusion for severe isolated congenital diaphragmatic hernia. Obstet Gynecol 2012;119:93–101.
8. Ruano R, Duarte SA, Pimenta EJ, et al. Comparison between fetal endoscopic tracheal occlusion using a 1.0-mm fetoscope and prenatal expectant management in severe congenital diaphragmatic hernia. Fetal Diagn Ther 2011;29:64–70.
9. Jani JC, Nicolaides KH, Gratacos E, et al. Fetal lung-to-head ratio in the prediction of survival in severe left-sided diaphragmatic hernia treated by fetal endoscopic tracheal occlusion (FETO). Am J Obstet Gynecol 2006;195:1646–50.

10. Dekoninck P, Gratacos E, Van Mieghem T, et al. Results of fetal endoscopic tracheal occlusion for congenital diaphragmatic hernia and the set up of the randomized controlled TOTAL trial. Early Hum Dev 2011;87:619–24.
11. Cannie MM, Jani JC, De Keyzer F, et al. Evidence and patterns in lung response after fetal tracheal occlusion: clinical controlled study. Radiology 2009;252:526–33.
12. Peralta CF, Jani JC, Van Schoubroeck D, et al. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. Am J Obstet Gynecol 2008;198:60 e61–5.
13. Levison J, Halliday R, Holland AJ, et al. A population-based study of congenital diaphragmatic hernia outcome in New South Wales and the Australian Capital Territory, Australia, 1992-2001. J Pediatr Surg 2006;41:1049–53.
14. Tsao K, Allison ND, Harting MT, et al. Congenital diaphragmatic hernia in the preterm infant. Surgery 2010;148:404–10.
15. Ali K, Grigoratos D, Cornelius V, et al. Outcome of CDH infants following fetoscopic tracheal occlusion – influence of premature delivery. J Pediatr Surg 2013;48:1831–6.
16. Done E, Gratacos E, Nicolaides KH, et al. Predictors of neonatal morbidity in fetuses with severe isolated congenital diaphragmatic hernia undergoing fetoscopic tracheal occlusion. Ultrasound Obstet Gynecol 2013;42:77–83.
17. Cordier AG, Jani JC, Cannie MM, et al. Stomach position in the prediction of survival in left-sided congenital diaphragmatic hernia with or without fetoscopic endoluminal tracheal occlusion. Ultrasound Obstet Gynecol 2015;46:155–61.
18. Cordier AG, Cannie MM, Guilbaud L, et al. Stomach position versus liver-to-thoracic volume ratio in left-sided congenital diaphragmatic hernia. J Matern Fetal Neonatal Med 2015;28:190–5.
19. Metkus AP, Filly RA, Stringer MD, et al. Sonographic predictors of survival in fetal diaphragmatic hernia. J Pediatr Surg 1996;31:148–51,discussion 151–42.
20. Jani J, Nicolaides KH, Keller RL, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. Ultrasound Obstet Gynecol 2007;30:67–71.
21. Britto IS, Araujo Junior E, Sangi-Haghpeykar H, et al. Reference ranges for 2-dimensional sonographic lung measurements in healthy fetuses: a longitudinal study. J Ultrasound Med 2014;33:1917–23.
22. Datin-Dorriere V, Walter-Nicolet E, Rousseau V, et al. Experience in the management of eighty-two newborns with congenital diaphragmatic hernia treated with high-frequency oscillatory ventilation and delayed surgery without the use of extracorporeal membrane oxygenation. J Intensive Care Med 2008;23:128–35.
23. Mitanchez D. Antenatal treatment of congenital diaphragmatic hernia: an update. Arch Pediatr 2008;15:1320–5.
24. Jani JC, Nicolaides KH, Gratacos E, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. Ultrasound Obstet Gynecol 2009;34:304–10.
25. Ruano R, Peiro JL, da Silva MM, et al. Early fetoscopic tracheal occlusion for extremely severe pulmonary hypoplasia in isolated congenital diaphragmatic hernia: preliminary results. Ultrasound Obstet Gynecol 2013;42:70–6.
26. Morini F, Goldman A, Pierro A. Extracorporeal membrane oxygenation in infants with congenital diaphragmatic hernia: a systematic review of the evidence. Eur J Pediatr Surg 2006;16:385–91.
27. Mugford M, Elbourne D, Field D. Extracorporeal membrane oxygenation for severe respiratory failure in newborn infants. Cochrane Database Syst Rev 2008;CD001340.
28. Reiss I, Schaible T, van den Hout L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. Neonatology 2010;98:354–64.

Improving the Prediction of Neonatal Outcomes in Isolated Left-Sided Congenital Diaphragmatic Hernia by Direct and Indirect Sonographic Assessment of Liver Herniation

Nicolas Sananes, MD, Ingrid Britto, MD, PhD, Adesola C. Akinkuotu, MD, Oluyinka O. Olutoye, MD, PhD, Darrell L. Cass, MD, Haleh Sangi-Haghpeykar, PhD, Timothy C. Lee, MD, Christopher I. Cassady, MD, Amy Mehollin-Ray, MD, Stephen Welty, MD, Caraciolo Fernandes, MD, Michael A. Belfort, MD, PhD, Wesley Lee, MD, Rodrigo Ruano, MD, PhD

Objectives—Liver herniation can be assessed sonographically by either a direct (liver-to-thoracic area ratio) or an indirect (stomach position) method. Our objective was to evaluate the utility of those methods to assess liver herniation for the prediction of neonatal outcomes in patients with isolated left-sided congenital diaphragmatic hernia (CDH).

Methods—We conducted a retrospective cohort study of all patients with CDH who had prenatal assessment and were delivered at Texas Children's Hospital between January 2004 and April 2014. The predictive value of sonographic parameters for mortality and the need for extracorporeal membrane oxygenation was evaluated by univariate, multivariate, and factor analysis and by receiver operating characteristics curves.

Results—A total of 77 fetuses with isolated left-sided CDH were analyzed. The lung-to-head ratio, liver-to-thorax ratio, and stomach position (according to the classifications of Kitano et al [*Ultrasound Obstet Gynecol* 2011; 37:277–282] and Cordier et al [*J Matern Fetal Neonatal Med* 2015; 28:190–195]) were significantly associated with both neonatal outcomes ($P < .03$). Significant correlations were observed between all of these sonographic parameters. A combination of the liver-to-thorax ratio and stomach position (Kitano) or stomach position (Cordier) with the lung-to-head ratio increased the area under the receiver operating characteristic curve of the lung-to-head ratio for mortality prediction (0.86 [95% confidence interval, 0.74–0.98], 0.83 [0.72–0.95], and 0.83 [0.74–0.92], respectively).

Conclusions—Sonographic measurements of liver herniation (liver-to-thorax ratio and stomach position) are predictive of neonatal outcomes in isolated left-sided congenital diaphragmatic hernia. Our study shows that the combination of those sonographic measurements of liver herniation and lung size improves the accuracy of predicting mortality in those fetuses.

Key Words—congenital diaphragmatic hernia; liver herniation; lung-to-head ratio; obstetric ultrasound; pulmonary hypoplasia; prenatal predictors; stomach position

Received July 10, 2015, from the Department of Obstetrics and Gynecology (N.S., I.S.W.B., H.S.-H., M.A.B., W.L., R.R.), Michael E. DeBakey Department of Surgery (A.C.A., O.O.O., D.L.C., T.C.L.), Department of Radiology (C.I.C., A.M.-R.), and Department of Pediatrics, Section of Neonatology (S.W., C.F.), Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas USA. Revision requested September 6, 2015. Revised manuscript accepted for publication October 14, 2015.

Dr Sananes was a recipient of a scholarship grant from the Fulbright Franco-American Commission for Education Exchange (process 68141003), and Dr Britto was a recipient of a postdoctoral scholarship grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo (process 2013/12493-1).

Address correspondence to Rodrigo Ruano, MD, PhD, Pavilion for Women, Texas Children's Fetal Center, 6651 Main St, Suite F1020, Houston, TX 77030 USA.

E-mail: ruano@bcm.edu, rodrigoruano@hotmail.com

Abbreviations
CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation

doi:10.7863/ultra.15.07020

Congenital diaphragmatic hernia (CDH) affects approximately 1 per 2500 live births and is associated with substantial morbidity and mortality, mostly because of pulmonary hypoplasia.[1–3] Accurate prenatal determination of

the severity of the CDH is necessary to counsel patients and to provide appropriate perinatal management, including selection of candidates for fetal endoscopic tracheal occlusion.[4–6]

Sonographic measurement of the contralateral lung-to-head ratio is commonly used to assess lung size in CDH.[7–9] Studies have shown that the amount of liver herniation can be measured on magnetic resonance imaging and is an independent prognostic factor.[10–12] A recent study demonstrated that the amount of liver herniation can be assessed directly on 2-dimensional sonography in the same image that is used to measure the lung-to-head ratio (lung size) by calculating the liver-to-thoracic area ratio.[13] Investigators have also shown that the stomach position in the fetal chest can be considered an indirect method to quantify the degree of liver herniation, which also correlates with prognosis.[14–16]

To our knowledge, there is no study comparing direct and indirect methods for quantifying liver herniation using conventional sonography. Conventional sonography is already widely used by maternal-fetal specialists to calculate the lung-to-head ratio, which is the measurement most often used to predict outcomes in fetuses with left-sided CDH.

Therefore, the primary objective of this study was to evaluate the utility of the direct and indirect methods used to quantify the amount of liver herniation by 2-dimensional sonography (liver-to-thorax ratio and stomach position) for predicting outcomes in fetuses with isolated left CDH. The secondary objectives were to evaluate the correlation between the methods and to evaluate the combination of these parameters with the lung-to-head ratio for the prediction of outcomes in these patients.

Materials and Methods

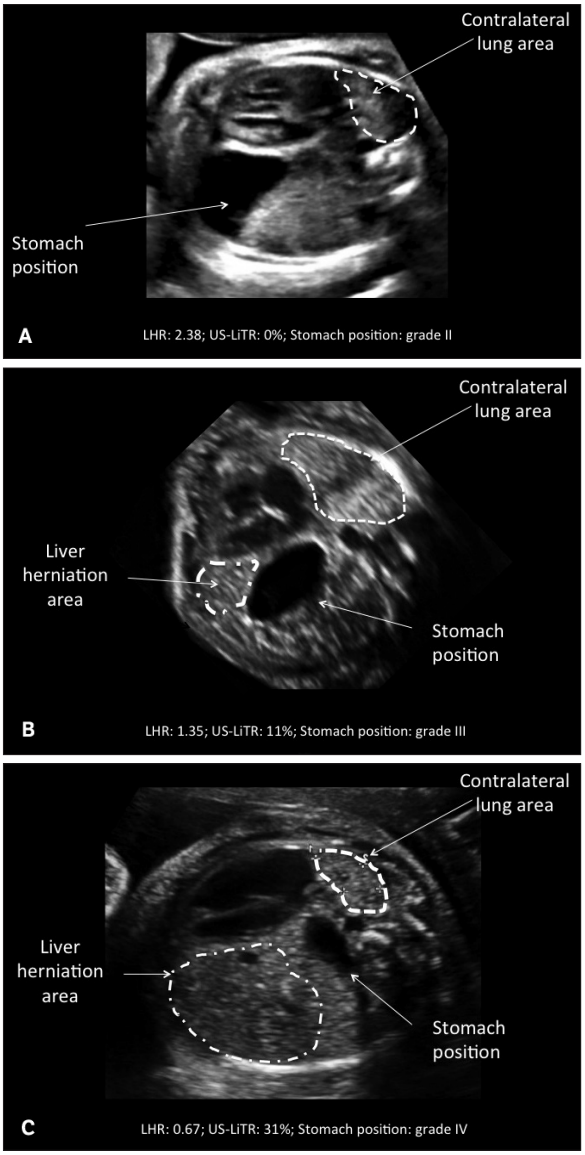
Study Design

We conducted a retrospective cohort study of all fetuses with isolated left-sided CDH who had a prenatal sonographic examination and delivered at Texas Children's Hospital between January 2004 and April 2014. We excluded cases with associated anomalies, fetal intervention, and right-sided CDH. The definition of isolated CDH was based on the absence of other structural anomalies on prenatal examination, a normal fetal karyotype, and absence of a cardiac anomaly on fetal echocardiography. The sonographic parameters were obtained from images derived from video clips of comprehensive fetal sonographic examinations. The local Institutional Review Board approved the study (H-29006).

Sonographic Measurements

Figure 1 shows all of the sonographic evaluations and measurements using single images. The lung-to-head ratio measurements were performed according to a standardized protocol by multiplying the two longest perpendicular diameters of the lung on a 4-chamber view of the heart.[7,17]

Figure 1. Sonograms of different fetuses with mild (**A**), moderate (**B**), and severe (**C**) forms of left-sided CDH at 24, 26, and 25 weeks' gestation, respectively. Stomach position is according to the Cordier classification. LHR indicates lung-to-head ratio; and US-LiTR, sonographic liver-to-thorax ratio.



On the same sonogram, the liver herniation area and the fetal thoracic area were measured, and the sonographic liver-to-thoracic area ratio was then calculated.[13] The stomach position was categorized as grades 0 to 3 on a coronal view according to the classification of Kitano et al[14] and as grades 1 to 4 on the 4-chamber view of the heart according to the classification of Cordier et al.[15] The stomach position according to the Cordier classification was evaluated on the same image on which the lung-to-head ratio and liver-to-thorax ratio were measured. Only 1 sonographic examination per patient (the first examination) at our center was considered for analysis. All evaluations of the stomach position were performed by a single investigator (N.S.), whereas the measurements of the lung-to-head ratio and liver-to-thorax ratio were performed by another investigator (I.B.), who were both well trained to perform prenatal sonographic scans and were blinded to postnatal outcomes.

Neonatal Protocol

All neonates were treated according to a standard perinatal protocol.[18] All neonates underwent immediate endotracheal intubation with orogastric decompression and avoidance of bag-mask ventilation by a staff neonatologist. Gentle mechanical ventilation using a permissive hypercapnia protocol was used. Escalation of ventilatory support to a maximal peak inspiratory pressure and positive end-expiratory pressure of 30 and 6 cm H_2O, respectively, and use of high-frequency oscillatory ventilation to a maximum mean airway pressure of 15 cm H_2O were based on preestablished criteria. The need for extracorporeal membrane oxygenation (ECMO) was determined by the presence of persistent hypoxia (oxygenation index >40 or oxygen pressure persistently <40 mm Hg) with persistent acidosis (pH <7.2) or inadequate tissue perfusion (arterial lactate >3 mmol/L and rising). The timing of surgical repair of the CDH was planned with consideration of the infant's physiologic stability and the presence or absence of pulmonary hypertension.

Statistical Analysis

Results were reported as mean ± standard deviation for continuous variables and as number and percentage for categorical variables. The outcomes in this study were survival at 6 months of life and the need for ECMO postnatally. Univariate comparisons were performed by a *t* test and χ^2 square test for continuous and categorical variables, respectively. Correlations between variables were evaluated by the Spearman test. The performances of the different predictors were evaluated by receiver operating characteristic curve analysis, with estimation of areas under the curve with 95% confidence intervals. Multivariable studies were performed using factor analysis (principal component analysis). Statistical comparisons between the areas under the curves of each sonographic variable and multivariate models were performed according to the tests of McNeil et al,[19] Hanley and McNeil,[20] and DeLong et al.[21] Statistical analyses were performed with the software SPSS version 19.0 software package (IBM Corporation, Armonk, NY) and the MedCalc version 11.6 software package (MedCalc, Mariakerke, Belgium). $P < .05$ was considered statistically significant.

Results

A total of 142 cases of CDH were prenatally evaluated and delivered in our institution during the study period. Sixty-five patients were excluded from the study because of associated anomalies (n = 39), right-sided CDH (n = 15), and fetal intervention (n = 11). There was no case of termination of pregnancy. Therefore, a total of 77 patients with isolated left-sided CDH were included in the study (68 patients were reported in our previous study[13]). In the previous study, we described the liver-to-thorax ratio in fetuses with left and right CDH. The mean gestational age at delivery ± SD was 37.6 ± 1.6 weeks (range, 32–40 weeks).

The survival rate within 6 months of life was 79.3%, since 1 fetus died in utero, and 15 died in the neonatal period. Among the 76 fetuses who were born alive, ECMO was needed in 26 cases (34.2%). Table 1 shows sample characteristics and prenatal findings according to mortality at 6 months of life and the need for ECMO. There was a significant association between the lung-to-head ratio, liver-to-thorax ratio, and stomach position (independent of the classification method) and neonatal mortality or need for ECMO ($P < .05$).

Spearman correlation coefficients between variables are reported in Table 2. Significant correlations were observed between the lung-to-head ratio, liver-to-thorax ratio, and stomach position. The liver-to-thorax ratio correlated more with the stomach position according to the Cordier classification than the Kitano classification.

Figures 2 and 3 show receiver operating characteristic curve analyses of all isolated parameters (lung-to-head ratio, liver-to-thorax ratio, and stomach position) to predict mortality and ECMO, respectively. Figures 4 and 5 show receiver operating characteristic curve analyses of different combinations of parameters to predict mortality and ECMO, respectively. Table 3 provides the areas under the curves for each predictor and each combination of parameters. The lung-to-head ratio had the best accuracy

to predict mortality compared to the other parameters when used singly, but the difference was not statistically significant ($P > .05$). The combination of the lung-to-head ratio and liver-to-thorax ratio was the most accurate predictor of mortality when compared to all other parameters when used singly or in combination ($P < .05$). The predictive accuracy of the combination of the lung-to-head ratio and liver-to-thorax ratio was not improved by the addition of stomach grading, whatever it was ($P > .05$). In considering prediction of the need for ECMO, all sonographic parameters when analyzed singly had similar accuracy, with no statistical difference. The combination of parameters slightly improved the accuracy to predict ECMO, but this finding was still not statistically significant.

Discussion

Principal Findings

Our study shows that direct and indirect sonographic assessments of liver herniation are useful tools for predicting mortality in fetuses with isolated left-sided CDH. The accuracy to predict outcomes in those fetuses by the direct or indirect method for quantifying liver herniation improves when combined with the lung-to-head ratio, especially the liver-to-thorax ratio. However, when used individually, all of these parameters were less accurate to predict the need for ECMO (accuracy of 70%), with only a slight improvement when used in combination (lung-to-head ratio and liver-to-thorax ratio: accuracy of 75%).

Strengths and Weaknesses

To our knowledge, a study that evaluated two sonographic methods to quantify fetal liver herniation (liver-to-thorax ratio and stomach position) and compared and combined them with the lung-to-head ratio has not been reported previously. In addition, we provide information about those measurements from one of the largest series. We also provide information about using these sonographic parameters to predict the need for ECMO. We acknowledge that our study had some limitations, such as the retrospective design. To avoid bias, the operator performed the measurements

Table 1. Sample Characteristics and Prenatal Findings of 77 Fetuses With Isolated Left-Sided CDH According to Outcome

	Mortality at 6 mo of Life			ECMO		
	Yes	No		Yes	No	
Parameter	(n = 16)	(n = 61)	*P*	(n = 26)	(n = 50)	*P*
Maternal age, y	27.9 ± 7.4	27.7 ± 6.0	.903	27.5 ± 6.1	27.2 ± 6.3	.78
GA at sonography, wk	26.7 ± 5.4	26.8 ± 5.0	.928	27.2 ± 5.7	26.6 ± 4.7	.631
Lung-to-head ratio	1.12 ± 0.39	1.87 ± 0.75	<.001	1.40 ± 0.68	1.89 ± 0.75	.007
Liver-to-thorax ratio, %	16.92 ± 8.52	8.20 ± 9.05	.001	13.78 ± 8.2	7.89 ± 9.7	.01
Stomach position (Kitano)			.002			.026
0	0 (0.0)	5 (8.2)		0 (0.0)	5 (10.0)	
1	4 (25.0)	30 (49.1)		8 (30.8)	26 (52.0)	
2	4 (25.0)	20 (32.9)		10 (38.4)	14 (28.0)	
3	8 (50.0)	6 (9.8)		8 (30.8)	5 (10.0)	
Stomach position (Cordier)			.018			.008
1	0 (0.0)	7 (11.5)		0 (0.0)	7 (14.0)	
2	1 (6.2)	19 (31.1)		3 (11.5)	17 (34.0)	
3	4 (25.0)	17 (27.9)		8 (30.8)	13 (26.0)	
4	11 (68.8)	18 (29.5)		15 (57.7)	13 (26.0)	
GA at delivery, wk	37.6 ± 1.0	37.6 ± 1.7	.967	37.8 ± 1.2	37.5 ± 1.8	.552

Data are presented as mean ± SD and number (percent). GA indicates gestational age.

Table 2. Correlations Between Lung-to-Head Ratio, Lung-to-Thoracic Ratio, and Stomach Position

Parameter	Lung-to-Head Ratio	Lung-to-Thoracic Ratio	Stomach Position (Kitano)	Stomach Position (Cordier)
Lung-to-head ratio	1.00	–0.54[a]	–0.51[a]	–0.55[a]
Lung-to-thorax ratio	–0.54[a]	1.00	0.49[a]	0.69[a]
Stomach position (Kitano)	–0.51[a]	0.49[a]	1.00	0.65[a]
Stomach position (Cordier)	–0.55[a]	0.69[a]	0.65[a]	1.00

[a]Significant at $P < .001$.

blinded to the neonatal outcome. We did not test the reproducibility of the measurements in this study, as that has been addressed in other publications.[13,15] In this study, we did not include patients who underwent fetal endoscopic tracheal occlusion. This fact could be considered a bias, since fetuses with less severe forms of CDH could have been excluded from the analysis. However, fetal endoscopic tracheal occlusion was performed in only 10 patients with isolated left-sided CDH during the study period, and we included fetuses with severe forms of CDH who did not undergo fetal intervention. In addition, fetal intervention may change the sonographic parameters (lung-to-head ratio, liver-to-thorax ratio, and stomach position), which can also lead to bias. Nevertheless, Cordier et al[16] showed that the stomach position remains a relevant prognostic factor even after fetal endoscopic tracheal occlusion. Further studies are necessary to investigate whether the amount of liver herniation and the stomach position classification change after fetal endoscopic tracheal occlusion.

Figure 2. Receiver operating characteristics curves for the prediction of mortality at 6 months of life using the lung-to-head ratio (LHR), sonographic liver-to-thorax ratio (US-LiTR), and stomach position according to the Kitano and Cordier classifications.

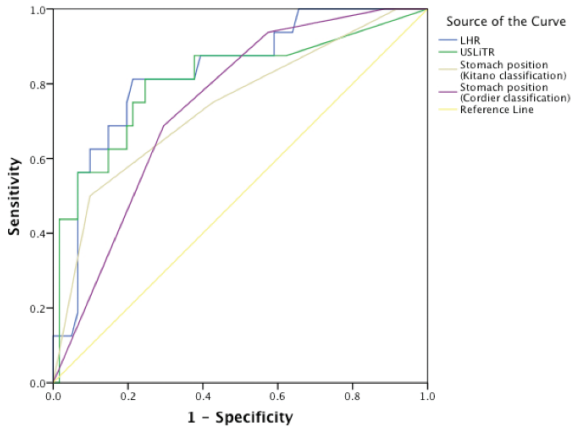


Figure 3. Receiver operating characteristics curves for the prediction of ECMO using the lung-to-head ratio (LHR), sonographic liver-to-thorax ratio (US-LiTR), and stomach position according to the Kitano and Cordier classifications.

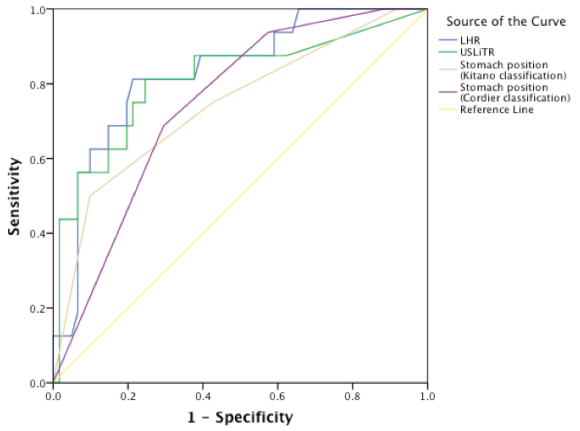


Figure 4. Receiver operating characteristics curves for the prediction of mortality at 6 months of life using combinations of the lung-to-head ratio (LHR), sonographic liver-to-thorax ratio (US-LiTR), and stomach position according to the Kitano and Cordier classifications.

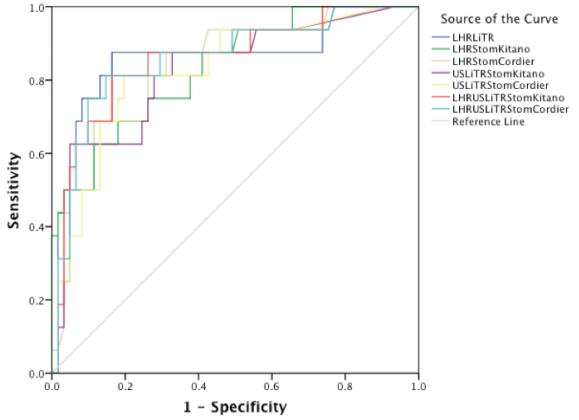


Figure 5. Receiver operating characteristics curves for the prediction of ECMO using combinations of the lung-to-head ratio (LHR), sonographic liver-to-thorax ratio (US-LiTR), and stomach position according to the Kitano and Cordier classifications.

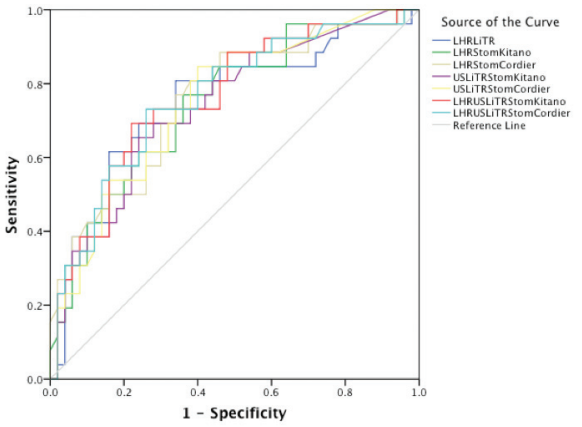


Table 3. Prediction of Neonatal Outcome Using Sonographic Parameters Alone and Combined

Parameter	Mortality at 6 mo of Life		ECMO	
	AUC	95% CI	AUC	95% CI
Lung-to-head ratio	0.80	0.65–0.92	0.72	0.60–0.84
Lung-to-thorax ratio	0.77	0.63–0.90	0.73	0.60–0.80
Stomach position (Kitano)	0.74	0.60–0.89	0.70	0.57–0.82
Stomach position (Cordier)	0.74	0.62–0.87	0.73	0.61–0.84
Lung-to-head ratio + lung-to-thorax ratio	0.86	0.74–0.98	0.76	0.63–0.87
Lung-to-head ratio + stomach position (Kitano)	0.83	0.72–0.95	0.74	0.63–0.86
Lung-to-head ratio + stomach position (Cordier)	0.83	0.74–0.92	0.76	0.64–0.87
Lung-to-thorax ratio + stomach position (Kitano)	0.82	0.70–0.91	0.74	0.62–0.86
Lung-to-thorax ratio + stomach position (Cordier)	0.83	0.71–0.93	0.75	0.64–0.86
Lung-to-head ratio + lung-to-thorax ratio + stomach position (Kitano)	0.86	0.73–0.93	0.76	0.65–0.88
Lung-to-head ratio + lung-to-thorax ratio + stomach position (Cordier)	0.86	0.76–0.93	0.76	0.65–0.88

AUC indicates area under the curve; and CI, confidence interval.

Interpretation

Quantification of liver herniation by magnetic resonance imaging is predictive of neonatal outcomes in left-sided CDH.[10–12] Our study confirms that sonographic assessment of liver herniation, either by a direct or an indirect method, can predict survival at 6 months of life and the need for ECMO.[13–16] Additionally, our results demonstrate that there is a good correlation (correlation coefficient of 0.69) between the liver-to-thorax ratio and evaluation of the stomach position according to Cordier et al.[15] This correlation was expected, since the Cordier method indirectly evaluates the degree of liver herniation. However, only weak correlations were observed between the lung-to-head ratio and liver-to-thorax ratio and between the lung-to-head ratio and stomach position. For these reasons, we recommend using the combination of the lung-to-head ratio (lung size) and one other method to measure the amount of liver herniation (liver-to-thorax ratio or stomach position), but there is no need to use the combination of all parameters. In our experience, the combination of the lung-to-head ratio and liver-to-thorax ratio was statistically more accurate than the lung-to-head ratio and stomach position; however, measuring the liver-to-thorax ratio is more demanding than evaluating the stomach position. Further studies are necessary to confirm our findings.

The ideal situation would be to obtain as much information as possible from as few images as necessary to facilitate the evaluation of a fetus with left-sided CDH. Three of the measurements evaluated can be performed on the exact same sonogram (in a cross section of the fetal chest at the level of the 4-chamber view of the heart). These measurements are the lung-to-head ratio, the liver-to-thorax ratio, and the stomach position according to Cordier et al.[15] Evaluation of the stomach position according to Kitano et al[14] requires a different sonogram; therefore, different sections of the fetus are necessary to obtain that measurement in addition to the lung-to-head ratio.

To test whether the combination of the parameters would improve the accuracy to predict outcomes, principal component analysis was used, instead of multivariate regression. The reason for performing such an analysis was because of the multicollinearity among the variables.

Conclusions

Fetal outcomes in left-sided CDH can be predicted by using a single sonogram by estimating both the lung size and the amount of liver herniation. Our results suggest the liver herniation can be quantified on sonography directly by the liver-to-thorax ratio or indirectly by assessing the stomach position. The combination of sonographic measurements of lung size (lung-to-head ratio) and assessment of the amount of liver herniation (liver-to-thorax ratio or stomach position in the fetal chest) improves the accuracy in predicting outcomes in those fetuses. Future prospective studies with larger numbers of patients are necessary to determine which combination will predict outcomes best in those fetuses.

References

1. Langham MR Jr, Kays DW, Ledbetter DJ, Frentzen B, Sanford LL, Richards DS. Congenital diaphragmatic hernia: epidemiology and outcome. *Clin Perinatol* 1996; 23:671–688.
2. Stege G, Fenton A, Jaffray B. Nihilism in the 1990s: the true mortality of congenital diaphragmatic hernia. *Pediatrics* 2003; 112:532–535.
3. Gallot D, Boda C, Ughetto S, et al. Prenatal detection and outcome of congenital diaphragmatic hernia: a French registry-based study. *Ultrasound Obstet Gynecol* 2007; 29:276–283.

4. Jani JC, Nicolaides KH, Gratacós E, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. *Ultrasound Obstet Gynecol* 2009; 34:304–310.
5. Peralta CF, Jani JC, Van Schoubroeck D, Nicolaides KH, Deprest JA. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. *Am J Obstet Gynecol* 2008; 198:60.e1–60.e5.
6. Ruano R, Duarte SA, Pimenta EJ, et al. Comparison between fetal endoscopic tracheal occlusion using a 1.0-mm fetoscope and prenatal expectant management in severe congenital diaphragmatic hernia. *Fetal Diagn Ther* 2011; 29:64–70.
7. Metkus AP, Filly RA, Stringer MD, Harrison MR, Adzick NS. Sonographic predictors of survival in fetal diaphragmatic hernia. *J Pediatr Surg* 1996; 31:148–152.
8. Lipshutz GS, Albanese CT, Feldstein VA, et al. Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. *J Pediatr Surg* 1997; 32:1634–1636.
9. Laudy JA, Van Gucht M, Van Dooren MF, Wladimiroff JW, Tibboel D. Congenital diaphragmatic hernia: an evaluation of the prognostic value of the lung-to-head ratio and other prenatal parameters. *Prenat Diagn* 2003; 23:634–639.
10. Cannie M, Jani J, Chaffiotte C, et al. Quantification of intrathoracic liver herniation by magnetic resonance imaging and prediction of postnatal survival in fetuses with congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2008; 32:627–632.
11. Lazar DA, Ruano R, Cass DL, et al. Defining "liver-up": does the volume of liver herniation predict outcome for fetuses with isolated left-sided congenital diaphragmatic hernia? *J Pediatr Surg* 2012; 47:1058–1062.
12. Ruano R, Lazar DA, Cass DL, et al. Fetal lung volume and quantification of liver herniation by magnetic resonance imaging in isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol* 2014; 43:662–669.
13. Werneck Britto IS, Olutoye OO, Cass DL, et al. Quantification of liver herniation in fetuses with isolated congenital diaphragmatic hernia using two-dimensional ultrasonography. *Ultrasound Obstet Gynecol* 2015; 46:150–154.
14. Kitano Y, Okuyama H, Saito M, et al. Re-evaluation of stomach position as a simple prognostic factor in fetal left congenital diaphragmatic hernia: a multicenter survey in Japan. *Ultrasound Obstet Gynecol* 2011; 37:277–282.
15. Cordier AG, Cannie MM, Guilbaud L, et al. Stomach position versus liver-to-thoracic volume ratio in left-sided congenital diaphragmatic hernia. *J Matern Fetal Neonatal Med* 2015; 28:190–195.
16. Cordier AG, Jani JC, Cannie MM, et al. Stomach position in the prediction of survival in left-sided congenital diaphragmatic hernia with or without fetoscopic endoluminal tracheal occlusion. *Ultrasound Obstet Gynecol* 2015; 46:155–161.
17. Jani JC, Peralta CF, Nicolaides KH. Lung-to-head ratio: a need to unify the technique. *Ultrasound Obstet Gynecol* 2012; 39:2–6.
18. Lazar DA, Cass DL, Rodriguez MA, et al. Impact of prenatal evaluation and protocol-based perinatal management on congenital diaphragmatic hernia outcomes. *J Pediatr Surg* 2011; 46:808–813.
19. McNeil BJ, Hanley JA, Funkenstein HH, Wallman J. Paired receiver operating characteristic curves and the effect of history on radiographic interpretation: CT of the head as a case study. *Radiology* 1983; 149:75–77.
20. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983; 148:839–843.
21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; 44:837–845.

7.6.5. Évaluation de la vascularisation pulmonaire

Original article

Longitudinal ultrasound assessment of lung vasculature in fetuses with congenital diaphragmatic hernia treated or not by endoscopic tracheal occlusion and controls

Short title: lung vasculature in CDH

I. BRITTO [1,2], N. SANANES [1,3], H. SANGI-HAGHPEYKAR [2], R. RUANO [1,2]

[1] Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, USA

[2] Department of Obstetrics and Gynecology, Texas Children's Hospital, Houston, Texas, USA

[3] Department of Obstetrics and Gynecology, Strasbourg teaching Hospital, Strasbourg, France

Corresponding author: Rodrigo Ruano, Pavilion for Women – Texas Children's Fetal Center, 6651 Main Street, Suite F1020, Houston, Texas, United States, 77030, ruano@bcm.edu, rodrigoruano@hotmail.com

Acknowledgements:

I.S.W.B. is a recipient of post-doctoral scholarship grant from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, process n. 2013/12493-1).

N.S. is a recipient of a scholarship grant from FULBRIGHT Franco-American Commission for Education Exchange (process n. 68141003).

ABSTRACT

Objective:

The primary objective of this study was to assess the fetal pulmonary vasculature response after fetal endoscopic tracheal occlusion (FETO) for congenital diaphragmatic hernia (CDH), and to compare with both untreated CDH cases and controls. The secondary objective was to evaluate the correlation between vasculature and survival.

Methods:

In this prospective observational longitudinal study, 3D power Doppler ultrasonography was performed in 33 fetuses divided in 3 groups: 10 normal fetuses, 13 fetuses with isolated left CDH with routine prenatal management and 10 fetuses treated with FETO. Using the same pre-settings for all fetuses, 3 vascular indices were measured throughout pregnancy: vascularization indices (VI), flow indices (FI), and vascularization-flow index (VFI).

Results:

Fetal pulmonary indices showed a constant distribution through pregnancy in normal fetuses and CDH with expectative management. FI and VFI were significantly lower in cases of CDH with no fetal intervention than in controls (p= 0.0006 and p= 0.018 respectively). FETO resulted in a significant improvement in VFI and VI when compared with controls (p=0.0003 and p<.0001 respectively). Among FETO cases, the vascular indices VFI and VI were significantly higher in cases that survived compared to fetuses who died (p= .0005 and p= .01 respectively).

Conclusion:

Vascular indices are altered in CDH in comparison to controls. FETO may restore vascular indices and this response after the procedure can predict neonatal survival.

Keywords: congenital diaphragmatic hernia; fetal endoscopic tracheal occlusion; lung perfusion; three-dimensional power doppler ultrasound; prognosis

INTRODUCTION

Congenital diaphragmatic hernia (CDH) occurs 1 in 2200 newborns and is associated with significant morbidity and mortality due to pulmonary hypoplasia and pulmonary hypertension[1]. Assessment of both lung size and vasculature is of interest, in order to predict accurately the outcome and therefore provide appropriate counseling and perinatal management, including fetal endoscopic tracheal occlusion (FETO) [2-4].

Several parameters have been developed to estimate lung size and predict outcome for isolated CDH, including contralateral lung-to-head ratio (LHR), observed to expected LRH (o/e LHR), o/e total lung volume (O/e TLV) and quantitative lung index (QLI) [5-15]. Even if lung size and vasculature may be partially correlated, accuracy to predict outcome still needs to be improved and direct assessment of pulmonary vasculature is of interest [16].

Pathology studies demonstrated that CDH is associated with decreased number of arterial branches and increased muscular thickness in the pulmonary vessels [18, 19]. Indeed, clinical studies showed that the sonographic fetal pulmonary vascular indices are significantly lower in cases with CDH than in controls [20].

FETO for severe isolated CDH have the potential to increase airway pressure, leading to proliferation, increase alveolar air space and maturation of pulmonary vasculature [21]. Surprinsingly, the only two human pathologic studies didn't show that FETO improved lung vasculature [22, 23]. The few clinical data suggest that pulmonary vasculature correlates with prognosis and that FETO may improve lung tissue perfusion [24, 25]. There is no study comparing the lung vasculature in CDH treated by FETO and lung vasculature in normal fetuses.

Additional studies are therefore necessary because of the paucity of data, and because of the inconsistency in pathology and clinical findings. Moreover, comparison of lung vasculature between FETO cases and controls still need to be investigated.

The primary objective of this study was to assess the fetal pulmonary vasculature response after FETO for CDH, and to compare with both untreated CDH cases and controls. The secondary objective was to evaluate the correlation between vasculature and survival.

METHODS

Study design

This was a single-center cohort prospective observational study conducted in Texas Children's Hospital, that is a tertiary Maternal Fetal Medicine unit. Inclusion period was from February 2011 to September 2014. All patients agreed to participate at this study. The Institutional Review Board from Texas Children's Hospital approved this protocol.

Participants

All consecutive cases of fetal isolated left CDH referred in our center during the study period were included, whether they underwent a FETO or not. The definition of isolated CDH was based on the absence of other structural anomalies on prenatal examination, normal fetal karyotype and absence of cardiac anomaly on fetal echocardiogram.

Assessment of lung vasculature was performed in the right lung using 3-dimensional power Doppler according to methods previously described [20], 24. 3D power Doppler has the advantage of assessing the volume and quantifying the power Doppler signal in the region of interest. A Voluson 730 ultrasound machine (G.E .Health-care, Kretztechnik, Zipf, Austria) was used for 3D volume scanning. Preestableshid settings were used for all cases: angio mode: cent; smooth: 4/5; FRQ: low; quality: 16; density: 6; enhance: 16; balance: G>150; filter: 2; actual power: 2dB; pulse repetition frequency: 0.9 20. In all cases, 3 D power Doppler was performed at the level of four chambers view of the fetal thorax with the pulmonary vessels located proximal to the transducer. The angle of the volumetric box was adjusted to scan entire fetal thorax and the angle of volume sampling varied with gestational age. After obtaining the multiplanar image of 3 orthogonal planes, the right lung volume was measured using VOCAL technique with 30°rotation. 3 D power Doppler histogram was used to determine vascular indices from computer algorithms. The vascular indices analyzed were: vascularization indices (VI), which is related to the color voxel/ total voxel ratio, indicating how many vessels can be detected within the lung; flow indices (FI), which refers to the weighted

color voxel and gives an amplitude value for the color signal, showing how many blood cells are in area of interest (pulmonary blood flow) and vascularization-flow index (VFI), which is related to the weighted color voxel / total voxel ratio, combining the information of vascularity and blood flow.

Each fetus was longitudinally evaluated every two to four weeks and the vascular indices were plotted against gestational age. In the CDH group treated with FETO, measurements were performed before and after the procedure. All measurements were performed by R.R.

Gestational age was established based on the date of the last period and on sonographic measurement of the crown-rump length in the first trimester. The LHR was calculated by measuring the ratio between the longest diameters of the contralateral lung and head circumference at the level of four chambers heart view as described by Metkus [10].

FETO was performed between 24 and 30 weeks using a previously described technique[26]. Briefly, fetal intervention was performed under maternal local anesthesia and fetal systemic anesthesia, using a curved operating sheath with a diameter of 1.3 mm and a 1.0 mm scope. Direct visualization of the fetal trachea was achieved in all cases prior to occlusion by a detachable balloon filled with saline (GOLDBAL 2 or 4; Balt, Montmorency, France). Prophylactic tocolysis and antibiotics were used during the procedure and for 24 hours after fetal intervention. Unplug was planned at around 34 weeks by either fetoscopic approach or ultrasound-guided puncture.

All neonates were managed according to a standard perinatal protocol [27]. All neonates underwent immediate endotracheal intubation with orogastric decompression and avoidance of bag-mask ventilation by a staff neonatologist. Gentle mechanical ventilation using a permissive hypercapnia protocol was used. Escalation of ventilatory support to a maximal peak inspiratory pressure and positive end expiratory pressure of 30 and 6 cm of H20 respectively, and use of high-frequency oscillatory ventilation to a maximum mean airway pressure of 15 were based on pre-established criteria. Need of ECMO was determined by the presence of persistent hypoxia (preductal SaO2<80%), persistent acidosis (pH<7.2), and/or inadequate tissue perfusion. The time of surgery repair was managed according to the infant physiologic stability.

Variables

Fetal pulmonary vasculature was assessed in the right lung using vascular indices (VI, FI, and VFI), which were expressed as continuous variables. The neonatal outcome studied was the 6-month survival.

Data collected included basic demographics and prognosis factors of survival in CDH.

Statistical analysis

Results were reported as mean ± standard deviation for continuous variables and as number and percentage for categorical variables. Vascular indices were plotted against gestational age by regression analysis. P value < 0.05 was considered statistically significant.

RESULTS

A total of 33 patients were included in the study. There were 23 cases of CDH (10 treated with FETO and 13 with expectative management) and 10 controls. The number of evaluations for each patient ranged from 1 to 9, and the median was 4.

Median values of VI, FI and VFI were respectively 37.4; 40.2; 15.1 in controls, 29.5; 29.4; 9.3 in CDH with normal prenatal management and 30.6; 36.3; 10.7 in CDH with FETO.

Fetal pulmonary indices showed a constant distribution throughout pregnancy in controls and CDH with conservative management (Figure 1, 2 and 3).

VFI and FI were significantly lower in cases of CDH with no fetal intervention than in controls (p= .018 and p= .0006 respectively) (Figure 1 and 2). VI indices were lower in cases of CDH with conservative management than in controls, but this result was not statistically significant (p= .06) (Figure 3).

FETO resulted in a significant improvement in VFI (Figure 1) and VI (Figure 3) when compared with controls (p=0.0003 and p<0.0001 respectively). The procedure improved slightly FI indices (Figure 2), however this result was not significant (p=0.26).

Figure 1: VFI indices throughout pregnancy in controls, untreated CDH fetuses, and CDH fetuses treated with FETO.

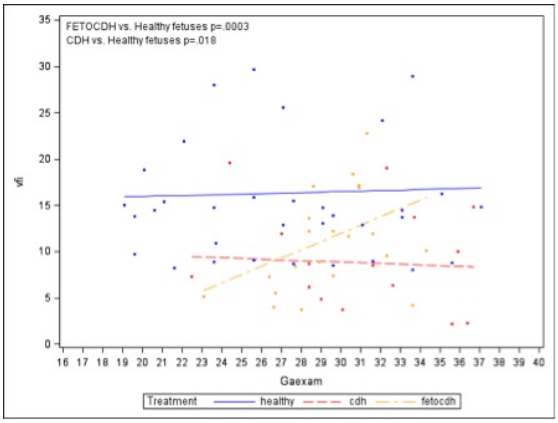


Figure 2: FI indices throughout pregnancy in controls, untreated CDH fetuses, and CDH fetuses treated with FETO.

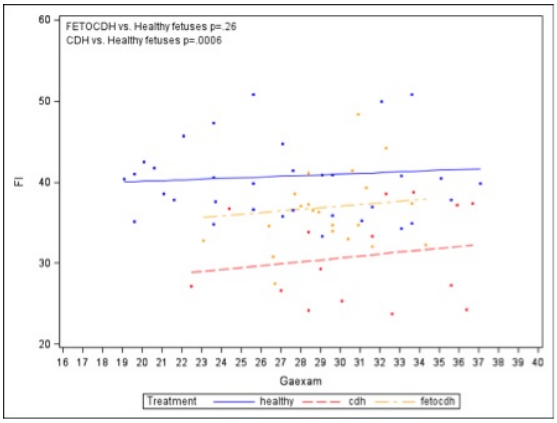
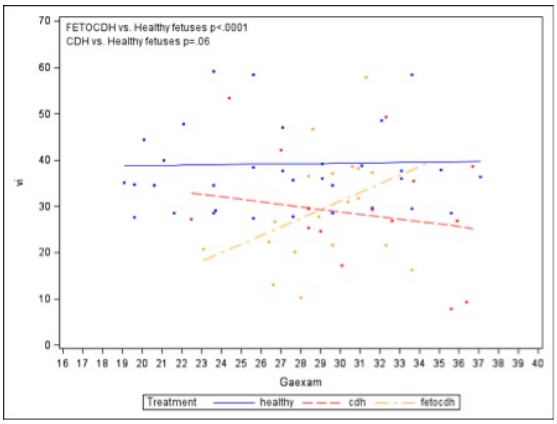


Figure 3: VI indices throughout pregnancy in controls, untreated CDH fetuses, and CDH fetuses treated with FETO.



Among 23 cases of CDH, survival rate was 60 % for FETO cases (6 /10 fetuses) and 76.9% for expectant management cases (10/13 fetuses). Maternal and fetal demographics are described in table 1.

Table 1. Baseline characteristics and prognostic factors according to survival, in 23 fetuses with isolated CDH treated by either FETO or expectant management

	FETO			Conservative management		
	Alive (N=6)	**Dead (N=4)**	***Total cases***	**Alive (N=10)**	**Dead (N=3)**	***Total cases***
Maternal age in years (mean ± SD)	30.7±5.6	30.2± 1.9		31.3±6.6	30.6±6.1	
GA at birth in weeks (mean ± SD)	35.0±1.4	37.1±2.1				
LHR <1.0	0.86±0.1	0.78±0.1	*10*	0.77±0.1	0.60±0.1	*2*
LHR 1.0-1.4			*0*	1.20±0.1	1.22±0.1	4
LHR>1.4			*0*	2.05±0.2		*7*

Among FETO cases, the vascular indices VFI and VI increase significantly in cases that survived compared to fetuses who died (p=0.01 and p=0.0005 respectively) (Figure 4 and 5). The same pattern was observed for FI, but the result was not significant (p=0.06).

Figure 4: FVI indices throughout pregnancy

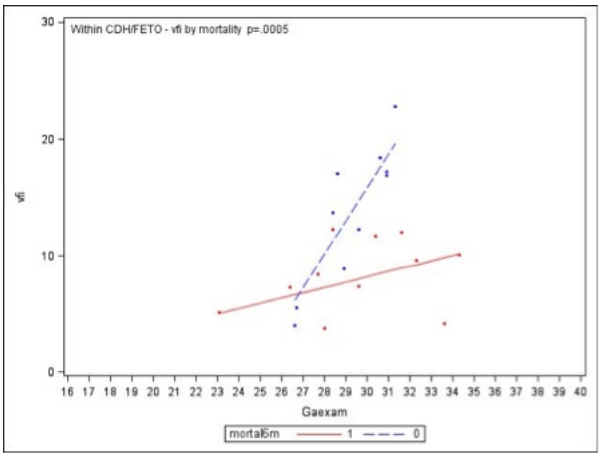
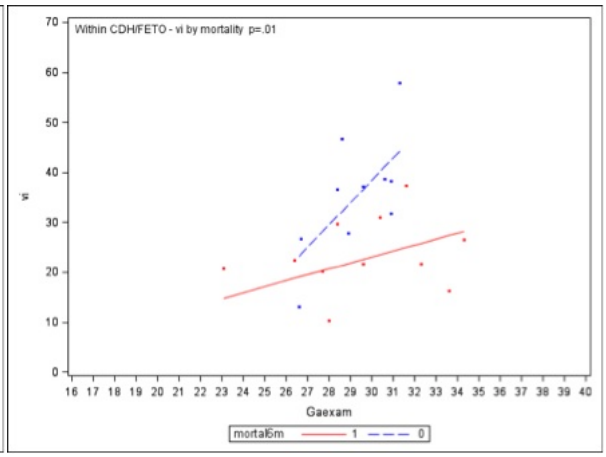


Figure 5: VI indices throughout pregnancy



DISCUSSION

Perfusion of the fetal lung can be an important predictor of lung function and neonatal outcome [28]. Many authors already proposed different techniques to study fetal pulmonary vasculature such as pulmonary artery diameter, acceleration time/ejection time ratio of pulmonary arteries, intrapulmonary arterial Doppler velocimetry [29-35]. These studies showed that Doppler of pulmonary artery is a useful tool to predict pulmonary hypoplasia and may help to refine the prediction of survival after FETO in severe CDH [34, 35].

Power Doppler is an interesting technique for analyzing low-velocity blood flows. Power Doppler images have been used by calculating the mean pixel intensity of the signal over the region of interest [28]. 3D power Doppler has the advantage of assessing the volume and quantifying the power Doppler signal in the region of interest [20]. Some limitations related to this technique include difficulty of adjusting gain, depth and attenuation in overlying layers that could interfere with the estimation of lung perfusion [28]. For this reason, pre-settings were determined in our study and each patient was evaluated longitudinally, considering the first evaluation as its own control. The objective of the study was not to define reference values for indices of vascularity, but to analyze the variation of these indices in each case and the response after fetal intervention.

Fractional moving blood volume estimation in the fetal lung using power Doppler is a relevant tool to evaluate blood perfusion. However, this technique also requires experienced operators, using a well-defined region of interest and standard settings to be considered as a reproducible method to quantify fetal lung blood perfusion [36]. Using this method, a study showed that fetuses with CDH have decreased lung tissue perfusion, which is associated with decreased lung growth [37].

Our first observation in this study was the confirmation that fetal pulmonary vascular indices present a constant distribution throughout gestation as already described in the literature [20]. In cases of CDH with conservative management, pulmonary blood flow was significantly lower than controls and remained constant during pregnancy.

Among all cases of CDH, our findings demonstrated that pulmonary vascularization and pulmonary blood flow were significantly lower in cases who died. Pulmonary flow is also reduced but not significantly. A previous study showed that pulmonary vascularization index could be considered as a good predictor of neonatal outcome in CDH cases not treated with fetal intervention [15].

FETO can stimulate lung growth in fetuses with CDH [38] and improve neonatal survival in severe cases [39], leading to changes in pulmonary vascularity and lung size [2]. However, the impact on lung growth occurs differently in each case. A study showed that pulmonary response can be used to predict neonatal outcome in fetuses that undergo fetal intervention[2]. Our study confirms that FETO resulted in a significant improvement of pulmonary vascularization and pulmonary blood flow, by increasing VI and VFI. Moreover, our findings showed that pulmonary response measured by these vascular indices after tracheal occlusion can also be used to predict postnatal outcome.

One of the limitations of our study is the limited number of cases and further longitudinal studies are necessary to evaluate the response of pulmonary indices after fetal intervention.

In conclusion, 3D power Doppler can be used to evaluate fetal pulmonary status. Minimal changes occur in lung vascular indices through pregnancy. Vascular indices are altered in CDH in comparison to controls. FETO may restore vascular indices and this response after the procedure can predict neonatal survival.

REFERENCES

1. R. Keijzer and P. Puri. Congenital diaphragmatic hernia. Seminars in pediatric surgery 2010; 19: 180-185. DOI 10.1053/j.sempedsurg.2010.03.001.
2. R. Ruano, M. M. da Silva, J. A. Campos, R. Papanna, K. Moise, Jr., U. Tannuri and M. Zugaib. Fetal pulmonary response after fetoscopic tracheal occlusion for severe isolated congenital diaphragmatic hernia. Obstet Gynecol 2012; 119: 93-101. DOI 10.1097/AOG.0b013e31823d3aea.
3. C. F. Peralta, J. C. Jani, D. Van Schoubroeck, K. H. Nicolaides and J. A. Deprest. Fetal lung volume after endoscopic tracheal occlusion in the prediction of postnatal outcome. Am J Obstet Gynecol 2008; 198: 60 e61-65. DOI 10.1016/j.ajog.2007.05.034.
4. J. C. Jani, K. H. Nicolaides, E. Gratacos, C. M. Valencia, E. Done, J. M. Martinez, L. Gucciardo, R. Cruz and J. A. Deprest. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. Ultrasound Obstet Gynecol 2009; 34: 304-310. DOI 10.1002/uog.6450.
5. M. S. Arkovitz, M. Russo, P. Devine, N. Budhorick and C. J. Stolar. Fetal lung-head ratio is not related to outcome for antenatal diagnosed congenital diaphragmatic hernia. Journal of pediatric surgery 2007; 42: 107-110; discussion 110-101. DOI 10.1016/j.jpedsurg.2006.09.010.
6. J. Jani, K. H. Nicolaides, R. L. Keller, A. Benachi, C. F. Peralta, R. Favre, O. Moreno, D. Tibboel, S. Lipitz, A. Eggink, P. Vaast, K. Allegaert, M. Harrison, J. Deprest and C. D. H. R. G. Antenatal. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2007; 30: 67-71. DOI 10.1002/uog.4052.
7. J. Jani, C. F. Peralta, A. Benachi, J. Deprest and K. H. Nicolaides. Assessment of lung area in fetuses with congenital diaphragmatic hernia. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2007; 30: 72-76. DOI 10.1002/uog.4051.
8. J. A. Laudy, M. Van Gucht, M. F. Van Dooren, J. W. Wladimiroff and D. Tibboel. Congenital diaphragmatic hernia: an evaluation of the prognostic value of the lung-to-head ratio and other prenatal parameters. Prenat Diagn 2003; 23: 634-639. DOI 10.1002/pd.654.
9. G. S. Lipshutz, C. T. Albanese, V. A. Feldstein, R. W. Jennings, H. T. Housley, R. Beech, J. A. Farrell and M. R. Harrison. Prospective analysis of lung-to-head ratio predicts survival for patients with prenatally diagnosed congenital diaphragmatic hernia. J Pediatr Surg 1997; 32: 1634-1636.
10. A. P. Metkus, R. A. Filly, M. D. Stringer, M. R. Harrison and N. S. Adzick. Sonographic predictors of survival in fetal diaphragmatic hernia. Journal of pediatric surgery 1996; 31: 148-151; discussion 151-142.
11. C. F. Peralta, P. Cavoretto, B. Csapo, H. Vandecruys and K. H. Nicolaides. Assessment of lung area in normal fetuses at 12-32 weeks. Ultrasound Obstet Gynecol 2005; 26: 718-724. DOI 10.1002/uog.2651.
12. R. A. Quintero, L. F. Quintero, R. Chmait, L. Gomez Castro, L. M. Korst, M. Fridman and E. V. Kontopoulos. The quantitative lung index (QLI): a gestational age-independent sonographic predictor of fetal lung growth. American journal of obstetrics and gynecology 2011; 205: 544 e541-548. DOI 10.1016/j.ajog.2011.07.031.
13. K. Tsukimori, K. Masumoto, S. Morokuma, T. Yoshimura, T. Taguchi, T. Hara, Y. Sakaguchi, S. Takahashi, N. Wake and S. Suita. The lung-to-thorax transverse area ratio at term and near term correlates with survival in isolated congenital diaphragmatic hernia. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 2008; 27: 707-713.
14. R. Ruano, M. C. Aubry, B. Barthe, Y. Dumez and A. Benachi. Three-dimensional ultrasonographic measurements of the fetal lungs for prediction of perinatal outcome in isolated congenital diaphragmatic hernia. J Obstet Gynaecol Res 2009; 35: 1031-1041. DOI 10.1111/j.1447-0756.2009.001060.x.
15. R. Ruano, E. Takashi, M. M. da Silva, J. A. Campos, U. Tannuri and M. Zugaib. Prediction and probability of neonatal outcome in isolated congenital diaphragmatic hernia using multiple ultrasound parameters. Ultrasound Obstet Gynecol 2012; 39: 42-49. DOI 10.1002/uog.10095.
16. O. Moreno-Alvarez, R. Cruz-Martinez, E. Hernandez-Andrade, E. Done, O. Gomez, J. Deprest and E. Gratacos. Lung tissue perfusion in congenital diaphragmatic hernia and association with the lung-to-head ratio and intrapulmonary artery pulsed Doppler. Ultrasound Obstet Gynecol 2010; 35: 578-582. DOI 10.1002/uog.7592.
17. A. P. Bos, D. Tibboel, V. C. Koot, F. W. Hazebroek and J. C. Molenaar. Persistent pulmonary hypertension in high-risk congenital diaphragmatic hernia patients: incidence and vasodilator therapy. Journal of pediatric surgery 1993; 28: 1463-1465.
18. K. K. Nobuhara and J. M. Wilson. The effect of mechanical forces on in utero lung growth in congenital diaphragmatic hernia. Clinics in perinatology 1996; 23: 741-752.
19. X. Roubliova, E. Verbeken, J. Wu, H. Yamamoto, T. Lerut, D. Tibboel and J. Deprest. Pulmonary vascular morphology in a fetal rabbit model for congenital diaphragmatic hernia. Journal of pediatric surgery 2004; 39: 1066-1072.
20. R. Ruano, M. C. Aubry, B. Barthe, D. Mitanchez, Y. Dumez and A. Benachi. Quantitative analysis of fetal pulmonary vasculature by 3-dimensional power Doppler ultrasonography in isolated congenital diaphragmatic hernia. Am J Obstet Gynecol 2006; 195: 1720-1728. DOI 10.1016/j.ajog.2006.05.010.

21. P. A. Khan, M. Cloutier and B. Piedboeuf. Tracheal occlusion: a review of obstructing fetal lungs to make them grow and mature. American journal of medical genetics Part C, Seminars in medical genetics 2007; 145C: 125-138. DOI 10.1002/ajmg.c.30127.
22. A. E. Heerema, J. T. Rabban, R. M. Sydorak, M. R. Harrison and K. D. Jones. Lung pathology in patients with congenital diaphragmatic hernia treated with fetal surgical intervention, including tracheal occlusion. Pediatr Dev Pathol 2003; 6: 536-546.
23. E. Danzer, M. G. Davey, P. A. Kreiger, E. D. Ruchelli, M. P. Johnson, N. S. Adzick, A. W. Flake and H. L. Hedrick. Fetal tracheal occlusion for severe congenital diaphragmatic hernia in humans: a morphometric study of lung parenchyma and muscularization of pulmonary arterioles. J Pediatr Surg 2008; 43: 1767-1775. DOI 10.1016/j.jpedsurg.2008.04.033.
24. R. Ruano, M. M. da Silva, J. A. Campos, R. Papanna, K. Moise, Jr., U. Tannuri and M. Zugaib. Fetal pulmonary response after fetoscopic tracheal occlusion for severe isolated congenital diaphragmatic hernia. Obstet Gynecol 2012; 119: 93-101. DOI 10.1097/AOG.0b013e31823d3aea.
25. R. Cruz-Martinez, O. Moreno-Alvarez, E. Hernandez-Andrade, M. Castanon, E. Done, J. M. Martinez, B. Puerto, J. Deprest and E. Gratacos. Contribution of intrapulmonary artery Doppler to improve prediction of survival in fetuses with congenital diaphragmatic hernia treated with fetal endoscopic tracheal occlusion. Ultrasound Obstet Gynecol 2010; 35: 572-577. DOI 10.1002/uog.7593.
26. R. Ruano, C. T. Yoshisaki, M. M. da Silva, M. E. Ceccon, M. S. Grasi, U. Tannuri and M. Zugaib. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2012; 39: 20-27. DOI 10.1002/uog.10142.
27. D. A. Lazar, D. L. Cass, M. A. Rodriguez, S. F. Hassan, C. I. Cassady, Y. R. Johnson, K. E. Johnson, A. Johnson, K. J. Moise, B. Belleza-Bascon and O. O. Olutoye. Impact of prenatal evaluation and protocol-based perinatal management on congenital diaphragmatic hernia outcomes. J Pediatr Surg 2011; 46: 808-813. DOI 10.1016/j.jpedsurg.2011.02.009.
28. T. Jansson, E. Hernandez-Andrade, G. Lingman and K. Marsal. Estimation of fractional moving blood volume in fetal lung using Power Doppler ultrasound, methodological aspects. Ultrasound in medicine & biology 2003; 29: 1551-1559.
29. R. Ruano, M. de Fatima Yukie Maeda, J. I. Niigaki and M. Zugaib. Pulmonary artery diameters in healthy fetuses from 19 to 40 weeks' gestation. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 2007; 26: 309-316.
30. S. Fuke, T. Kanzaki, J. Mu, K. Wasada, M. Takemura, N. Mitsuda and Y. Murata. Antenatal prediction of pulmonary hypoplasia by acceleration time/ejection time ratio of fetal pulmonary arteries by Doppler blood flow velocimetry. American journal of obstetrics and gynecology 2003; 188: 228-233.
31. R. Chaoui, K. Kalache, C. Tennstedt, F. Lenz and M. Vogel. Pulmonary arterial Doppler velocimetry in fetuses with lung hypoplasia. European journal of obstetrics, gynecology, and reproductive biology 1999; 84: 179-185.
32. R. Cruz-Martinez, M. Castanon, O. Moreno-Alvarez, R. Acosta-Rojas, J. M. Martinez and E. Gratacos. Usefulness of lung-to-head ratio and intrapulmonary arterial Doppler in predicting neonatal morbidity in fetuses with congenital diaphragmatic hernia treated with fetoscopic tracheal occlusion. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2013; 41: 59-65. DOI 10.1002/uog.11212.
33. R. Cruz-Martinez, E. Hernandez-Andrade, O. Moreno-Alvarez, E. Done, J. Deprest and E. Gratacos. Prognostic value of pulmonary Doppler to predict response to tracheal occlusion in fetuses with congenital diaphragmatic hernia. Fetal diagnosis and therapy 2011; 29: 18-24. DOI 10.1159/000320249.
34. R. Cruz-Martinez, O. Moreno-Alvarez, E. Hernandez-Andrade, M. Castanon, E. Done, J. M. Martinez, B. Puerto, J. Deprest and E. Gratacos. Contribution of intrapulmonary artery Doppler to improve prediction of survival in fetuses with congenital diaphragmatic hernia treated with fetal endoscopic tracheal occlusion. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2010; 35: 572-577. DOI 10.1002/uog.7593.
35. S. Yoshimura, H. Masuzaki, K. Miura, K. Muta, H. Gotoh and T. Ishimaru. Diagnosis of fetal pulmonary hypoplasia by measurement of blood flow velocity waveforms of pulmonary arteries with Doppler ultrasonography. American journal of obstetrics and gynecology 1999; 180: 441-446.
36. E. Hernandez-Andrade, A. Thuring-Jonsson, T. Jansson, G. Lingman and K. Marsal. Fractional moving blood volume estimation in the fetal lung using power Doppler ultrasound: a reproducibility study. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2004; 23: 369-373. DOI 10.1002/uog.1003.
37. O. Moreno-Alvarez, R. Cruz-Martinez, E. Hernandez-Andrade, E. Done, O. Gomez, J. Deprest and E. Gratacos. Lung tissue perfusion in congenital diaphragmatic hernia and association with the lung-to-head ratio and intrapulmonary artery pulsed Doppler. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2010; 35: 578-582. DOI 10.1002/uog.7592.
38. J. C. Jani, K. H. Nicolaides, E. Gratacos, H. Vandecruys, J. A. Deprest and F. T. Group. Fetal lung-to-head ratio in the prediction of survival in severe left-sided diaphragmatic hernia treated by fetal endoscopic tracheal occlusion (FETO). Am J Obstet Gynecol 2006; 195: 1646-1650. DOI 10.1016/j.ajog.2006.04.004.
39. R. Ruano, C. T. Yoshisaki, M. M. da Silva, M. E. Ceccon, M. S. Grasi, U. Tannuri and M. Zugaib. A randomized controlled trial of fetal endoscopic tracheal occlusion versus postnatal management of severe isolated congenital diaphragmatic hernia. Ultrasound Obstet Gynecol 2012; 39: 20-27. DOI 10.1002/uog.10142.

7.6.6. Étude de l'impact pronostique du côté de la hernie

Les hernies diaphragmatiques congénitales droites sont-elles de mauvais pronostic ?

Etude de cohorte nationale française

Anne Pinton, Nicolas Sananès, Centre de Référence des Hernies Diaphragmatiques.

Introduction

L'incidence des hernies de coupole diaphragmatique (HCD) se situe entre 1/2000 et 1/5000 naissances (1,2). Dans 80% des cas elle se situe à gauche et dans 20% des cas à droite (3,4). Le taux de survie globale de la HCD est estimé actuellement entre 60% et 80% (5,6).

Le côté droit de la HCD est souvent considéré comme un facteur de mauvais pronostic car de nombreuses études ont rapporté une survie des HCD droites inférieure à celle des HCD gauches (7–12). Cependant des problèmes de puissance statistique et de potentiels biais sont à signaler : (i) Le côté de la hernie est rarement étudié comme critère de jugement principal. (ii) Les HCD droites étant plus rares, elles sont le plus souvent rapportées dans des études avec un faible effectif ou alors au sein d'analyses multi-centriques avec une prise en charge hétérogène (11). (iii) Enfin les HCD droites sont potentiellement moins souvent diagnostiquées en prénatal que les HCD gauches et ne bénéficient donc pas forcément aussi souvent d'une prise en charge périnatale adaptée (13).

Des études plus récentes ont retrouvé que la survie des HCD droites est équivalente à celles des HCD gauches (14–16) et certaines concluaient même que le côté droit de la hernie était un facteur de bon pronostic (17–20). Actuellement, les données sont contradictoires sur la survie des HCD droites.

Notre hypothèse était que le côté droit de la HCD n'est pas un facteur pronostique indépendant de survie. L'objectif de notre étude était d'évaluer si le côté de la HCD était un facteur pronostique de survie néonatale.

Matériel et méthodes

Il s'agit d'une étude de cohorte à partir de la base de données nationale française du du Centre de Référence des hernies diaphragmatiques (CRHD) (http://fr.ap-hm.fr/site/hernie-diaphrag). Le Centre de Référence regroupe les principaux centres pluridisciplinaires de diagnostic prénatal français. Les données sont enregistrées de façon prospective et comprennent des renseignements sur la survie et la morbidité des enfants à la naissance et pendant le suivi pédiatrique. L'enregistrement des cas est réalisé par l'intermédiaire d'un questionnaire sécurisé en ligne, renseigné par les obstétriciens, les pédiatres et les chirurgiens qui avaient la charge de chaque cas.

Cette étude a reçu l'accord du comité scientifique du centre national des maladies rares (références du comité de protection des personnes 14e044).

Critères d'inclusion et d'exclusion

Toutes les patientes enceintes dont le fœtus était porteur d'une HCD de Bochdaleck droite ou gauche, découverte en prénatal ou en postnatal immédiat et dont la naissance de l'enfant avait eu lieu entre le 1er janvier 2008 et le 31 décembre 2016 ont été inclues dans l'étude. La période de suivi de ces fœtus commençait à la date du diagnostic (qu'il soit effectué durant la période prénatale ou postnatale) et se poursuivait jusqu'à la date de sortie de l'hospitalisation en réanimation.

Nous avons exclu les fœtus porteurs d'une anomalie chromosomique et/ou d'un syndrome polymalformatif (que le diagnostic soit fait en prénatal ou en postnatal), les interruptions médicales de grossesse, les morts fœtales in utéro et les fœtus ayant bénéficié de la pose d'un ballonnet intra-trachéal in utero. Un syndrome polymalformatif était défini par la présence d'une autre anomalie majeure en plus de la HCD et nécessitant un traitement médical ou chirurgical immédiat.

Prise en charge

Le suivi de la hernie diaphragmatique en prénatal était réalisé dans une des unités de médecine fœtale référente. Une amniocentèse était proposée au couple pour rechercher une

anomalie du caryotype et d'autres malformations associées. Une analyse par CGH array n'était pas réalisée en routine lors de la période de l'étude. Un suivi échographique était mis en place avec une mesure du lung-to-head ratio (LHR) (21) et l'évaluation de la position du foie (22). L'accouchement avait lieu dans une maternité de niveau 3 et tous les enfants bénéficiaient du même protocole de prise en charge standardisé décrit par le centre de prise en charge des maladies rares (23,24). Les nouveau-nés étaient intubés en salle de naissance et étaient admis en réanimation néonatale. Un support ventilatoire était mis en place. Une ventilation haute fréquence était mise place si nécessaire, parfois de façon systématique selon les centres.

L'intervention chirurgicale (avec ou sans mise en place de prothèse) avait lieu dès que l'enfant était stable hémodynamiquement, le plus souvent dans les 48 premières heures de vie.

Données et variables

Le recueil des données comprenait des données maternelles, obstétricales, échographiques, radiologiques et néonatales. L'âge gestationnel était établi selon la mesure de la longueur cranio-caudale mesurée entre 11 et 14 semaines d'aménorrhée (SA) (25).

Le LHR était réalisée de façon systématique en cas d'HCD lors de l'échographie du deuxième trimestre, c'est-à-dire entre 22 et 24 SA (26). Cependant la mesure pouvait être effectuée plus tardivement si le diagnostic d'HCD était porté après le deuxième trimestre. Le observed on expected lung-to-head ratio (LHR o/e) était calculé en divisant le LHR observé sur le LHR attendu (21). Il n'était pas réalisé systématiquement d'IRM pour évaluer la présence de foie en position intra-thoracique.

Le critère principal de jugement était la survie à la sortie de l'hospitalisation en réanimation.

Les critères de jugement secondaires incluaient la durée de l'oxygénothérapie, l'utilisation de la ventilation par oscillations à haute fréquence (HFO), la présence de complications respiratoires post-opératoires et l'utilisation de l'extracorporeal membrane oxygenation (ECMO). Concernant les données chirurgicales, la mise en place d'une prothèse et la mise en place d'un silo ont été étudiées car ils constituent des marqueurs de sévérité (27).

Analyse statistique

Les données quantitatives sont exprimées en moyenne ± écart-type et les données qualitatives en effectifs et en pourcentage. Des analyses comparatives univariées entre les HCD droites et gauches ont été réalisées. Pour les variables qualitatives, un test de Chi2 était réalisé. Pour les variables quantitatives un test de Student était effectué après vérification de la normalité de la distribution des données et l'égalité des variances entre les deux groupes. Un test était considéré comme significatif lorsque son degré de signification, noté p, était inférieur au seuil de significativité $p<0,05$.

Dans un second temps, une analyse multivariée a été conduite par régression logistique sur le critère de jugement principal. Étaient entrées dans le modèle les variables associées au critère de jugement principal en analyse univariée avec un $p < 0.10$. Pour prendre en compte un potentiel effet centre nous avons aussi réalisé un ajustement sur la taille du centre défini par le nombre de cas d'HCD par an et sur l'année de prise en charge en forçant ces deux données dans le modèle d'entrée pour le critère de jugement principal. Ensuite, une analyse par pas à pas descendant a permis de ne garder que les variables significatives avec $p<0,05$. Concernant le côté de la hernie, la variable était forcée dans le modèle. Les résultats sont exprimés en odd ratio avec un intervalle de confiance à 95%. L'analyse des données a été réalisée en utilisant le logiciel RStudio® (Version 0.98.1091).

Résultats

Population

Au total 894 cas d'HCD ont été recensés dans la base de données du 1er janvier 2008 au 31 décembre 2016. Après application des critères d'exclusion, il restait un total de 525 HCD dont 70 HCD droites et 455 HCD gauches (figure 1) avec un taux de survie de 57% pour les HCD droites et 77% pour les HCD gauches ($p<0,01$).

Analyse univariée

Le tableau 1 rapporte l'analyse univariée des données maternelles, obstétricales et échographiques, en fonction du côté de la hernie. Le LHR o/e plus souvent inférieur à 45% dans les HCD droites par rapport aux gauches : 61 vs 16%, p<0,01. Le foie est plus souvent ascensionné dans les HCD droites que gauches : 52 vs 22%, p<0.01. On ne note pas de différence significative entre les deux groupes concernant le taux de diagnostic prénatal (84% pour les deux groupes, p = 1).

Le tableau 2 rapporte l'analyse univariée des données néonatales en fonction du côté de la hernie. Parmi les 535 HCD, 19 sont décédés en salle de naissance (5 droites et 14 gauches) et et 131 sont décédés en réanimation (25 droites et 93 gauches). Un total de 37 nouveaux-nés n'ont pas pu être opérés car décédés avant la possibilité d'une chirurgie. Finalement, 488 nouveau-nés ont bénéficié d'une prise en charge chirurgicale.

L'âge gestationnel à l'accouchement était significativement plus précoce dans les HCD droites que gauches (37,6 SA vs 38,7 SA, p <0,01), tout comme les poids de naissance (2,8kg vs 3,0kg, p<0,01). On ne retrouve pas de différence significative entre les deux groupes concernant la prise en charge médicale néonatale. Concnernant la prise en charge chirurgicale, le côté droit de la hernie était associé à une plus fréquente nécessité de la pose d'un silo, de la réduction du foie et de la pose d'un patch

Multivariate analysis

L'analyse multivariée des facteurs prénataux a été réalisée sur 247 cas d'HCD parmi les 441 cas diagnostiqués en prénatal, en raison de données manquantes concernant au moins une variable devant être entrée dans le modèle (Tableau 3). Après analyse par pas à pas descendante, on retrouve que seuls l'ascension du foie dans le thorax (OR : 2,27 ; IC 95% [1,07-4,76] ; p=0,03) et la mesure du LHR o/e (OR : 2,99 ; IC 95% [1,41-6,36] ; p<0,01) sont significativement associés au décès. Le côté de la hernie n'est pas un facteur significativement associé au décès en analyse multivariée (OR 1,87 ; IC 95% [0,61-5,51], p=0,26).

Discussion

Principaux résultats

Le taux global de survie de la hernie droite est inférieur à celui de la hernie gauche. Cela est vraisemblablement dû au fait que le o/e LHR est en moyenne inférieur en cas de hernie droite et que le foie est plus souvent ascensionné. Après ajustement sur ces facteurs, le côté de la hernie n'apparait pas comme un facteur prédictif indépendant de survie.

Forces et faiblesses

Cette étude présente le point fort d'être une analyse d'une importante série, issue d'un recueil de données national, incluant des données prénatales, néonatales et chirurgicales. Ce recueil de données s'intègre dans un plan national de soin avec une prise en charge standardisée par un protocole de soins national (23,24).

Cette base de données inclut la majorité des cas de hernies en France. En effet, sur une période de neuf ans avec une incidence d'HCD de 0,21 pour 1000 naissances vivantes on aurait dû obtenir autour de 1470 cas d'HCD (28) et nous en comptons 894 dans la base de données. En revanche, on déplore un taux élevé de données manquantes sur certaines variables, avec pour conséquence la limitation des effectifs utilisés pour l'analyse multivariée.

Enfin, notre période d'étude s'étend sur neuf ans et est multicentrique. Pour ne pas induire un biais lors de l'analyse multivariée, nous avons ajusté sur le temps et sur les centres..

Interprétation

Le taux de survie des HCD dans notre étude est de 55%, c'est-à-dire dans la fourchette basse des chiffres retrouvés dans la littérature, *i.e.* entre 55% et 79% (6,13). Cela s'explique par le fait que nous avons inclus toutes les hernies nées vivantes, y compris celles qui n'ont pas été diagnostiquées in utero et y compris celles qui n'ont pas pu avoir de prise en charge chirurgicale (31). À noter que le taux de survie des HCD droites prises en charge chirurgicalement dans notre étude est de 80%, ce qui est similaire à ce qui est retrouvé dans l'étude de Duess et al. (31).

Concernant le caractère pronostique ou pas du côté de la HCD, plusieurs études récentes se sont intéressées à cette problématique. Tout d'abord l'étude unicentrique de Duess et al. retrouvait une mortalité post-opératoire plus importante dans les HCD droites que dans les HCD gauches (21,9% vs 8,2% p=0,02). Cependant, cette étude s'est intéressée uniquement aux HCD opérées et il n'a pas eu d'analyse différentielle prenant en compte le caractère ascensionné ou non du foie, ni de la mesure du o/e LHR. L'étude de Fisher et al. retrouvait une survie inférieure des HCD droites par rapport aux gauches (55% vs 77%, p<0,01) mais, là encore, les données prénatales n'ont pas été analysées. Quatre études avec un design proche la nôtre ne retrouvaient pas de différence significative entre les HCD droites et gauches concernant mortalité à la sortie de l'hospitalisation (13,20,30,32). Un seule seulement de ces études n'a inclus une analyse avec ajustement sur le volume pulmonaire et la position du foie (20). Enfin, compte-tenu des faibles effectifs des HCD droites dans chacune des études, l'absence de différence de mortalité entre les HCD droites et gauches est potentiellement en lien avec un manque de puissance.

Concernant toujours le pronostic des hernies droites, il serait intéressant d'étudier d'autres critères que la mortalité : morbidité respiratoire, morbidité gastro-intestinale et morbidité neurologique à moyen et plus long terme. Enfin, la question de la prise en charge prénatale de la HCD est pertinente. Seule l'étude de DeKoning *et al.* s'est spécifiquement penchée sur cette problématique, en évaluant l'intérêt d'une occlusion trachéale fœtale par endoscopie sur 48 cas de HCD droite (4). La survie était plus importante en cas d'occlusion trachéale, mais sans que cette différence ne soit significative.

Notre étude retrouve un taux de détection prénatal global de 83% ce qui est cohérent avec les données de la littérature concernant des études multicentriques (13,30). De façon intéressante, nous n'avons pas retrouvé de différence de diagnostic prénatal en fonction du côté de la hernie, contrairement à certaines données publiées (11,13,32,33). Cela est intéressant à noter puisque la moins bonne détection prénatale des hernies droites est un des facteurs avancés pour expliquer un potentiel moins bon pronostic.

Concernant l'évaluation prénatale des HCD dans le cas de notre étude, la réalisation d'une IRM n'était pas systématique, ni pour évaluer le volume pulmonaire, ni pour évaluer la position du foie. Ces deux paramètres peuvent cependant être évalués en échographie.

L'utilisation du o/e LHR est une technique de référence pour l'évaluation pulmonaire (35,36), même si cette mesure est opérateur dépendant et n'est sans doute pas réalisée de façon aussi standardisée dans tous les établissements (37). Nous avons choisis le seuil de 45% pour nos analyses statistiques en se basant sur l'étude de DeKonink *et al.*(4), même si ce seuil n'est pas validé dans les HCD droites autant que dans les gauches.

L'utilisation de l'ECMO est réputée plus fréquente dans la prise en charge des HCD droites que dans les HCD gauches (32). Dans notre étude, l'utilisation de l'ECMO n'est pas significativement différente entre les deux groupes. Cependant dans notre étude, tout comme dans celle de Beaumier et al., le taux d'ECMO est très faible. Cela s'explique car sa pratique reste controversée devant une augmentation de la morbidité (20).

En ce qui concerne la prise en charge chirurgicale on retrouve significativement plus souvent l'utilisation d'une prothèse, d'un silo et d'un drain thoracique pour les HCD droites. Cela est probablement lié à un défect plus large du diaphragme dans les HCD droites par rapport aux HCD gauches. (13,38). Cela peut aussi expliquer le recours plus fréquent, dans notre étude, à une deuxième chirurgie dans les HCD droites (13).

Conclusion

Le côté de la hernie n'apparait pas comme un facteur prédictif indépendant de survie.

Figure 1 : Diagramme de flux

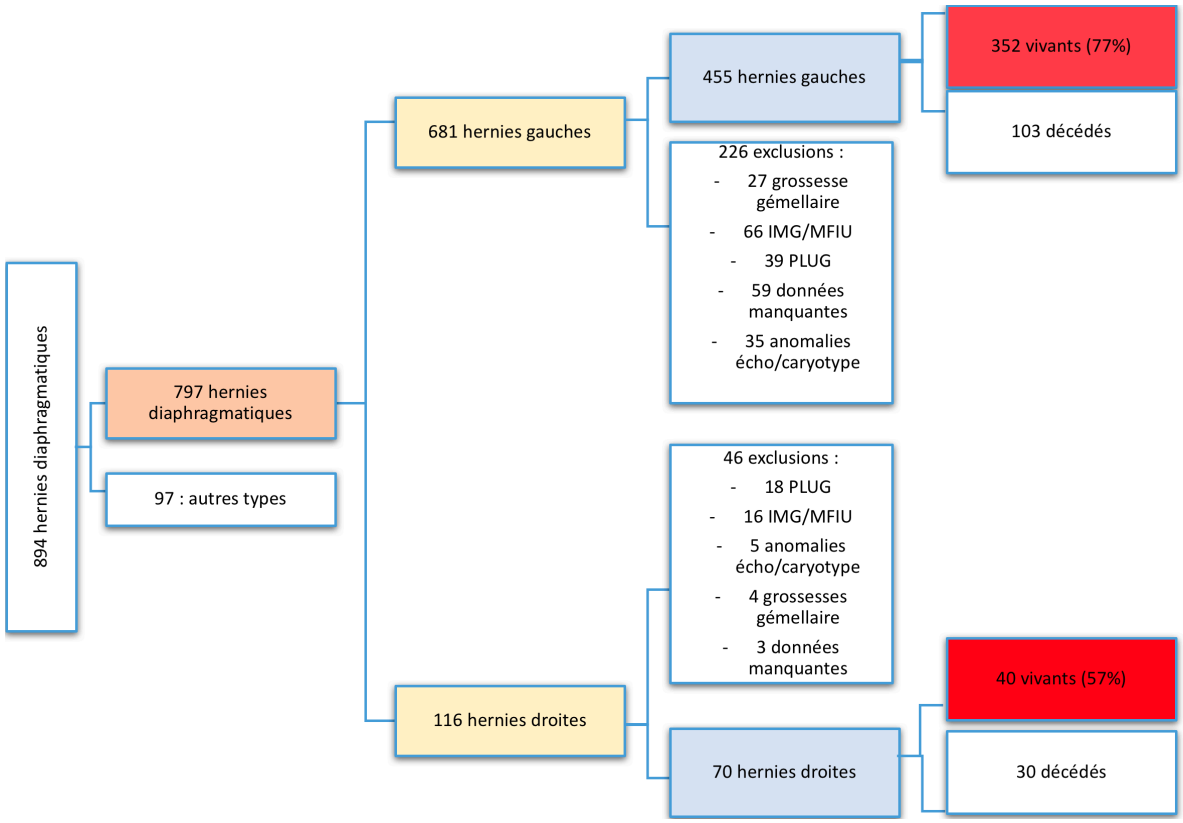


Tableau 1 : Analyse univariée des données maternelles et prénatales selon le côté de la hernie

	Hernies droites	**Hernies gauches**	**p**
Données maternelles et obstétricales	**N=70**	**N=455**	
Age de la patiente **- < 30 ans** **- ≥ 30 ans**	 59% 41%	 51% 48%	0,15
Parité **- Nulliparité** **- Multiparité**	 21% 79%	 22% 78%	0,89
Diagnostic prénatal **- Oui** - **Non**	 84% 16%	 84% 16%	1
Données échographiques	***N= 59***	***N=383***	
LHR o/e **- < 45 %** **- > 45 %**	 61% 39%	 16% 84%	<0,01
Foie ascensionné **- Oui** **- Non**	 52% 47%	 22% 78%	<0,01
Age gestationnel au diagnostic **- 1er trimestre** **- 2ème trimestre** **- 3ème trimestre**	 6% 68% 26%	 13% 61% 26%	0,33
Quantité de liquide amniotique **- Normale** **- Augmentée**	 14% 86%	 15% 85%	1

Tableau 2 : Analyses univariée des données néonatales selon le côté de la hernie

	HCD droites	HCD gauches	P
Données à la naissance	***N= 70***	***N=455***	
Mode d'accouchement **- Césarienne** **- Voie basse**	 36% 64%	 36% 64%	 1
Poids à la naissance (kg)	2,8 ± 0,8	3,0 ± 0,6	<0,01
Terme à l'accouchement (SA)	37,6 ± 3,5	38,7 ± 2,3	<0,01
pH à la naissance (mm Hg)	7,2 ± 0,2	7,2 ± 0,1	0,47
Ventilation immédiate - **Oui** - **Non**	 70% 30%	 71% 29%	 0,88
Moment intubation (min)	11,9 ± 36,0	21,6 ± 83,5	0,41
Données de la prise en charge respiratoire en réanimation	***N= 65***	***N=441***	
Utilisation de la ventilation par HFO - **Non** - **Oui** - **Systématique**	 40% 39% 21%	 49% 26% 25%	 0,09
Utilisation de l'ECMO - **Oui** - **Non**	 2% 98%	 5% 95%	 0,36
HTAP 48 heures - **Oui** - **Non**	 51% 49%	 47% 53%	 0,67
Oxygénothérapie 28 jours - **Oui** - **Non**	 31% 69%	 21% 79%	 0,13
Durée de la prise en charge en réanimation **- < 30 jours** **- ≥ 30 jours**	 71% 29%	 78% 22%	 0,21

Données sur la chirurgie	**N=46**	**N=442**	
Pose de prothèse			
- Oui	36%	21%	0,03
- Non	64%	79%	
Réduction du foie			
- Oui	39%	20%	0,03
- Non	64%	80%	
Mise en place d'un silo			
- Oui	20%	6%	<0,01
- Non	80%	94%	
Présence d'un sac herniaire			
- Oui	20%	18%	0,69
- Non	80%	82%	
Drain post-opératoire			
- Oui	56%	20%	<0,01
- Non	44%	80%	

Tableau 3 : Facteurs associés en analyse univariée et multivariée

	Hernies vivantes	Hernies décédées	p (univarié)	p (multivarié)
Mode d'accouchement				
- Césarienne	32%	47%	<0,01	NS
- Voie basse	67%	53%		
Age gestationnel à la naissance (SA)	38,9 ± 2,0	37,1 ± 3,4	<0,01	NS
Age gestationnel au diagnostic				
- 1er trimestre	9%	18%		
- 2ème trimestre	58%	71%	<0,01	NS
- 3ème trimestre	33%	11%		
LHR o/e				
- inf. 45%	24%	45%	<0,01	<0,01
- ≥ 45%	76%	55%		
Côté de la hernie				
- Gauche	90%	77%	<0,01	0,39
- Droit	10%	23%		
Foie ascensionné				
- Oui	22%	54%	<0,01	0,02
- Non	78%	45%		
Année de prise en charge				
-avant 2012	54%	55%	0,91	NS
- 2012 - 2016	46%	45%		
Lieu prise en charge				
- Moins de 5 hernies par an	17%	13%		
- Entre 6 – 15 hernies par an	51%	61%	0,09	NS
- Plus de 15 hernies par an	32%	26%		

8. AUTRES THÉMATIQUES DE RECHERCHE

8.1. Pathologies des grossesses monochoriales

8.1.1. Série de 200 lasers pour STT

J Gynecol Obstet Hum Reprod 46 (2017) 175–181

Disponible en ligne sur ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

Article original

Photocoagulation laser par fœtoscopie pour syndrome transfuseur-transfusé : analyse d'une série consécutive unicentrique de 200 cas

Fetoscopic laser coagulation in 200 consecutive monochorionic pregnancies with twin-twin transfusion syndrome

L. Lecointre [a,b,*], N. Sananès [a,b,c], A.S. Weingertner [a], A. Gaudineau [a,b], C. Akladios [b], V. Cavillon [a], B. Langer [b], R. Favre [a]

[a] *Pôle de gynécologie-obstétrique, département d'échographie et de médecine fœtale, CMCO-HUS, 19, rue Louis-Pasteur, 67300 Schiltigheim/Strasbourg, France*
[b] *Pôle de gynécologie-obstétrique, département d'obstétrique, hôpital de Hautepierre, hôpitaux universitaires de Strasbourg, 1, avenue Molière, 67098 Strasbourg cedex, France*
[c] *Inserm, UMR-S 1121, « Biomatériaux et Bioingénierie », 11, rue Humann, 67085 Strasbourg cedex, France*

INFO ARTICLE

Historique de l'article :
Reçu le 8 mai 2016
Reçu sous la forme révisée le 9 octobre 2016
Accepté le 10 octobre 2016
Disponible sur Internet le 30 janvier 2017

Mots clés :
Coagulation laser fœtoscopique
Syndrome transfuseur-transfusé
Grossesses monochoriales

RÉSUMÉ

But. – Rapporter les données préopératoires, caractéristiques chirurgicales, complications et le devenir périnatal des grossesses gémellaires compliquées de syndrome transfuseur-transfusé (STT) traitées par coagulation laser fœtoscopique. Analyser les facteurs prédictifs de survie néonatale et comparer les 100 cas les plus récents avec les 100 les plus anciens.
Matériels et méthodes. – Étude de cohorte observationnelle monocentrique de 200 grossesses gémellaires monochoriales biamniotiques compliquées de STT prises en charge entre janvier 2004 et décembre 2014 et traitées par coagulation laser fœtoscopique.
Résultats. – Parmi les 200 patientes traitées, on note 49 stades I, 88 stades II, 55 stades III et 8 stades IV. L'âge gestationnel moyen au laser était de 20,1 ± 3,0 semaines d'aménorrhée (SA) tandis que l'âge gestationnel à l'issue de grossesse était en moyenne de 31,6 SA ± 5,4. Le taux de survie néonatal global était de 68,0 % avec la survie d'au moins un enfant dans 84,0 % des cas. Une rupture prématurée des membranes est survenue dans 39 cas en moyenne à 28,8 SA ± 4,6. Les paramètres prédictifs d'avoir au moins un enfant vivant étaient le stade de Quintero et l'âge gestationnel à l'accouchement. Dans la période la plus récente, il y avait significativement plus de STT stade I de Quintero traités par laser, plus de coagulations selon Solomon et un nombre de vaisseaux coagulés plus important.
Conclusion. – La survie néonatale en cas de STT est améliorée par la photocoagulation laser fœtoscopique, idéalement selon une technique de Solomon. L'intérêt d'une prise en charge active des stades I est à l'étude.

Keywords:
Fetoscopic laser therapy
Twin-twin transfusion syndrome
Monochorionic twins

ABSTRACT

Objectives. – To report preoperative data, surgical characteristics, complications and perinatal outcome of twin-twin transfusion syndrome (TTTS) managed with laser ablation surgery, to analyze predictors of neonatal survival and to compare the 100 most recent cases with the older 100.
Materials and methods. – Observational cohort moncentric study of 200 cases of TTTS consecutively treated with fetoscopic laser coagulation between January 2004 and December 2014.

* Auteur correspondant.
Adresse e-mail : liselecointre@hotmail.fr (L. Lecointre).

http://dx.doi.org/10.1016/j.jogoh.2016.10.004
2468-7847/

Results. – There were 49 stage I, 88 stage II, 55 stage III and eight stage IV. Median gestation at time of laser was 20.1 ± 3.0 weeks' gestation (WG) whereas median gestation at delivery was 31.6 ± 5.4 WG. Overall perinatal survival rate was 68.0% and 84.0% have one or more surviving twins. Preterm premature rupture of membranes occurred in 39 cases with and the median gestational age for this complication was 28.8 ± 4.6 SA. Predictive factors to have at least one living birth were Quintero stage and gestational age at delivery. In the most recent period, there were significantly more TTTS Quintero stage I treated with laser, more coagulation by the Solomon technique and a larger number of coagulated vessels.
Conclusion. – The neonatal survival of TTTS is improved by fetoscopic laser coagulation, preferely by using Solomon tecnhique. The use of active management of stage I is currently on research.

Introduction

Le syndrome transfuseur-transfusé (STT) est une complication spécifique des grossesses multiples monochoriales ; sa prévalence est estimée entre 10 et 15 % [1]. Le diagnostic de STT peut être établi, au deuxième trimestre de la grossesse le plus souvent, par l'association d'un oligoamnios chez le donneur et d'un polyhydramnios chez le co-jumeau receveur [2,3]. La sévérité du STT est stadifiée selon la classification proposée par Quintero [4] (Tableau 1). Le pronostic global du STT est sévère en l'absence de traitement.

La coagulation laser fœtoscopique des anastomoses vasculaires s'est imposée comme le traitement de référence des STT. Plusieurs auteurs ont montré l'intérêt de la coagulation laser fœtoscopique par rapport à l'amniodrainage dans le traitement de cette complication [5–9]. L'essai clinique randomisé multicentrique Eurofoetus [5] a démontré la supériorité de la coagulation laser fœtoscopique avec un bénéfice significatif sur la survie d'au moins un jumeau (76 % vs 56 % ; $p < 0{,}05$) ainsi que sur l'âge gestationnel à l'accouchement et sur la morbidité neurologique à court et long terme [10]. Cependant, la mortalité néonatale reste non négligeable, tout comme le risque de lésions cérébrales sévères (3 à 16 % des cas) [5,11–13] et d'anomalies du développement neurologique (8 à 18 % des cas) [14]. À noter aussi que la coagulation laser fœtoscopique est associée à un risque de rupture prématurée des membranes.

En France, la prise en charge du STT est organisée autour d'un centre de référence parisien et de 11 centres de compétence régionaux. Le premier cas de photocoagulation laser à Strasbourg a eu lieu en 2004. Depuis, certaines indications sont discutées (prise en charge des stades précoces), la qualité des endoscopes s'est améliorée et les techniques ont évolué (bichorionisation complète).

L'objectif principal de cette étude est de rapporter les données préopératoires, les caractéristiques chirurgicales, les complications et le devenir périnatal des STT traités par laser dans notre centre. L'objectif secondaire est d'analyser les facteurs prédictifs de survie néonatale et de comparer les 100 cas les plus récents avec les 100 les plus anciens.

Matériel et méthodes

Il s'agit d'une étude de cohorte observationnelle portant sur des données recueillies de façon prospective entre janvier 2004 et décembre 2014. Les critères d'inclusion étaient les grossesses gémellaires monochoriales biamniotiques compliquées de STT et traitées par coagulation laser fœtoscopique au centre médico-chirurgical et obstétrical des hôpitaux universitaires de Strasbourg, Schiltigheim, France. Nous avons exclu les grossesses triples, les grossesses gémellaires compliquées de mort fœtale lors de la première échographie diagnostique, ou lorsqu'il existait des anomalies chromosomiques ou congénitales fœtales. Cette étude a été approuvée par la Commission nationale française de l'informatique et des libertés (CNIL) sous le numéro 1879796.

Tableau 1
Classification de Quintero.
The Quintero staging.

Stade 1	Poly/oligomanios avec vessie du donneur encore visible
Stade 2	Vessie du donneur non visible
Stade 3	Anomalies doppler avec flux diastolique nul chez le fœtus donneur ou *reverse flow* du Ductus Venosus chez le fœtus receveur ou flux veineux pulsatile
Stade 4	Anasarque d'un jumeau
Stade 5	Decès d'un jumeau

Le diagnostic de chorionicité était fait à l'échographie du premier trimestre par l'absence de signe du lambda. Le STT était diagnostiqué en échographie par l'association d'un oligoamnios (avec une plus grande citerne inférieure à 2 cm) chez le donneur et d'un polyhydramnios (avec une plus grande citerne supérieure à 8 cm si la grossesse est âgée de moins de 20 SA ou supérieure à 10 cm au-délà de 20 SA) chez le co-jumeau receveur [2,3]. La gravité du STT était évaluée selon la classification de Quintero [4]. Dans notre pratique, la coagulation laser fœtoscopique était systématiquement proposée pour tous les stades dits sévères de II à IV et pour les stades I en cas d'hydramnios massif du receveur et/ou de mauvaise tolérance maternelle de cet hydramnios.

La coagulation laser fœtoscopique était réalisée sous sédation maternelle par de l'Hypnovel® 5 mg et de l'Atarax® 100 mg per os une heure avant l'intervention. Une anesthésie locale cutanée et de la paroi utérine à la Xylocaine® 1 % était réalisée en début d'intervention. Le geste était réalisé à l'aide d'un fœtoscope Storz® 1,3 à 2 mm avec une optique droite ou inclinée à 30° au travers d'une chemise droite ou courbe selon la localisation placentaire. Par ailleurs, selon la taille du fœtoscope, des trocarts de huit à 12 frenchs étaient utilisés. La coagulation était réalisée à l'aide d'une fibre laser de 400 μm, d'une puissance maximale de 40 Watts, passée au travers de la chemise du fœtoscope. En début de geste, les anastomoses vasculaires en surface placentaires étaient identifiées, puis coagulées, après repérage de la membrane inter-amniotique. Une nouvelle cartographie en fin de geste permettait de contrôler que toutes les anastomoses étaient bien coagulées et ne se s'étaient pas reperméabilisées.

De 2003 à 2009, la méthode utilisée était la coagulation sélective pure des anastomoses vasculaires placentaires identifiées. De 2009 à 2012, les patientes étaient randomisées dans le cadre d'un essai randomisé pour la technique de photocoagulation : soit sélective pure décrite par Quintero et al. [15], soit la technique Solomon décrite par l'équipe de Lopriore et al. [16]. Depuis 2012, toutes les patientes bénéficient de la technique Solomon où tout l'équateur vasculaire est coagulé. Toute l'intervention était réalisée sous contrôle échographique. Une antibioprophylaxie par Clamoxyl® 1 g i.v. était administrée en peropératoire. En fin de geste, un amniodrainage était effectué afin de réduire la plus grande citerne du receveur à 5 cm ou moins. Aucune tocolyse systématique n'était

utilisée. Un traitement parentéral par antispasmodique était administré en cas de douleurs pelviennes. Un contrôle de l'évolution du STT, de la vitalité fœtale et des critères hémodynamiques en échographie et doppler étaient réalisés à 24 et 48 heures du geste. En cas d'évolution favorable, un contrôle à une semaine était programmé, puis le suivi classique tous les 15 jours. L'accouchement était programmé vers 36 SA.

Les caractéristiques cliniques des patientes, la classification des STT, les données de la coagulation laser fœtoscopique, les complications ainsi que les issues et le devenir néonataux ont été analysées. Les variables continues ont été représentées par leurs moyennes et la déviation standard, les variables catégorielles par l'effectif et le pourcentage. Les comparaisons en fonction de la survie néonatale (au moins un survivant) et de la période de traitement (100 premiers lasers contre les 100 suivants) ont été réalisées par un test exact de Fisher, un test du χ^2 ou un test *t* de Student, en fonction des effectifs et du type de variable. Des modèles de prédiction de la survie néonatale ont été développés en utilisant une régression logistique multivariée avec une sélection des variables selon une procédure « stepwise » (seuil d'entrée : $p = 0{,}10$; seuil de sortie : $p = 0{,}05$). Les variables qui expliquaient la survie (d'au moins un jumeau vivant ou des deux vivants) ont été exprimées par un *odds ratio* (OR) et l'intervalle de confiance (IC) à 95 %. Un degré de signification inférieur à 0,05 était considéré comme significatif. Le logiciel SAS version 9.3 (SAS Institute Inc., Cary, NC, États-Unis) a été utilisé pour toutes les analyses statistiques.

Résultats

Description de la population

La répartition d'après la classification de Quintero des 200 STT traités par laser dans notre centre était la suivante : 49 stades I (24,5 %), 88 stades II (44,0 %), 55 stades III (27,5 %) et 8 stades IV (4,0 %). L'âge gestationnel moyen au moment de la procédure était de 20,1 ± 3,0 semaines d'aménorrhée (SA), le nombre moyen d'anastomoses coagulées était de 10 ± 5 et une technique de coagulation sélective a été réalisée dans 140 cas (70,0 %), tandis que la technique Solomon a été pratiquée chez 60 patientes (30,0 %). L'ensemble des caractéristiques maternelles, chirurgicales, obstétricales et néonatales est reporté dans le Tableau 2.

Concernant les complications peropératoires fœtales, 2 hémorragies intra-ovulaires sont survenues au cours du geste. Aucune complication maternelle n'est survenue pendant le geste.

Par ailleurs, 10 gestes supplémentaires ont été réalisés, dont 8 pour une récidive de STT et 2 en raison de la survenue d'un TAPS iatrogène. Parmi ces dix coagulations laser fœtoscopiques itératives, 8 coagulations selon Solomon et 2 selon une technique sélective ont été réalisées.

Issues obstétricales et périnatales

Vingt-deux grossesses (11 %) se sont compliquées d'une fausse-couche avant 24 SA. Une rupture prématurée des membranes (RPM) est survenue dans 39 cas (19,5 %), en moyenne à 28,8 SA ± 4,6. Sept cas (3,5 %) de rupture prématurée des membranes sont survenus avant 24 SA, 9 cas entre 24 et 28 SA et 14 cas entre 28 et 32 SA. L'âge gestationnel à l'issue de grossesse était en moyenne de 31,6 SA ± 5,4, le poids moyen du donneur était de 1262 g ± 791 et celui du receveur de 1543 g ± 849. Sur les 400 enfants attendus, 272 étaient vivants après une semaine (68,0 %), parmi lesquels 132 donneurs (48,5 %) et 140 receveurs (51,5 %). Sur les 200 grossesses, il y avait 105 cas avec deux enfants survivants (52,5 %), 62 avec un enfant survivant (31,0 %) et 33 sans aucun survivant (16,5 %).

Tableau 2
Caractéristiques maternelles, chirurgicales, obstétricales et néonatales.
Maternal and obstetric characteristics, laser data, obstetric outcomes and live birth.

Caractéristiques maternelles et obstétricales	
Âge de la patiente (années)	29,9 ± 5,5
Parité	76 (38,0 %)
0	
≥ 1	124 (62,0 %)
Stade Quintero	
I	49 (24,5 %)
II	88 (44,0 %)
III	55 (27,5 %)
IV	8 (4,0 %)
Longueur cervicale (mm)	39,5 ± 11,3
Placenta antérieur	83 (41,5 %)
Caractéristiques chirurgicales	
Âge gestationnel au moment du laser (semaines)	20,1 ± 3,0
Endoscope utilisé	
1,3 mm	30 (15,0 %)
2 mm	170 (85,0 %)
Nombre de vaisseaux coagulés	10 ± 5
Technique	
Sélective	140 (70,0 %)
Solomon	60 (30,0 %)
Amniodrainage (mL)	1721 ± 1111
Durée du laser (min)	27,4 ± 10,5
Caractéristiques obstétricales et néonatales	
Rupture prématurée des membranes	39 (19,5 %)
Âge gestationnel à l'accouchement (semaines)	31,6 ± 5,4
Voie d'accouchement	
Voie basse	92 (46,0 %)
Césarienne	108 (54,0 %)
Poids (g)	
Donneur	1262 ± 791
Receveur	1543 ± 849
Survie	
Survie globale	272 (68,0 %)
Deux enfants survivants	105 (52,5 %)
Au moins un enfant survivant	168 (84,0 %)
Un enfant survivant	62 (31,0 %)
Aucun enfant survivant	33 (16,5 %)

Les données sont représentées par la moyenne et la déviation standard pour les variables continues et par l'effectif et le pourcentage pour les variables catégorielles.

Les taux de survie néonatale après laser en fonction du stade de Quintero sont illustrés sur la Fig. 1. Les nombres de cas avec 2 survivants étaient 32 (65,3 %), 44 (50,0 %), 27 (49,1 %) et 2 (25 %), pour les stades de Quintero I, II, III et IV. Les nombres de cas avec un survivant étaient respectivement 14 (28,6 %), 29 (33,0 %), 15 (27,3 %) et 4 (50 %), tandis que les nombres de cas sans aucun survivant étaient respectivement 3 (6,1 %), 15 (17,0 %), 13 (23,6 %) et 2 (25,0 %).

Les comparaisons des caractéristiques maternelles, chirurgicales, obstétricales et néonatales, en fonction de s'il y avait au moins un enfant survivant, sont rapportées dans le Tableau 3. En analyse multivariée, les paramètres prédictifs de la naissance d'au moins un enfant vivant étaient le stade de Quintero (OR 0,30 pour chaque incrément de stade, 95 % IC [0,10–0,86], $p = 0{,}02$) et l'âge gestationnel à l'accouchement (OR 2,2 pour chaque semaine de grossesse supplémentaire, 95 % IC [1,61–3,05], $p < 0{,}0001$). Les variables associées significativement à la survie des 2 jumeaux étaient de même le stade de Quintero (OR 0,69, 95 % IC [0,48–1,00], $p = 0{,}05$) et l'âge gestationnel à l'accouchement (OR 1,18, 95 % IC [1,10–1,26], $p < 0{,}0001$).

Concernant la morphologie fœtale, 7,5 % d'anomalies morphologiques ont été diagnostiquées chez les donneurs et 6,0 % chez les receveurs.

Parmi les anomalies morphologiques identifiées chez les donneurs, on comptait 6 (40,0 %) anomalies morphologiques vraies (microcéphalie, chylothorax, tétralogie de Fallot, communication inter-ventriculaire, hypoVG) et 9 (60 %) anomalies diagnostiquées après le laser et potentiellement imputables au

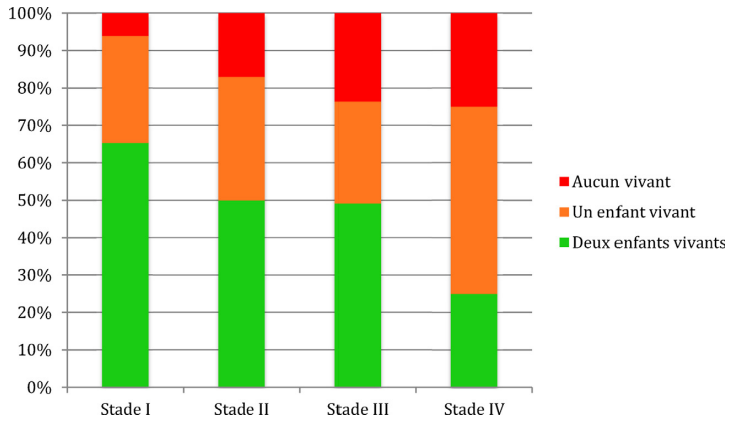


Fig. 1. Taux de survie néonatale après laser en fonction du stade de Quintero.
Neonatal survival after laser therapy according to TTTS stage.

STT et/ou à son traitement (porencéphalie, hémorragie intraventriculaire, dilatation ventriculaire droite, hypoVD, anasarque, maladie des brides amniotiques). Chez les receveurs, il y avait 2 (16,7 %) anomalies morphologiques vraies (une tétralogie de Fallot et une malformation adénomatoïde kystique du poumon type III) et 10 (83,3 %) anomalies potentiellement imputables au STT. Parmi ces dernières, deux cardiomyopathies dilatées étaient déjà présentes avant le laser, les autres malformations (porencéphalie, hémorragie intraventriculaire, sténose pulmonaire, hypoVD et thrombose fémorale gauche) ayant été diagnostiquées après le laser.

Comparaisons des 100 premières procédures avec les 100 suivantes

Les comparaisons des caractéristiques maternelles, chirurgicales, obstétricales et néonatales, entre les 100 premiers cas de laser et les 100 cas suivants, sont présentées dans le Tableau 4. Il y avait significativement plus de STT stade I de Quintero traités par lasers

Tableau 3
Comparaisons des caractéristiques maternelles, chirurgicales, obstétricales et néonatales, en fonction de la survie.
Comparisons of maternal, surgical, obstetric and neonatal characteristics, according to survival.

	Au moins un enfant vivant	Aucun enfant vivant	*p*
Caractéristiques maternelles et obstétricales			
Âge de la patiente (années)	30,2 ± 5,4	28,7 ± 6,2	0,22
Parité			
0	60 (78,9 %)	16 (21,1 %)	0,18
≥ 1	107 (86,3 %)	17 (13,7 %)	
Stade Quintero			
I	46 (93,9 %)	3 (6,1 %)	0,01
II	73 (83,0 %)	15 (17,0 %)	
III	42 (76,4 %)	13 (23,6 %)	
IV	6 (75,0 %)	2 (25,0 %)	
Longueur cervicale (mm)	40,5 ± 10,4	34,6 ± 14,1	0,006
Placenta antérieur			
Oui	62 (74,7 %)	21 (25,3 %)	0,005
Non	105 (89,7 %)	12 (10,3 %)	
Caractéristiques chirurgicales			
Âge gestationnel au moment du laser (semaines)	20,3 ± 3,0	19,3 ± 2,9	0,07
Endoscope utilisé			
1,3 mm	25 (83,3 %)	5 (16,7 %)	0,91
2 mm	142 (83,5 %)	28 (16,5 %)	
Nombre de vaisseaux coagulés	9,7 ± 4,5	10,7 ± 4,6	0,26
Technique			
Sélective	116 (82,9 %)	24 (17,1 %)	0,50
Solomon	51 (85,0 %)	9 (15,0 %)	
Amniodrainage (mL)	1728 ± 1128	1687 ± 1035	0,84
Durée du laser (min)	27,3 ± 10,2	27,9 ± 12,1	0,78
Caractéristiques obstétricales et néonatales			
RPM			
Oui	32 (82,1 %)	7 (17,9 %)	0,71
Non	135 (83,8 %)	26 (16,2 %)	
Âge gestationnel à l'accouchement (semaines)	33,5 ± 3,4	22,4 ± 3,9	< 0,0001
Voie d'accouchement			
Voie basse	62 (67,4 %)	30 (32,6 %)	< 0,0001
Césarienne	105 (97,2 %)	3 (2,8 %)	

Les données sont représentées par la moyenne et la déviation standard pour les variables continues et par l'effectif et le pourcentage pour les variables catégorielles.

Tableau 4
Comparaisons des caractéristiques maternelles, chirurgicales, obstétricales et néonatales, entre les 100 premiers cas et les 100 suivants.
Comparisons of maternal, surgical, obstetric and neonatal characteristics, between the first 100 cases and the 100 following.

	100 premiers lasers	100 derniers lasers	*p*
Caractéristiques maternelles et obstétricales			
Âge de la patiente (années)	29,4 ± 5,8	30,5 ± 5,2	0,19
Parité			
0	43 (56,7 %)	33 (43,4 %)	0,15
≥ 1	57 (46,0 %)	67 (54,0 %)	
Stade Quintero			
I	10 (20,4 %)	39 (79,6 %)	< 0,0001
II	48 (54,6 %)	40 (45,4 %)	
III	38 (69,1 %)	17 (30,1 %)	
IV	4 (50,0 %)	4 (50,0 %)	
Longueur cervicale (mm)	37,9 ± 9,6	41,1 ± 12,6	0,05
Placenta antérieur			
Oui	45 (54,2 %)	38 (45,8 %)	0,32
Non	55 (47,0 %)	62 (53,0 %)	
Caractéristiques chirurgicales			
Âge gestationnel au moment du laser (semaines)	20,5 ± 3,1	19,8 ± 2,8	0,08
Endoscope utilisé	13 (43,3 %)	17 (56,7 %)	0,43
1,3 mm	87 (51,2 %)	83 (48,8 %)	
2 mm			
Nombre de vaisseaux coagulés	9,1 ± 4,3	10,6 ± 4,6	0,02
Technique			
Sélective	98 (70,0 %)	42(30,0 %)	< 0,0001
Solomon	2 (3,3 %)	58 (96,7 %)	
Amniodrainage (mL)	2009 ± 1199	1432 ± 937	0,0002
Durée du laser (min)	26,8 ± 9,9	28,0 ± 11,1	0,41
Caractéristiques obstétricales et néonatales			
RPM			
Oui	16 (40,0 %)	24 (60,0 %)	0,15
Non	84 (52,8 %)	75 (47,2 %)	
Âge gestationnel à l'accouchement (semaines)	31,8 ± 5,3	31,5 ± 5,5	0,66
Voie d'accouchement			
Voie basse	44 (47,8 %)	48 (52,2 %)	0,57
Césarienne	56 (51,9 %)	52 (48,1 %)	
Survie			
Deux enfants survivants	51 (48,1 %)	55 (51,9 %)	0,57
Au moins un enfant survivant	85 (50,6 %)	83 (49,4 %)	0,70
Un enfant survivant	34 (54,8 %)	28 (45,2 %)	0,36
Aucun enfant survivant	15 (46,9 %)	17 (53,1 %)	0,70

Les données sont représentées par la moyenne et la déviation standard pour les variables continues et par l'effectif et le pourcentage pour les variables catégorielles.

pendant la deuxième période que pendant la première : 39 (79,6 %) contre 10 (20,4 %), $p < 0,0001$. Dans la période la plus récente, il y avait plus de coagulations selon Solomon que selon une technique sélective (58 [97,2 %] contre 2 [3,3 %], $p < 0,0001$) et le nombre de vaisseaux coagulés était plus important (10,6 ± 4,6 contre 9,1 ± 4,3, $p = 0,02$). Les taux de survie sont comparables, quel que soit le critère retenu.

Discussion

Nous rapportons, le devenir de 200 grossesses gémellaires monochoriales biamniotiques compliquées de STT pris en charge par fœtoscopie avec coagulation des anastomoses vasculaires placentaires au laser, au CHU de Strasbourg. Les facteurs prédictifs de survie néonatale étaient le stade de Quintero et l'âge gestationnel à l'accouchement. Dans la deuxième période de l'étude, les STT étaient pris en charge volontiers plus précocement et la technique de coagulation Solomon était prépondérante.

Notre expérience montre que dans 84,0 %, la coagulation laser permet la survie d'au moins un des deux jumeaux avec un taux de survie néonatal global de 68,0 % et une survie des deux jumeaux dans 52,5 %. Ces résultats sont concordants avec ceux de la littérature. Dans une étude rétrospective récente portant sur 340 grossesses, Peeters et al. rapportent une survie néonatale d'au moins un jumeau dans 86,0 % des cas et des deux jumeaux dans 59,0 % des cas [17]. Dans notre étude, les facteurs prédictifs de survie néonatale étaient le stade de Quintero et l'âge gestationnel à l'accouchement. D'autres séries ont mis en évidence d'autres facteurs pronostics tels que la localisation placentaire, la longueur du col et la fonction cardiaque du receveur [18–20].

Dans notre série, nous avons relevé les anomalies cérébrales fœtales et néonatales mais n'avons pas étudié le pronostic neurodéveloppemental à long terme. Il est important de noter que des lésions cérébrales sont diagnostiquées en anténatal chez 5 à 14 % des jumeaux après une coagulation laser fœtoscopique [11,21]. Les deux jumeaux sont à risque de lésions neurologiques quel que soit leur statut anténatal de donneur ou de receveur. Parmi les 128 grossesses incluses dans l'essai Eurofœtus entre 1999 et 2002, 120 enfants étaient survivants à l'âge de six mois, ont pu être suivis jusqu'à l'âge de six ans et avaient des scores de développement neurologique meilleurs en cas de traitement par laser qu'en cas d'amniodrainage itératif [10]. Ces résultats sont concordants avec ceux décrit par Spruijt et al. rapportant un risque de lésions cérébrales graves dans les STT traités par laser similaire à celui d'un groupe témoin de jumeaux dichorioniques appariés sur l'âge gestationnel à la naissance et ceux de Rossi et al. retrouvant seulement 11,1 % d'atteinte neurologique dans les STT traités par laser qui se manifeste essentiellement pendant la petite enfance [22,23]. Ces résultats imposent, de ce fait, l'instauration d'un suivi pédiatrique rigoureux au long cours.

Nous n'avons pas mis en évidence de différence significative entre les deux techniques de coagulation (sélective ou Solomon) pour ce qui est du taux de survie (82,9 % versus 85,0 %, $p = 0,50$). Ces résultats sont similaires à ceux décrits dans l'essai contrôlé

randomisé récent de Slaghekke et al. portant sur 274 grossesses dans lequel ils comparent les taux de survie selon la technique de coagulation sélectionnée : technique Solomon versus technique conventionnelle. Ces derniers rapportent une survie néonatale d'au moins un jumeau dans 85,0 % versus 87,0 % des cas (OR 0,85, IC 95 % 0,43–1,68), des deux jumeaux dans 64,0 % versus 60,0 % des cas (OR 1,16, IC 95 % 0,71–1,89), pour une survie globale de 74,0 % versus 73,0 % (OR 1,04, IC 95 % 0,66–1,63) [24]. L'intérêt de la bichorionisation complète du placenta est avant tout de diminuer significativement le risque de Twin Anemia Polycythemia Sequence (TAPS) post-laser (3,0 % versus 16,0 % pour la technique conventionnelle ; OR 0,16, IC 95 % 0,05–0,49) ainsi que la récidive de STT (1,0 % versus 7,0 % ; OR 0,21, IC 95 % 0,04–0,98) [24]. Seuls Ruano et al. retrouvaient que l'utilisation de la technique de Solomon améliorait la survie néonatale [20]. Concernant la morbidité néonatale, en particulier neurologique, Slaghekke et al. n'ont pas retrouvé un bénéfice de la technique Solomon, probablement en raison d'un manque de puissance.

La réalisation d'un geste thérapeutique dans les STT de stade I est controversé, en raison de leur évolution naturelle souvent satisfaisante. Dans notre expérience, une coagulation laser fœtoscopique était réalisée en cas de stade I avec un hydramnios majeur et/ou une mauvaise tolérance maternelle secondaire à cet hydramnios. Dans notre étude, 24,5 % des procédures ont en effet étaient réalisées en cas de stade I de Quintero avec une survie néonatale d'au moins un jumeau dans 93,9 % des cas. D'après les données de la littérature, il semblerait qu'environ 70,0 % des stades I pourraient évoluer favorablement avec 28,0 % de stabilité et 41,0 % de régression spontanée et seraient associés à un bon devenir périnatal [25]. Néanmoins, l'évolution des stades I reste peu connue et certains peuvent évoluer vers des formes plus sévères de moins bon pronostic et nécessitant un traitement par laser [26,27]. Dans une revue de la littérature publiée en 2013, Rossi et al. rapportent un taux de survie néonatale global comparable en cas de traitement conservateur et de coagulation laser fœtoscopique (86,0 % et 85,0 %), avec un taux de progression en stade plus avancé de 15,0 % en cas de prise en charge conservatrice alors qu'aucun cas de progression n'a été diagnostiqué dans le groupe laser [26]. À noter que ce travail est critiquable de part l'hétérogénéité des études qui le composent mais aussi et surtout le potentiel biais lié au design rétrospectif. D'après une étude récente du réseau américain de chirurgie fœtale, 53 % des STT stades 1 s'aggravent en l'absence de geste thérapeutique et le fait de réaliser une coagulation laser permet globalement d'améliorer le pronostic [28]. Une étude française coordonnée par l'équipe de Y. Villes et évaluant l'intérêt de la prise en charge des stades I est actuellement en cours (ClinicalTrials.gov Identifier : NCT01220011).

Par ailleurs, il faut noter les limites de la classification de Quintero pour les stades précoces puisqu'elle ne prend en compte les anomalies doppler qu'à partir du stade III. Or, même en cas de stade précoce, il existe un risque de cardiopathie spécifique pour le receveur et la prise en compte de paramètres cardiovasculaires pourraient être intéressante. Le score de Children's Hospital of Philadelphia (CHOP) et le Myocardial Performance Index (MPI) constitueraient en effet des facteurs prédictifs de perte de fœtale dans les stades précoces [29–31].

Dans notre expérience, les résultats en termes de survie sont comparables entre les 100 cas les plus récents et les 100 plus anciens. Ceci suggère, que l'expérience n'améliore pas significativement ces résultats, comme cela avait été évoqué dans une revue systématique de la littérature [32]. Cette constatation doit cependant être pondérée par le critère d'analyse qui est retenu, à savoir la survie néonatale ou la morbidité à plus long terme telle que le développement neurologique. Van Klink et al., en comparant deux cohortes de patientes de 2000 à 2005 puis de 2008 à 2010, ont retrouvé une augmentation du taux de survie de 70,0 à 80,0 % et une diminution des anomalies neurologiques de 18,0 à 6,0 %. Ils expliquent leurs résultats par l'amélioration des connaissances sur les complications potentielles des STT, une courbe d'apprentissage optimale et l'utilisation de la technique Solomon depuis 2008 [33,34].

Conclusion

La survie néonatale en cas de STT est améliorée par la photocoagulation laser fœtoscopique, idéalement selon une technique de Solomon. L'intérêt d'une prise en charge active des stades I est à l'étude. La morbidité neurodéveloppementale à long terme ne doit pas être négligée.

Déclaration de liens d'intérêts

Les auteurs déclarent ne pas avoir de liens d'intérêts.

Références

[1] Fisk NM, Duncombe GJ, Sullivan MHF. The basic and clinical science of twin-twin transfusion syndrome. Placenta 2009;30:379–90.
[2] van Mieghem T, Baud D, Devlieger R, Lewi L, Ryan G, De Catte L, et al. Minimally invasive fetal therapy. Best Pract Res Clin Obstet Gynaecol 2012;26:711–25.
[3] Gratacós E, Deprest J. Current experience with fetoscopy and the Eurofoetus registry for fetoscopic procedures. Eur J Obstet Gynecol Reprod Biol 2000; 92:151–9.
[4] Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. J Perinatol 1999;19:550–5.
[5] Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351:136–44.
[6] Middeldorp JM, Lopriore E, Sueters M, Klumper FJCM, Kanhai HHH, Vandenbussche FPHA, et al. Twin-to-twin transfusion syndrome after 26 weeks of gestation: is there a role for fetoscopic laser surgery? BJOG Int J Obstet Gynaecol 2007;114:694–8.
[7] Robyr R, Quarello E, Ville Y. Management of fetofetal transfusion syndrome. Prenat Diagn 2005;25:786–95.
[8] Moise KJ, Dorman K, Lamvu G, Saade GR, Fisk NM, Dickinson JE, et al. A randomized trial of amnioreduction versus septostomy in the treatment of twin-twin transfusion syndrome. Am J Obstet Gynecol 2005;193:701–7.
[9] De Lia JE, Kuhlmann RS, Harstad TW, Cruikshank DP. Fetoscopic laser ablation of placental vessels in severe previable twin-twin transfusion syndrome. Am J Obstet Gynecol 1995;172:1202–8 [discussion 1208–11].
[10] Salomon LJ, Ortqvist L, Aegerter P, Bussieres L, Staracci S, Stirnemann JJ, et al. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2010;203. 444. e1–7.
[11] Lopriore E, van Wezel-Meijler G, Middeldorp JM, Sueters M, Vandenbussche FP, Walther FJ. Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. Am J Obstet Gynecol 2006;194:1215–20.
[12] Lenclen R, Paupe A, Ciarlo G, Couderc S, Castela F, Ortqvist L, et al. Neonatal outcome in preterm monochorionic twins with twin-to-twin transfusion syndrome after intrauterine treatment with amnioreduction or fetoscopic laser surgery: comparison with dichorionic twins. Am J Obstet Gynecol 2007;196. 450.e1–7.
[13] Cincotta RB, Gray PH, Gardener G, Soong B, Chan FY. Selective fetoscopic laser ablation in 100 consecutive pregnancies with severe twin-twin transfusion syndrome. Aust N Z J Obstet Gynaecol 2009;49:22–7.
[14] Van Klink JMM, Koopman HM, Oepkes D, Walther FJ, Lopriore E. Long-term neurodevelopmental outcome in monochorionic twins after fetal therapy. Early Hum Dev 2011;87:601–6.
[15] Quintero RA, Comas C, Bornick PW, Allen MH, Kruger M. Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2000;16:230–6.
[16] Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;201. 66.e1–4.
[17] Peeters SHP, Van Zwet EW, Oepkes D, Lopriore E, Klumper FJ, Middeldorp JM. Learning curve for fetoscopic laser surgery using cumulative sum analysis. Acta Obstet Gynecol Scand 2014;93:705–11.
[18] Robyr R, Boulvain M, Lewi L, Huber A, Hecher K, Deprest J, et al. Cervical length as a prognostic factor for preterm delivery in twin-to-twin transfusion syndrome treated by fetoscopic laser coagulation of chorionic plate anastomoses. Ultrasound Obstet Gynecol 2005;25:37–41.

[19] Gapp-Born E, Sananes N, Weingertner AS, Guerra F, Kohler M, Fritz G, et al. Predictive value of cardiovascular parameters in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2014;44:427–33.
[20] Ruano R, Rodo C, Peiro JL, Shamshirsaz AA, Haeri S, Nomura ML, et al. Fetoscopic laser ablation of placental anastomoses in twin-twin transfusion syndrome using "Solomon technique". Ultrasound Obstet Gynecol 2013;42:434–9.
[21] Quarello E, Molho M, Ville Y. Incidence, mechanisms, and patterns of fetal cerebral lesions in twin-to-twin transfusion syndrome. J Matern-Fetal Neonatal Med 2007;20:589–97.
[22] Spruijt M, Steggerda S, Rath M, van Zwet E, Oepkes D, Walther F, et al. Cerebral injury in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstet Gynecol 2012;120:15–20.
[23] Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstet Gynecol 2011;118:1145–50.
[24] Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, et al. Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet Lond Engl 2014;383:2144–51.
[25] O'Donoghue K, Cartwright E, Galea P, Fisk NM. Stage I twin-twin transfusion syndrome: rates of progression and regression in relation to outcome. Ultrasound Obstet Gynecol 2007;30:958–64.
[26] Rossi AC, D'Addario V. Survival outcomes of twin-twin transfusion syndrome stage I: a systematic review of literature. Am J Perinatol 2013;30:5–10.
[27] Wagner MM, Lopriore E, Klumper FJ, Oepkes D, Vandenbussche FPHA, Middeldorp JM. Short- and long-term outcome in stage 1 twin-to-twin transfusion syndrome treated with laser surgery compared with conservative management. Am J Obstet Gynecol 2009;201. 286.e1–6.
[28] Emery SPHS, Catov JM, Miller RS, Moon-Grady AJ. The North American Fetal Therapy Network: intervention versus expected management for stage I twin-twin transfusion syndrome. Am J Obstet Gynecol 2016;S38.
[29] Rychik J, Tian Z, Bebbington M, Xu F, McCann M, Mann S, et al. The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease. Am J Obstet Gynecol 2007;197. 392.e1–8.
[30] Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function – a study in normals and dilated cardiomyopathy. J Cardiol 1995;26:357–66.
[31] Gapp-Born E, Sananes N, Guerra F, Kohler M, Weingertner AS, Fritz G, et al. Predictive value of cardiovascular parameters in stages 1 and 2 of twin-to-twin transfusion syndrome. Prenat Diagn 2014;34:908–14.
[32] Ahmed S, Luks FI, O'Brien BM, Muratore CS, Carr SR. Influence of experience, case load, and stage distribution on outcome of endoscopic laser surgery for TTTS – a review. Prenat Diagn 2010;30:314–9.
[33] van Klink JMM, Koopman HM, van Zwet EW, Middeldorp JM, Walther FJ, Oepkes D, et al. Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. Am J Obstet Gynecol 2014;210. 540.e1–7.
[34] Papanna R, Biau DJ, Mann LK, Johnson A, Moise KJ. Use of the learning curve-cumulative summation test for quantitative and individualized assessment of competency of a surgical procedure in obstetrics and gynecology: fetoscopic laser ablation as a model. Am J Obstet Gynecol 2011;204. 218.e1–9.

8.1.2. Pronostic neurologique après laser pour STT

Original article

Evaluation of long-term neurodevelopment in twin-twin transfusion syndrome after laser therapy

Running head: Neurodevelopment in twin-twin transfusion syndrome after laser

Word count: 2 229; Table count: 4; Figure count: 1

Authors: Nicolas Sananès [1,2], Victor Gabriele [1], Anne Sophie Weingertner[1], Rodrigo Ruano [3], Magdalena Sanz-Cortes [3], Adrien Gaudineau [1], Bruno Langer [1], Israël Nisand [1], Chérif Youssef Akladios [1], and Romain Favre [1]

Institutions:

[1] Department of obstetrics and gynecology, Strasbourg University Hospital, France

[2] INSERM, UMR-S 1121, "Biomatériaux et Bioingénierie", Strasbourg, France

[3] Texas Children's Fetal Center and Baylor College of Medicine, Houston, Texas, USA

Corresponding author: Nicolas Sananès

Service de gynécologie obstétrique,

Hôpitaux Universitaires de Strasbourg, 67091 Strasbourg cedex, France.

Tel: +33 3 88 12 78 39

E-mail: nicolas.sananes@chru-strasbourg.fr

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.4950

Funding source: none.

Conflict of interest: none

What's already none about this topic?

Laser surgery improves neonatal survival and neurological outcomes in case of twin-twin transfusion syndrome (TTTS). There are only few studies on neurologic morbidity after laser, especially with a long-term follow-up setting.

What does this study add?

This study shows that laser surgery in case of TTTS is associated with 13.5% of abnormal Ages and Stages Questionnaires at 3.6 years. It also suggests that a birth weight below the fifth percentile might be associated with neurodevelopmental abnormalities.

ABSTRACT

Objective: The primary objective of our study was to evaluate the long-term neurodevelopment outcome after laser surgery for twin-twin transfusion syndrome (TTTS). The secondary objective was to identify perinatal prognostic factors associated with neurodevelopmental impairment.

Method: This was a single-center cohort prospective study carried out in pregnancies complicated by TTTS and treated by laser. Neurodevleopmental assesment included the administration of Ages and Stages Questionnaires® (ASQ), for the infants between two and five years of age.

Results: A total of 187 patients underwent a laser for TTTS between 2004 and 2013. Significant brain lesions were detected in 8 (2.9%) cases by ultrasound and/or MRI including intraventricular hemorrhage, periventricular leukomalacia and porencephaly. Questionnaires were administered to 126 children (50.4%) at 24 months or older at the moment of testing. There were 13.5% of those infants who had an abnormal ASQ (established as 1 area or more scoring<2SD) at 3.6 years +/- 1.3 follow-up. There was a higher rate of abnormal ASQ among the infants with a birth weight below the fifth percentile (p=0.036).

Conclusion: TTTS is associated with a risk of abnormal neurological development, even in case of laser surgery. Further studies are necessary to identify the risk factors for neurological impairment.

Key words: Twin-twin transfusion syndrome, laser therapy, fetal surgery, prognostic factors, Ages and Stages Questionnaires (ASQ), neurodevelopmental outcome.

INTRODUCTION

Twin-twin transfusion syndrome (TTTS) occurs in 10 to 15% of monochorionic pregnancies and results from an unbalanced blood flow between the donor and the recipient twin [1, 2]. Fetoscopic laser photocoagulation of vascular anastomoses improves both neonatal survival and neurological outcomes [3, 4]. Even so, there is still a risk of severe cerebral injury and neurodevelopmental impairment in cases of TTTS treated with laser surgery [5].

There are only few studies on neurologic morbidity after laser for TTTS, and even fewer with a long-term follow-up, whereas neurodevelopmental impairments may become obvious only several years after birth [6, 7]. Moreover, there is a paucity of data concerning perinatal factors to predict poor neurologic outcome, as pointed out by a recent meta-analysis [5]. Nevertheless, this assessment is crucial for appropriate parents counseling and children follow-up.

The primary objective of our study was to evaluate the neurodevelopment outcome after laser surgery for TTTS for 24 months to 5 years. The secondary objective was to identify perinatal prognostic factors associated with neurodevelopmental outcome.

METHODS

Study design and setting

This was a single-center prospective cohort study carried out in monochorionic diamniotic twin pregnancies complicated by TTTS and treated by laser at the Centre Médico-Chirurgical et Obstétrical of the Strasbourg University Hospitals, France. Recruitment period was from January 2004 to July 2013, whereas follow-up of children ended in July 2016. All data were collected prospectively using the computerized medical dossier system DIAMM® (Micro6®). All the patients gave their informed consent and this study was approved by the French Data Protection Authority (CNIL) under number 1837195.

Participants and methods of follow-up

All consecutive cases of TTTS treated by laser were included. Our hospital is a referral center for the diagnosis and the treatment for TTTS in the east of France. Chorionicity was assessed by first trimester ultrasound and monochorionic placentation was established by absence of the lambda sign at that scan. TTTS was diagnosed on echography by the association of an oligohydramnios (largest cisternal pocket less than 2 cm) in the donor and polyhydramnios (deepest vertical pocket more than 8 cm if the pregnancy was less than 20 weeks or greater than 10 cm if more than 20 weeks) in the recipient co-twin [8, 9]. The severity of TTTS was graded using Quintero's classification [10]. Fetoscopic laser coagulation was routinely offered for stages II to IV, and in stage I only in the event of massive polyhydramnios and/or poor maternal tolerance (uterine contractions, abdominal pain and breathing discomfort).

Laser surgery was performed by two experienced operators, according to a technique previously described [11]. Photocoagulation was achieved according to either a pure selective technique as described by Quintero *et al.* [12], or the Solomon technique described by Lopriore *et al*. [13] in which the entire vascular equator is coagulated. From 2004 to 2008, pure selective technique was performed. Between 2008 and 2012, selective technique or Solomon technique were equally used since our center was participating in the Solomon trial. After 2012, every laser was performed according to Solomon technique. After the surgery, the patients were subjected to weekly and then bi-monthly ultrasound monitoring.

Neurologic examination of neonates was systematically performed at birth. Prenatal or postnatal cerebral imaging by ultrasound and/or MRI was offered only in case of suspected abnormal prenatal findings and/or abnormal neurologic examination after birth. Further neurodevelopmental assessment was performed with the Ages and Stages Questionnaires® (ASQ) test in their French version. Questionnaires were systematically mailed to parents for assessment of their children (or child) every year until the age of five years. These questionnaires have been validated against the Bayley Scales of Infant Development as a screening tool for abnormal neurologic development at 24 months of age [14, 15]. It has been previously already used for developmental follow-up of live-born twins after laser for TTTS [16, 17]. This parent-completed child-monitoring system is a series of 20 questionnaires that correspond to age intervals from birth to 6 years. Each questionnaire assesses five areas of child development, which are considered abnormal if the score is below 2 standard deviations lower than the mean.

Variables

The primary outcome measurement was an abnormal ASQ score, which was defined by at least one abnormal area. Questionnaires were administered from 24 months after birth so no age adjustment was needed. If several questionnaires were completed for one child, then only the latest one was taken into account for the analysis. Infants presenting with an abnormal test were informed about it and were counseled to contact their general pediatrician for further assessment.

Clinical features of the patients, TTTS classification, fetoscopic laser coagulation data, complications and neonatal outcomes were collected and analyzed. Fetal loss was defined by either intra-uterine fetal death or miscarriage. Neonatal death was defined by the death of the neonate within 1 month after birth. Gestational age at delivery and birth weight were analyzed as both continuous and categorical variables according to validated thresholds [18-20].

Statistical methods

Descriptive data were presented as mean and standard deviation for continuous variables and as numbers and percentages for categorical variables. Comparisons for normally distributed continuous variables were made using Student's *t*-tests and for categorical variables using χ^2-tests and Fisher exact-tests. Univariate analyses were performed in order to compare children with follow-up versus those with not, and to identify parameters associated with an abnormal ASQ score. A p value < 0.05 was considered as statistically significant. SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA) was used for all data analyses.

RESULTS

Population

A total of 187 patients underwent laser coagulation for TTTS during the inclusion period: 42 (22.4%) pregnancies were classified as Quintero stage I, 80 (42.8%) as stage II, 57 (30.5%) as stage III, and 8 (4.3%) as stage IV. The descriptive data of the population are given in table 1. Among the 374 fetuses, there were 103 (27.5%) fetal losses and 21 (7.7%) neonatal deaths. The survival rates after laser were as follow: 94/187 (50.3%) with two survivors, 62/187 (33.1%) with one survivor, 31/187 (16.6%) with no survivor, *i.e.* 250/374 (66.8%) survivors. Parents of a total of 124 (49.6%) children did not complete the ASQ after 2 years. Conversely, 126 (50.4%) children among the 250 alive at one month of age had an ASQ completed. A flow-chart of the patients selection is illustrated in figure 1.

The parents of 126 (50.4%) children completed the questionnaires. There were slightly more Quintero stage I in the group lost to follow-up and more Quintero stage III in the group of assessed children (p=0.004). Otherwise, there was no statistical significant difference between the assessed population and those lost to follow-up, regarding fetal intervention, obstetrical, and neonatal characteristics, as shown in table 2.

Ultrasound and MRI findings

There were 8 (2.9%) cases that were diagnosed with significant brain lesions as detected by ultrasound and/or MRI including intraventricular hemorrhage, periventricular leukomalacia and porencephaly. In all the 3 cases diagnosed prenatally, termination of pregnancy was requested by parents and performed as allowed by the

French law, after patients counseling and authorization of the maternal fetal medicine board. Among the 5 cases diagnosed after birth, all of them were born prematurely (before 30 weeks of gestation) and 4 died within the first month of life. Only one survived and he's now 3-years old with a moderate cerebral palsy (able to walk with the assistance of aids).

Results of the Ages and Stages Questionnaires

The repartition of ages for filling ASQ was as follows: 49 (38.9%) at 2 years of age, 29 (23.0%) at 4 years of age, and 48 (38.1%) at 5 years of age. The mean age at the moment of testing was 3.6 years +/- 1.3, with 61.1% of the ASQ filled for 4 and 5 year-old children. Results of the questionnaires are reported in table 3. A total of 17 (13.5%) children had an abnormal ASQ. There was 1 abnormal area in 12 (9.5%) children and 2 or more abnormal areas in 5 (4.0%) cases. Rates of abnormality for each area were as follow: 4.0% for communication, 5.6% for gross motor, 4.0% for fine motor, 5.6% for problem solving, 3.2% for personal and social skills. There was no specific field of neurodevelopment in abnormalities, neither differences between donors and recipients.

Table 4 reports the comparisons of TTTS features and outcomes in the group with abnormal ASQ versus the controls. There were significantly more cases with abnormal ASQ among the Quintero stage I than the other stages (p=0.021). There was a higher rate of abnormal ASQ among the neonates with a birth weight below the fifth percentile (p=0.036). No other parameter was significantly associated with an abnormal ASQ, especially there was no significant difference regarding the donor or recipient status, nor the gestational age at birth

DISCUSSION

Main findings

There were 13.5% of the infants treated by laser for TTTS who had an abnormal ASQ (defined as 1 or more areas < 2SD) at 3.6 years +/- 1.3 follow-up. In our study a birth weight below the fifth percentile was associated with abnormal neurodevelopment.

Strengths and weaknesses

This study reports long-term neurological follow-up of a large series of infants who were treated by laser for TTTS. The follow-up of at least 2 years allows avoiding the correction based on prematurity. Limitations of this study include the lack of a control group in order to better interpret the findings in that cohort of high-risk pregnancies. Ages and Stages Questionnaires are easily administered and have been validated against the Bayley Scales of Infant Development as a screening tool for abnormal neurologic development [14, 15]. However, since ASQ are filled out by the parents, child capabilities might have been under- or over-estimated. Unfortunately, we do not have the data regarding the neurological examination of the children, even those who had an abnormal ASQ. Also, ASQ is a screening test that may assess some important neurodevelopmental capacities but may not detect some subtle developmental deficits. Moreover, although we followed up this cohort until 5 years of age, there may be some developmental deficits that may not be detected until later on in the infant's life once they start becoming more academically challenged at school age. It should be kept in mind that some important factors such as the infant's environment represented by parental educational level or socioeconomic status has not been taken in consideration in this study, and this could constitute a source of potential bias. Another limitation of

this study is the high rate of lost-to-follow-up but, except regarding the Quintero stage, there was no statistical difference between the children assessed and the ones who were lost-to-follow-up. Because, of the missing data regarding a subgroup of patients with a higher proportion of Quintero stage 1, *i.e.* who may have a better outcome, rate of abnormal ASQ might be over-estimated.

Interpretation

The incidence of neurological abnormalities in TTTS survivors after laser therapy ranges between 6 and 25% in the literature [16, 17, 21-26]. These differences are partly due to the definition used, and the type and time for assessment. It has been also suggested that the incidence of neurological impairment reduces over time, with more and more experienced operators [27]. Our study was not sufficiently powered to conduct such analyses. It has been suggested that some technical improvements, such has the Solomon technique, might improve neurological morbidity. However, survival without neurodevelopmental impairment between Solomon and standard laser techniques, so as neurodevelopmental impairment in long-term survivors, are not statistically different according to the 2-year outcomes from the "Solomon trial" [28]. We did not show either any difference in ASQ depending on the laser technique used. To be noted at last that in our series we report 5 cases of termination of pregnancy in cases of high-risk of neurological impairment (brain lesions diagnosed by fetal MRI and/or demise of the co-twin). According to a recent study, prenatal brain damage after laser for TTTS, as assessed by third trimester-MRI, occurs in 2% of cases and is associated with incomplete surgery [29].

Identifying risk factors for abnormal neurodevelopment is of interest for appropriate parental counseling and guided developmental monitoring throughout

childhood. In our series, Quintero stage I was associated with a higher rate of abnormal ASQ than more severe stages. This may be biased because we offered laser surgery in Quintero stage I TTTS only in the event of massive polyhydramnios. Moreover, stage I TTTS is not a single condition since recipient can present a myocardial dysfunction and the donor may have an absent urine production [30,31]. A large multicenter study showed that Quintero staging was significantly associated with neurodevelopmental impairments in univariate analysis and an important trend for each increment in stage (OR 2.9, 95%CI 0.9-9.2) [24]. We found that a birth weight below the fifth percentile was associated with a higher frequency of abnormal neurolodevelopmental scores. We did not find any significant association between gestational age at birth and abnormal ASQ but this may be due to a lack of power. Indeed, the multicenter study from Lopriore *et al.* showed that in multivariable analysis, lower gestational age at birth was the only factor independently associated with neurodevelopmental impairment (OR 1.3 for each week, 95%CI 1.1-1.7) [24].

Conclusion

Laser surgery for TTTS is associated with 13.5% of abnormal Ages and Stages Questionnaires at 3.6 years. In our series, infants born with a birth weight below the fifth percentile were more to present an abnormal neurodevelopment. Further studies are necessary to identify the risk factors for neurological impairment.

REFERENCES

1. Fisk NM, Duncombe GJ, Sullivan MH. The basic and clinical science of twin-twin transfusion syndrome. Placenta 2009; 30:379-90.
2. Lewi L, Gucciardo L, Van Mieghem T, *et al.* Monochorionic diamniotic twin pregnancies: natural history and risk stratification. Fetal diagnosis and therapy 2010; 27:121-33.
3. Rossi AC, D'Addario V. Laser therapy and serial amnioreduction as treatment for twin-twin transfusion syndrome: a metaanalysis and review of literature. American journal of obstetrics and gynecology 2008; 198:147-52.
4. Senat MV, Deprest J, Boulvain M, *et al.* Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. The New England journal of medicine 2004; 351:136-44.
5. Rossi AC, Vanderbilt D, Chmait RH. Neurodevelopmental outcomes after laser therapy for twin-twin transfusion syndrome: a systematic review and meta-analysis. Obstetrics and gynecology 2011; 118:1145-50.
6. Escobar GJ, Littenberg B, Petitti DB. Outcome among surviving very low birthweight infants: a meta-analysis. Archives of disease in childhood 1991; 66:204-11.
7. Ornstein M, Ohlsson A, Edmonds J, *et al.* Neonatal follow-up of very low birthweight/extremely low birthweight infants to school age: a critical overview. Acta paediatrica Scandinavica 1991; 80:741-8.
8. Gratacos E, Deprest J. Current experience with fetoscopy and the Eurofoetus registry for fetoscopic procedures. European journal of obstetrics, gynecology, and reproductive biology 2000; 92:151-9.
9. van Mieghem T, Baud D, Devlieger R, *et al.* Minimally invasive fetal therapy. Best practice & research Clinical obstetrics & gynaecology 2012; 26:711-25.
10. Quintero RA, Morales WJ, Allen MH, *et al.* Staging of twin-twin transfusion syndrome. Journal of perinatology : official journal of the California Perinatal Association 1999; 19:550-5.
11. Lecointre L, Sananes N, Weingertner AS, *et al.* Fetoscopic laser coagulation for twin-twin transfusion syndrome before 17 weeks' gestation: laser data, complications and neonatal outcome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2014; 44:299-303.
12. Quintero RA, Comas C, Bornick PW, *et al.* Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2000; 16:230-6.
13. Lopriore E, Slaghekke F, Middeldorp JM, *et al.* Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. American journal of obstetrics and gynecology 2009; 201:66 e1-4.
14. Squires J, Bricker D, Potter L. Revision of a parent-completed development screening tool: Ages and Stages Questionnaires. Journal of pediatric psychology 1997; 22:313-28.
15. Gollenber AL, Lynch CD, Jackson LW, *et al.* Concurrent validity of the parent-completed Ages and Stages Questionnaires, 2nd Ed. With the Bayley Scales of Infant Development II in a low-risk sample. Child Care Health Dev 2010;36(4):485-90.

16. Salomon LJ, Ortqvist L, Aegerter P, *et al.* Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. American journal of obstetrics and gynecology 2010; 203:444 e1-7.
17. Tosello B, Blanc J, Haumonte JB, *et al.* Short and medium-term outcomes of live-born twins after fetoscopic laser therapy for twin-twin transfusion syndrome. Journal of perinatal medicine 2014; 42:99-105.
18. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet 2008; 371:261-9.
19. Ego A. [Definitions: small for gestational age and intrauterine growth retardation]. Journal de gynecologie, obstetrique et biologie de la reproduction 2013; 42:872-94.
20. Leroy B, Lefort F, Neveu P, *et al.* Intrauterine growth charts for twin fetuses. Acta geneticae medicae et gemellologiae 1982; 31:199-206.
21. Banek CS, Hecher K, Hackeloer BJ, *et al.* Long-term neurodevelopmental outcome after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology 2003; 188:876-80.
22. Graef C, Ellenrieder B, Hecher K, *et al.* Long-term neurodevelopmental outcome of 167 children after intrauterine laser treatment for severe twin-twin transfusion syndrome. American journal of obstetrics and gynecology 2006; 194:303-8.
23. Lopriore E, Middeldorp JM, Sueters M, *et al.* Long-term neurodevelopmental outcome in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology 2007; 196:231 e1-4.
24. Lopriore E, Ortibus E, Acosta-Rojas R, *et al.* Risk factors for neurodevelopment impairment in twin-twin transfusion syndrome treated with fetoscopic laser surgery. Obstetrics and gynecology 2009; 113:361-6.
25. Lopriore E, van Wezel-Meijler G, Middeldorp JM, *et al.* Incidence, origin, and character of cerebral injury in twin-to-twin transfusion syndrome treated with fetoscopic laser surgery. American journal of obstetrics and gynecology 2006; 194:1215-20.
26. Sutcliffe AG, Sebire NJ, Pigott AJ, *et al.* Outcome for children born after in utero laser ablation therapy for severe twin-to-twin transfusion syndrome. BJOG : an international journal of obstetrics and gynaecology 2001; 108:1246-50.
27. van Klink JM, Koopman HM, van Zwet EW, *et al.* Improvement in neurodevelopmental outcome in survivors of twin-twin transfusion syndrome treated with laser surgery. American journal of obstetrics and gynecology 2014; 210:540 e1-7.
28. van Klink JM, Slaghekke F, Balestriero MA, *et al.* Neurodevelopmental outcome at 2 years in twin-twin transfusion syndrome survivors randomized for the Solomon trial. American journal of obstetrics and gynecology 2016; 214:113 e1-7.
29. Stirnemann J, Chalouhi G, Essaoui M, *et al.* Fetal brain imaging following laser surgery in twin-to-twin surgery. BJOG : an international journal of obstetrics and gynaecology 2016 Jun 27. doi: 10.1111/1471-0528.14162. [Epub ahead of print]
30. Gapp-Born E, Sananes N, Guerra F, *et al.* Predictive value of cardiovascular parameters in stages 1 and 2 of twin-to-twin transfusion syndrome. Prenatal diagnosis 2014; 34:908-14.
31. Yamamoto M, Essaoui M, Nasr B, *et al.* Three-dimensional sonographic assessment of fetal urine production before and after laser surgery in twin-to-twin transfusion syndrome. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2007; 30:972-6.

Table 1: Baseline characteristics of patients and pregnancies

Characteristics			**n**
Patient age (years)		29.9 +/- 5.5	187
Parity	0	71 (38.0%)	187
	≥ 1	116 (62.0%)	187
Quintero stage	I	42 (22.4%)	187
	II	80 (42.8%)	187
	III	57 (30.5%)	187
	IV	8 (4.3%)	187
Anterior placenta		80 (42.8%)	187
Gestational age at laser (weeks)		20.3 +/- 2.9	187
Technique	Selective	136 (72.7%)	187
	Solomon	51 (27.3%)	187
Fetal loss of one twin		49 (26.2%)	187
Fetal loss of both twins		27 (14.4%)	187
PPROM < 34 weeks		35 (18.7%)	187
Gestational age at birth (weeks)		33.0 +/- 3.5	271
Gestational age at birth < 28 weeks		24	271
Gestational age at birth < 32 weeks		69	271
Gestational age at birth < 34 weeks		46	271
Gestational age at birth ≥ 34 weeks		132	271
Birth weight (g)		1666 +/- 693	271
Birth weight ≤ 5th percentile		36 (13.3%)	271
Neonatal death		21 (7.7%)	271
Overall survival		250 (66.8%)	374

Data are presented as mean and standard deviation for continuous variables and as numbers and percentages for categorical variables.

PPROM: preterm premature rupture of membranes

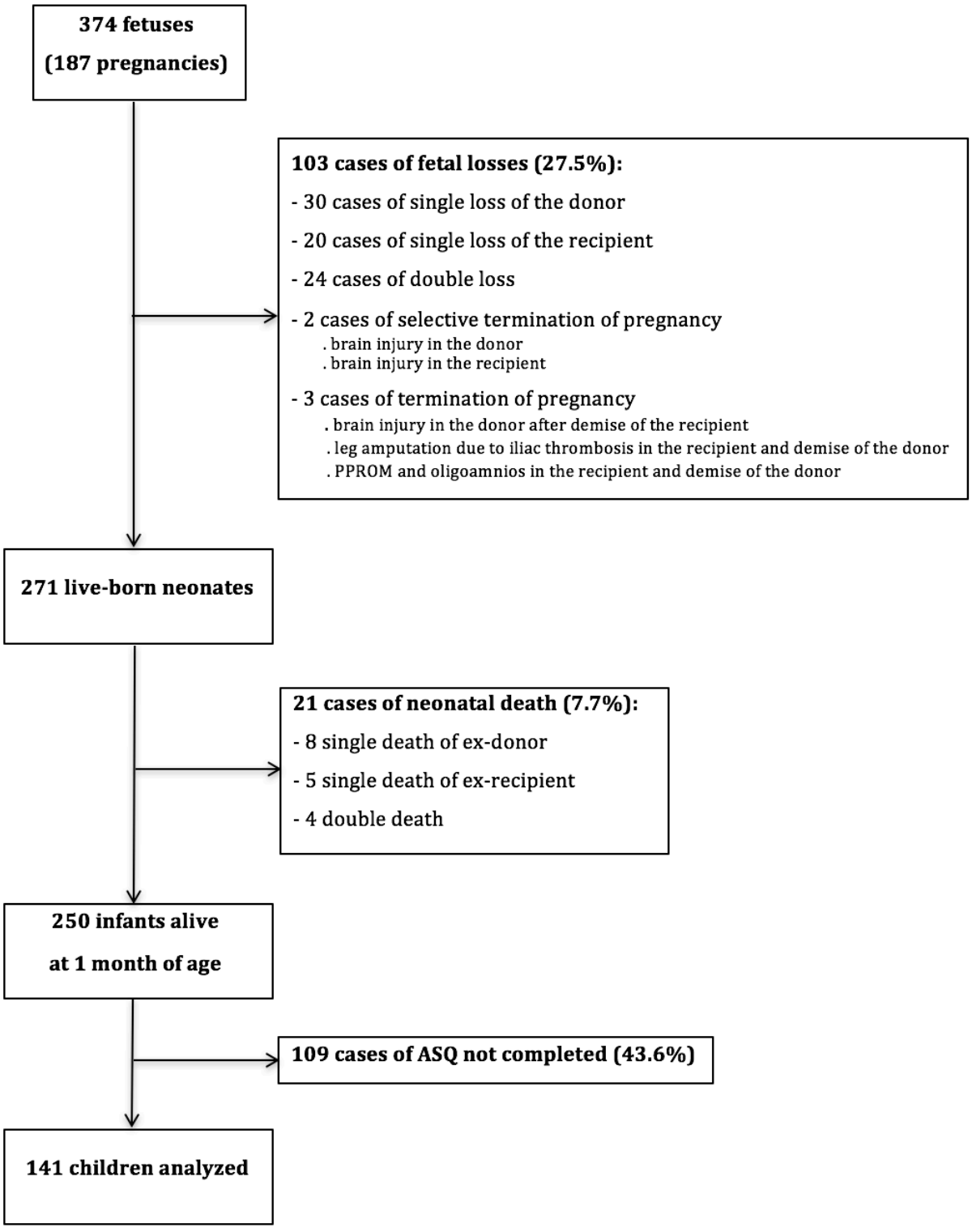

374 fetuses
(187 pregnancies)
103 cases of fetal losses (27.5%):
- 30 cases of single loss of the donor
- 20 cases of single loss of the recipient
- 24 cases of double loss
- 2 cases of selective termination of pregnancy
. brain injury in the donor
. brain injury in the recipient
- 3 cases of termination of pregnancy
. brain injury in the donor after demise of the recipient
. leg amputation due to iliac thrombosis in the recipient and demise of the donor
. PPROM and oligoamnios in the recipient and demise of the donor
271 live-born neonates
21 cases of neonatal death (7.7%):
- 8 single death of ex-donor
- 5 single death of ex-recipient
- 4 double death
250 infants alive
at 1 month of age
109 cases of ASQ not completed (43.6%)
141 children analyzed

Table 2: Comparison of children with follow-up versus those without ASQ filled.

Characteristics		**ASQ filled n = 126**	**ASQ not filled n = 124**	**p-value**
Quintero stage	I	23 (36.5%)	40 (63.5%)	0.004
	II	55 (50.5%)	54 (49.5%)	
	III	43 (61.4%)	27 (38.6%)	
	IV	5 (62.5%)	3 (37.5%)	
Anterior placenta		48 (51.6%)	45 (48.4%)	0.768
Gestational age at laser (weeks)		20.1 +/- 2.9	20.7 +/- 3.1	0.101
Technique	Selective	97 (54.2%)	82 (45.8%)	0.057
	Solomon	29 (40.8%)	42 (59.2%)	
Co-twin fetal loss		30 (60%)	20 (40%)	0.174
Premature rupture of membranes		25 (56.8%)	19 (43.2%)	0.486
Gestational age at birth (weeks)		33.6 +/- 3.4	33.3 +/- 3.1	0.485
Gestational age at birth < 28 weeks		7 (53.8%)	6 (46.2%)	0.799
Gestational age at birth < 32 weeks		36 (51.4%)	34 (48.6%)	0.840
Gestational age at birth < 34 weeks		56 (48.3%)	60 (51.7%)	0.532
Gestational age at birth ≥ 34 weeks		70 (52.2%)	64 (47.8%)	
Birth weight (g)		1791 +/-630	1803 +/-602	0.879
Birth weight ≤ 5th percentile		39 (60.9%)	25 (39.1%)	0.051

Data are presented as mean and standard deviation for continuous variables and as numbers and percentages for categorical variables.

Table 3: Results of the ASQ from our cohort

ASQ assessment	n = 126	Donor n = 60	Recipient n = 66
Abnormal ASQ	17 (13.5%)	9 (15.0%)	8 (12.1%)
1 abnormal area	12 (9.5%)	5 (8.3%)	7 (10.6%)
2 abnormal areas	1 (0.8%)	0 (0%)	1 (1.5%)
3 abnormal areas	2 (1.6%)	2 (3.3%)	0 (0%)
4 abnormal areas	2 (1.6%)	2 (3.3%)	0 (0%)
5 abnormal areas	0 (0%)	0 (0%)	0 (0%)
Communication < 2SD	5 (4.0%)	3 (5.0%)	2 (3.0%)
Gross motor < 2SD	7 (5.6%)	4 (6.7%)	3 (4.5%)
Fine motor < 2SD	5 (4.0%)	4 (6.7%)	1 (1.5%)
Problem solving < 2SD	7 (5.6%)	5 (8.3%)	2 (3.0%)
Personal and social skills < 2SD	4 (3.2%)	3 (5.0%)	1 (1.5%)

Table 4: Comparison of the TTTS features and outcomes in the group with abnormal ASQ versus the controls.

Variables		**Abnormal ASQ (n=17)**	**Normal ASQ (n=109)**	**p**
Quintero stage	I	8 (47.1%)	15 (13.8%)	0.021
	II	5 (29.4%)	50 (45.9%)	
	III	3 (17.6%)	40 (36.7%)	
	IV	1 (5.9%)	4 (3.6%)	
Anterior placenta	Yes	5 (29.4%)	43 (39.4%)	0.430
	No	12 (70.6%)	66 (60.6%)	
GA at laser (weeks)		21.2 ± 2.2	19.9 ± 3.0	0.103
Technique	Selective	15 (88.2%)	82 (75.2%)	0.356
	Solomon	2 (11.8%)	27 (24.8%)	
Donor		9 (52.9%)	51 (46.8%)	0.638
Recipient		8 (47.1%)	58 (53.2%)	
Co-twin fetal loss	Yes	3 (17.6%)	27 (24.8%)	0.761
	No	14 (82.4%)	82 (75.2%)	
PPROM < 34 weeks	Yes	1 (5.9%%)	24 (22.0%)	0.122
	No	16 (94.1%)	85 (78.0%)	
GA at birth (weeks)		32.2 ± 3.6	33.7 ± 3.4	0.650
GA at birth < 28 weeks		2 (11.8%)	5 (4.6%)	0.239
GA at birth < 32 weeks		5 (29.4%)	31 (28.4%)	0.999
GA at birth < 34 weeks		6 (35.3%)	50 (45.9%)	0.416
Birth weight (g)		1586 ± 606	1823 ± 631	0.150
Birth weight ≤ 5th perc		9 (52.9%)	30 (27.5%)	0.036

GA: gestational age; perc: percentile; PPROM: preterm premature rupture of membranes

Ultrasound Obstet Gynecol 2014; **44**: 427–433
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.13351

Predictive value of cardiovascular parameters in twin-to-twin transfusion syndrome

E. GAPP-BORN*, N. SANANES*†‡, A. S. WEINGERTNER*, F. GUERRA*, M. KOHLER*, G. FRITZ*, B. VIVILLE*, A. GAUDINEAU*, B. LANGER*, E. SAULEAU†, I. NISAND* and R. FAVRE*

**Department of Obstetrics and Gynaecology, Strasbourg University Teaching Hospital, Strasbourg, France; †Department of Biostatistics, University Teaching Hospital, Strasbourg, France; ‡INSERM, UMR-S 1121, 'Biomatériaux et Bioingénierie', Strasbourg, France*

KEYWORDS: CHOP score; MPI; prognostic factors; Quintero classification; Tei index; twin-to-twin transfusion syndrome

ABSTRACT

Objective *To evaluate the prognostic value of the Children's Hospital Of Philadelphia (CHOP) cardiovascular score and the modified myocardial performance index (MPI), in determining the risk of recipient fetal loss in twin-to-twin transfusion syndrome (TTTS).*

Methods *This cohort study was based on data collected prospectively from 105 pregnancies complicated by TTTS (Quintero stages I–IV) and treated with laser photocoagulation between May 2008 and February 2013. Fetuses underwent detailed anatomical and Doppler ultrasonography with cardiac assessment as part of routine care. CHOP score and right MPI were calculated and cut-offs selected using receiver–operating characteristics curve analysis. These were compared according to loss of recipient fetus, using univariate and multivariate logistic regression. The correlation between CHOP score, MPI and Quintero stage was determined and we investigated differences in MPI before and after laser coagulation in a cohort of 90 recipient fetuses.*

Results *Rates of recipient fetal loss were significantly higher when the CHOP score was ≥ 3 (39.5% vs 12.9%, P = 0.002) and when MPI z-score was > 1.645 (34.5% vs 10.6%, P = 0.004). After adjustment for Quintero stage, the risk of recipient fetal loss remained significantly higher when the CHOP score was ≥ 3 (odds ratio, 3.09; 95% CI, 1.035–9.21). There was a positive correlation between CHOP score, MPI and Quintero stage. MPI was significantly lower after compared with before laser coagulation.*

Conclusion *CHOP score and MPI are predictors of recipient fetal loss in TTTS and may be used to supplement Quintero's classification.*

INTRODUCTION

Twin–twin transfusion syndrome (TTTS) complicates 10% of monochorionic diamniotic twin pregnancies[1–3]. It accounts for 12% of perinatal mortality and 17% of perinatal morbidity in twin pregnancy[3–5]. Fetoscopy-guided laser photocoagulation of placental anastomoses significantly improves survival and is currently the preferred treatment[6]. It is essential to have reliable prognostic factors in order to be able to identify, from among cases of TTTS, those pregnancies most likely to benefit from laser treatment.

Quintero's classification is the most widely used system for categorizing TTTS[7]. However, it appears to have significant shortcomings and its prognostic value is contentious[6,8], especially because it does not include early-stage cardiovascular parameters; fetal cardiovascular modifications may occur in the early stages of the disease, in particular in the recipient fetus[8–12], which can develop specific cardiomyopathy due to the increased post-load and transplacental anastomotic passage of angiotensin (produced by the donor), giving rise to poorer myocardial compliance[12,13].

Other factors have been developed to evaluate fetal cardiac function in TTTS[11,14–19]. The Children's Hospital Of Philadelphia (CHOP) cardiovascular score comprises 12 parameters (Table 1) and is expressed as a score ranging from 0 to 20, with a higher score indicating poorer cardiac function[11]. The myocardial performance index (MPI) evaluates global, systolic and diastolic myocardial function[20] and has the advantage of being independent of ventricular geometry and fetal heart rate[13,20]. MPI increases with ventricular dysfunction[17]. Its major advantage is that it allows straightforward non-invasive assessment of right heart function. This is crucial[11,17], since the right ventricle is responsible for the

Correspondence to: Dr E. Gapp-Born, Department of Obstetrics and Gynaecology, Strasbourg University Teaching Hospital, 67091 Strasbourg cedex, France (e-mail: elodie.gapp@free.fr)

Accepted: 18 February 2014

Table 1 Cardiovascular parameters used in the Children's Hospital of Philadelphia (CHOP) score

Variable/Parameter	*Finding*	*Score*
Donor		
Umbilical artery	Normal	0
	Decreased diastolic blood flow	1
	Absent/reversed diastolic blood flow	2
Recipient		
Ventricular hypertrophy	None	0
	Present	1
Cardiac dilation	None	0
	Mild	1
	> mild	2
Ventricular dysfunction	None	0
	Mild	1
	> mild	2
Tricuspid valve regurgitation	None	0
	Mild	1
	> mild	2
Mitral valve regurgitation	None	0
	Mild	1
	> mild	2
Tricuspid valve inflow	Double-peak	0
	Single-peak	1
Mitral valve inflow	Double-peak	0
	Single-peak	1
Ductus venosus	Antegrade flow	0
	Absent diastolic blood flow	1
	Reversed diastolic blood flow	2
Umbilical vein	No pulsations	0
	Pulsations	1
Right-sided outflow tract	Pulmonary artery > aorta	0
	Pulmonary artery = aorta	1
	Pulmonary artery < aorta	2
	Right ventricular outflow obstruction	3
Pulmonary regurgitation	None	0
	Present	1

CHOP score (range, 0–20) is produced by adding the 12 individual scores, with a higher score indicating poorer cardiac function[11].

bulk of perfusion during fetal life[17]. Only a few studies have evaluated the prognostic value of the CHOP score and MPI in TTTS.

The principal objective of our study was to evaluate the prognostic value of the CHOP cardiovascular score and the modified MPI with respect to the risk of recipient fetal loss in TTTS. The secondary objectives were to study differences in MPI after compared with before laser coagulation, as well as the correlation between Quintero's classification, MPI and the CHOP cardiovascular score.

METHODS

This was a single-center cohort study carried out in monochorionic diamniotic twin pregnancies complicated by TTTS and treated by laser photocoagulation at the Centre Medico-Chirurgical et Obstetrical in Strasbourg, France, between July 2008 and October 2012. Referred patients and those recruited from our own center underwent detailed anatomical, including Doppler, ultrasonography using a GE Voluson E8 or Voluson 130 (GE Medical Systems, Zipf, Austria) ultrasound machine. All patients met Quintero's criteria for TTTS[7]. It should be noted that among Quintero stage-1 TTTS cases, laser photocoagulation was performed only if there was poor maternal clinical tolerance of polyhydramnios (uterine contractions, abdominal pain and/or respiratory distress).

Cardiac assessment comprised measurement of the CHOP cardiovascular score and MPI. All measurements were performed by a single operator just before laser coagulation. Estimation of the CHOP score was based on the sum of the 12 cardiovascular parameters defined by Rychik *et al.* in 2007[11]. For the MPI analysis, we took care to record measurements systematically, both before laser and 24 h after the procedure. We took account solely of the MPI values for the right heart in the recipient fetus, i.e. measuring the MPI modified according to Hernandez-Andrade *et al.*[16]: the Doppler gate was placed in such a way that tricuspid and pulmonary valve movements were obtained separately as distinct images. The isovolumetric contraction time (ICT) was obtained by measuring the time lapse between the end of the tricuspid valve's closing click and the beginning of the pulmonary valve's opening click, the ejection time (ET) between the beginning of the pulmonary valve's opening click and the end of its closing click, and the isovolumetric relaxation time (IRT) between the end of the pulmonary valve's closing click and the beginning of the tricuspid valve's opening click. MPI, expressed in ms, was then calculated by applying the formula: (ICT + IRT)/ET.

Data were collected prospectively as part of routine care. All the clinical and ultrasonographic data were recorded in a computerized medical dossier system (DIAMM®, Micro6, Nancy, France).

Immediately after delivery, placentae were obtained in order to examine them for any possible residual anastomoses, using a technique described previously[21–24].

The principal assessment criterion was loss of the recipient fetus, including intrauterine fetal death and early neonatal mortality (within 7 days of delivery). Secondary assessment criteria were differences in MPI after compared with before laser photocoagulation, as well as the correlation between the different study factors (CHOP cardiovascular score, MPI and Quintero stage).

Quantitative variables are displayed as mean and SD, and categorical variables as percentages. MPI values were transformed into *z*-scores using data from Van Mieghem *et al.*[25], according to gestational age. The optimal cut-off was then selected using the receiver–operating characteristics (ROC) curve, according to the Youden index. The optimal cut-off for the CHOP score was also selected according to ROC curve analysis. Means of quantitative variables were compared using Student's *t*-test and categorical variables using the χ^2 test. Univariate analysis was performed in order to identify variables which might explain the outcome. Multivariate

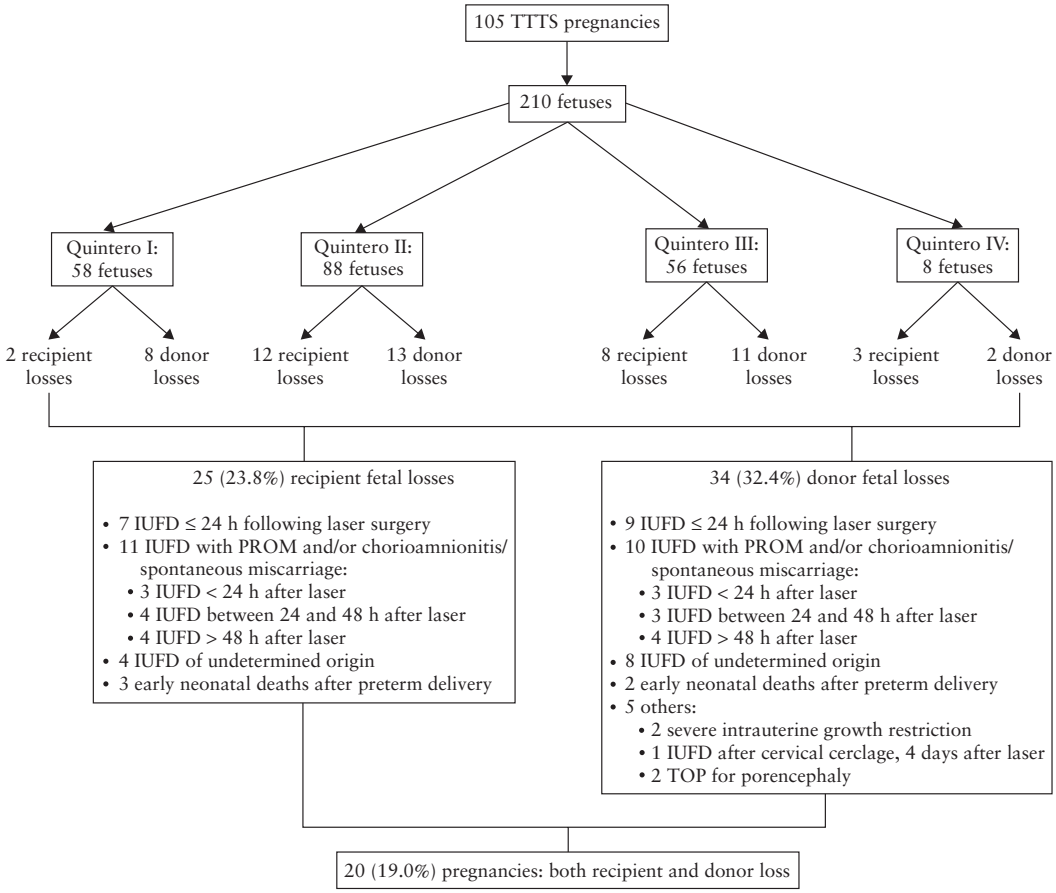


Figure 1 Flow chart showing outcome of 105 patients with monochorionic diamniotic twin pregnancy complicated by twin–twin transfusion syndrome (TTTS) and treated by laser photocoagulation. IUFD, intrauterine fetal death; PROM, premature rupture of membranes; TOP, termination of pregnancy.

logistic regression models were then built after manual selection of variables that were significant in the univariate analysis. Two models were built, one for MPI and one for the CHOP score (these two variables could not be entered in the same logistic regression model because of their colinearity). Odds ratios (OR) and their associated confidence intervals were computed for each predictor. For analysis of correlation, a non-parametric analysis was performed using Spearman's correlation test. Statistical analysis was performed with SPSS software (version 16, SPSS Inc., Chicago, IL, USA). All analyses were carried out based on a Type I or α risk set at 5%.

This study was approved by the French Data Protection Authority (CNIL) under number 1672926 v 0.

RESULTS

This study included 105 pregnancies complicated by TTTS: 29 (27.6%) Quintero stage I, 44 (41.9%) Quintero stage II, 28 (26.7%) Quintero stage III and four (3.8%) Quintero stage IV. Twenty-five (23.8%) recipient fetuses and 34 (32.4%) donor fetuses died; among these both donor and recipient died in 20 (19%) cases. Study population details and outcomes are reported in Figure 1.

Characteristics of the population based on the outcome of the recipient fetus (fetal loss/survival) are shown in Table 2. Both populations were comparable in terms of maternal age and gestational age at the time of laser photocoagulation. A higher Quintero stage and anterior location of the placenta were associated with a significantly higher recipient mortality rate.

After conversion of the modified MPI values into *z*-scores according to gestational age, the optimal cut-off was determined from the ROC curve to be 1.574 (Figure 2a). For the purposes of simplification, we used a cut-off *z*-score of 1.645, which corresponds to the 95th percentile (this did not alter the distribution of cases). The optimal cut-off for the CHOP cardiovascular score was 2.5 according to the ROC curve (Figure 2b). We chose to use a threshold of 3 for statistical analysis. The risk of recipient death was significantly higher when the MPI *z*-score was > 1.645 (34.5% *vs* 10.6, $P = 0.004$) and when the CHOP cardiovascular score was ≥ 3 (39.5% *vs* 12.9%, $P = 0.002$) (Table 2).

Table 2 Univariate comparison between recipient fetuses which survived and those which did not in 105 monochorionic diamniotic twin pregnancies complicated by twin–twin transfusion syndrome and treated by laser photocoagulation

Variable	*Recipient fetus survival (n = 80)*	*Recipient fetal loss (n = 25)*	P
Maternal age (years)	30.3 ± 5.1	29.8 ± 6.2	0.672
Gestational age at laser (weeks)	20 + 0/7 ± 3	19 + 4/7 ± 3	0.648
Placental location			
Anterior	28 (65.1)	15 (34.9)	0.024
Posterior	52 (83.9)	10 (16.1)	
Quintero stage			
I	27 (93.1)	2 (6.9)	0.004
II	32 (72.7)	12 (27.3)	
III	20 (71.4)	8 (28.6)	
IV	1 (25)	3 (75)	
Right MPI *z*-score			
≤ 1.645	42 (89.4)	5 (10.6)	0.004
> 1.645	38 (65.5)	20 (34.5)	
CHOP score			
< 3	54 (87.1)	8 (12.9)	0.002
≥ 3	26 (60.5)	17 (39.5)	
Ductus venosus			
Negative A-wave	9 (64.3)	5 (35.7)	
Positive A-wave	71 (78)	20 (22)	0.26
Normal PI	56 (76.7)	17 (23.3)	
PI > 95th percentile	24 (75)	8 (25)	0.85
Residual anastomoses			
None	32 (84.2)	6 (15.8)	0.304
≥ 1	15 (75)	5 (25)	

Data are given as mean ± SD or *n* (%). CHOP score, Children's Hospital Of Philadelphia score; MPI, modified myocardial performance index; PI, pulsatility index.

On multivariate analysis, the risk of recipient fetal death was significantly higher when the CHOP score was ≥3 (OR, 3.09 (95% CI, 1.035–9.21), $P = 0.043$). The risk of recipient fetal death was higher when the MPI *z*-score was > 1.645, but the difference was not statistically significant (OR, 2.96 (95% CI, 0.94–9.30), $P = 0.062$). Univariate and multivariate logistic regression results are reported in Table 3.

Concerning the correlation between the cardiovascular parameters and Quintero stage, it was found that the higher the Quintero stage the larger the proportion of abnormal MPI values (MPI *z*-score >1.645) and CHOP values (score ≥ 3) (Table 4). There was a positive correlation between Quintero stage and CHOP score, with a rho correlation coefficient of 0.56 ($P < 0.001$), and between Quintero stage and MPI, with a rho correlation coefficient of 0.33 ($P = 0.01$). There was also an interparametric correlation between the CHOP cardiovascular score and MPI, with a rho correlation coefficient of 0.40 ($P < 0.001$).

Differences in MPI after compared with before laser photocoagulation were studied in a cohort of 90 recipient fetuses, comprising those surviving the first 24 h following the laser procedure. The mean (± SD) MPI was significantly lower after the procedure: 0.39 (± 0.14) *vs* 0.44 (± 0.16) ms, $P = 0.012$.

DISCUSSION

Data are discordant concerning the independence of MPI from gestational age. Some authors have found that there is no correlation between them[15,26], while others have found that MPI is dependent on gestational age[19,25]. For our analyses we decided to transform the modified MPI into *z*-scores according to gestational age using data from Van Mieghem *et al.*[25,27], to overcome any potential variation of MPI with gestational age.

We found the CHOP score and MPI cardiovascular parameters to be predictive factors for recipient fetal loss in laser-treated TTTS pregnancies. After adjustment for Quintero stage and placental location, only the CHOP score remained an independent risk factor.

There are very few published studies investigating the CHOP cardiovascular score[11,28,29]. To our knowledge, only a single study[29] has tested the prognostic value of this score in a population treated by laser photocoagulation, but it failed to find a significant link between fetal survival rate (both global and of the recipient fetus) and the CHOP score. A study on exclusively Quintero stage 1 pregnancies did not find a correlation between a high CHOP score and risk of disease progression or a need to perform amnioreduction[28]. On the other hand, the gestational age at delivery appeared to be 2.2 weeks earlier when the CHOP score was > 5. In our study, the mean gestational age at delivery was 1.7 weeks earlier when the CHOP score was ≥ 3 (data not shown). Based on the 12 parameters that it measures in the recipient fetus, the CHOP score would probably allow analysis of the chronology of fetal cardiac damage. However, the score is difficult to establish because it requires an experienced ultrasonographer and a considerable amount of time: its total completion time has been estimated at 60 min, though this time can be reduced to 30–45 min with training and experience[11]. It is therefore necessary, before making it a feature of current practice, that its application and utility in the management of TTTS be justified.

Studies evaluating the utility of the MPI in TTTS are also scarce. To our knowledge, our cohort is one of the largest to date. Many of the studies in the literature have been concerned primarily with modifications of cardiac function in the recipient fetus[27,30,31] and have generally failed to evaluate the prognostic value of MPI with respect to obstetric outcome[11,17,32]. Some have examined the obstetric outcome, but did not find any genuine correlation[32]. Most of these studies agree on several points: MPI is a new Doppler index evaluating systolic and diastolic cardiac function[20]; it is technically easy to obtain and offers a high degree of reproducibility[16,26]; it has a major advantage in that it allows ready assessment of fetal right heart function, which has hitherto been difficult[11,17]; its value is significantly higher in the recipient fetus than in the donor fetus when TTTS is present compared with the non-pathological situation[9,17].

Most studies have evaluated global fetal cardiac function based on right and left heart MPI values[9,12,27,31]; a few authors have investigated the MPI of the left

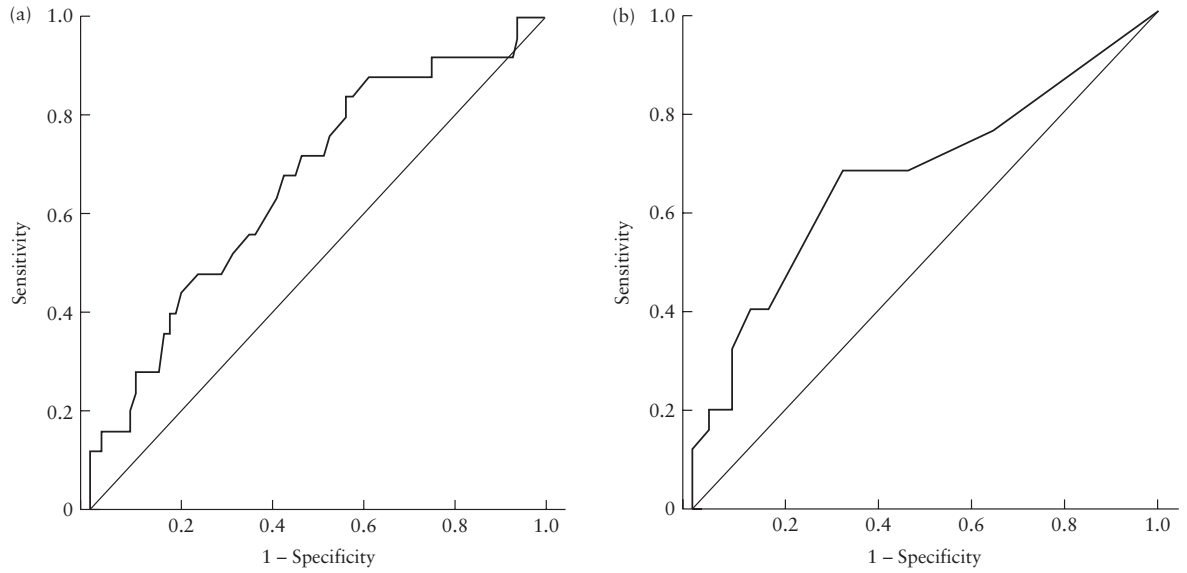


Figure 2 Receiver–operating characteristics curves for prediction of fetal loss in twin–twin transfusion syndrome by: (a) modified myocardial performance index (*z*-score cut-off: 1.574) and (b) Children's Hospital Of Philadelphia (CHOP) cardiovascular score (cut-off: 3).

Table 3 Prediction of recipient fetal loss in monochorionic diamniotic twin pregnancies complicated by twin–twin transfusion syndrome and treated by laser photocoagulation, using univariate and multivariate models

Models predictive of recipient fetal loss	*Odds ratio*	*95% CI*	P
Univariate models			
CHOP score	4.41	1.69–11.55	0.002
MPI *z*-score	4.42	1.51–12.95	0.004
Quintero stage	2.25	1.26–4.03	0.006
Placental location	2.79	1.11–7.01	0.030
Multivariate models			
Model 1			
MPI *z*-score	2.96	0.94–9.30	0.062
Quintero stage	0.56	0.301–1.05	0.07
Placental location	2.25	0.848–5.97	0.103
Model 2			
CHOP score	3.09	1.035–9.21	0.043
Quintero stage	0.64	0.33–1.22	0.176
Placental location	2.58	0.97–6.86	0.058

MPI *z*-score: ≤ 1.645 *vs* > 1.645; CHOP score: < 3 *vs* ≥ 3. CHOP score, Children's Hospital Of Philadelphia score; MPI, modified myocardial performance index.

ventricle alone[25,33]. Studies evaluating the right MPI are scarce and only a few have established normal values of fetal MPI[26], although essentially in singleton pregnancy. In our study, we sought to evaluate the MPI of the right heart in the recipient fetus in TTTS. The literature has clearly shown the importance of studying fetal right heart function[11,17].

We did not asses inter- and intraobserver reliability of MPI measurements in this study. Eidem *et al.*[15] reported reproducibility of MPI values. Some authors[16,18] modified the measurement of MPI parameters to improve the reproducibility. Recently, Ghawi *et al.*[26] found that inter- and intraobserver variability were both around 5% for right MPI and around 6% and 4%, respectively, for left MPI; apparently the variability is not much higher for the right compared with the left MPI, despite the necessity of obtaining two separate images for this measurement.

In 55.2% of the recipient fetuses in our cohort, the right MPI value was abnormal, reflecting the high prevalence of vascular repercussions, as observed by Habli *et al.*[31]. The mean MPI value in our cohort was 0.46 ms, which is smaller than that reported in the literature[30,11,17], probably because the gestational age at ultrasonography was close to 20 gestational weeks, earlier than that in other studies[11,17,30,31,33,34].

Table 4 Modified myocardial performance index (MPI) and Children's Hospital of Philadelphia (CHOP) score according to Quintero stage in 105 monochorionic diamniotic twin pregnancies complicated by twin–twin transfusion syndrome and treated by laser photocoagulation

	MPI z-score		*CHOP score*	
Quintero classification	*≤ 1.645 (normal)*	*> 1.645 (abnormal)*	*< 3*	*≥ 3*
Stage I	19 (65.5)	10 (34.5)	23 (79.3)	6 (20.7)
Stage II	21 (47.7)	23 (52.3)	33 (75)	11 (25)
Stage III	7 (25)	21 (75)	6 (21.4)	22 (78.6)
Stage IV	0 (0)	4 (100)	0 (0)	4 (100)
Total	47 (44.8)	58 (55.2)	62 (59)	43 (41)

Data are given as *n* (%).

With regard to the correlation between CHOP score and Quintero stage, our data are comparable to those in the literature[29], with a gradual rise in the proportion of CHOP scores ≥ 3 as a function of Quintero stage (Table 4) and a positive correlation between these two factors.

Our study also showed a linear increase in the percentage of recipient fetuses with abnormal MPI *z*-score > 1.645 as a function of Quintero stage: one-third of MPI values in fetuses at Quintero stage I were abnormal, while 100% were abnormal at Stage IV. This correlation between the right MPI value in the recipient fetus and Quintero stage has been described previously, although to a somewhat lesser degree[11].

Finally, with regard to the association between the right MPI value in the recipient fetus and CHOP score, a study[11] found results comparable to ours.

To our knowledge, our cohort is one of the largest to date investigating right MPI differences after compared with before laser photocoagulation[30,35]. As in other reports[27,30,35,36], our study revealed a fall in MPI in the recipient fetus after laser treatment (from mean ± SD, 0.44 ± 0.16 ms to 0.39 ± 0.14 ms). Nevertheless, both before- and after-laser MPI values in our study were lower than those reported in other studies, such as that of Papanna *et al.*[30] (0.62 ± 0.12 ms to 0.58 ± 0.07 ms). This difference may be linked to the distribution of the cohort population in terms of Quintero staging: of the 90 pregnancies in our cohort in which before- and after-laser MPI values were measured, 71% were in Quintero stages I and II (30% Stage I and 41% Stage II) and 29% were in Stages III and IV (26% Stage III and 3% Stage IV), whereas Papanna *et al.*[30] studied a population consisting essentially of subjects in Quintero stages III and IV (65%), i.e. with more cases of advanced TTTS. It may be that this factor accounts for their higher MPI values. Laser photocoagulation can therefore reduce the proportion of abnormal MPI values from nearly 50% before laser photocoagulation to close to one-third thereafter.

In conclusion, Quintero's classification appears to be deficient for determining the prognosis of TTTS, since it does not incorporate cardiovascular parameters, which may deteriorate precipitately. The CHOP score and MPI are predictors of recipient fetal loss in TTTS and may be used to supplement Quintero's classification.

REFERENCES

1. Feldstein VA, Filly RA. Complications of monochorionic twins. *Radiol Clin North Am* 2003; **41**: 709–727.
2. Trevett T, Johnson A. Monochorionic twin pregnancies. *Clin Perinatol* 2005; **32**: 475–94, viii.
3. Harkness UF, Crombleholme TM. Twin-twin transfusion syndrome: where do we go from here? *Semin Perinatol* 2005; **29**: 296–304.
4. Sebire NJ, Snijders RJ, Hughes K, Sepulveda W, Nicolaides KH. The hidden mortality of monochorionic twin pregnancies. *Br J Obstet Gynaecol* 1997; **104**: 1203–1207.
5. Steinberg LH, Hurley VA, Desmedt E, Beischer NA. Acute polyhydramnios in twin pregnancies. *Aust N Z J Obstet Gynaecol* 1990; **30**: 196–200.
6. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. *N Engl J Med* 2004; **351**: 136–144.
7. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin-twin transfusion syndrome. *J Perinatol* 1999; **19**: 550–555.
8. Taylor MJ, Govender L, Jolly M, Wee L, Fisk NM. Validation of the Quintero staging system for twin-twin transfusion syndrome. *Obstet Gynecol* 2002; **100**: 1257–1265.
9. Raboisson MJ, Fouron JC, Lamoureux J, Leduc L, Grignon A, Proulx F, Gamache S. Early intertwin differences in myocardial performance during the twin-to-twin transfusion syndrome. *Circulation* 2004; **110**: 3043–3048.
10. Barrea C, Alkazaleh F, Ryan G, McCrindle BW, Roberts A, Bigras JL, Barrett J, Seaward GP, Smallhorn JF, Hornberger LK. Prenatal cardiovascular manifestations in the twin-to-twin transfusion syndrome recipients and the impact of therapeutic amnioreduction. *Am J Obstet Gynecol* 2005; **192**: 892–902.
11. Rychik J, Tian Z, Bebbington M, Xu F, McCann M, Mann S, Wilson RD, Johnson MP. The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease. *Am J Obstet Gynecol* 2007; **197**: 392.e1–8.
12. Michelfelder E, Gottliebson W, Border W, Kinsel M, Polzin W, Livingston J, Khoury P, Crombleholme T. Early manifestations and spectrum of recipient twin cardiomyopathy in twin–twin transfusion syndrome: relation to Quintero stage. *Ultrasound Obstet Gynecol* 2007; **30**: 965–971.
13. Tei C, Dujardin KS, Hodge DO, Kyle RA, Tajik AJ, Seward JB. Doppler index combining systolic and diastolic myocardial performance: clinical value in cardiac amyloidosis. *J Am Coll Cardiol* 1996; **28**: 658–664.
14. Shah AD, Border WL, Crombleholme TM, Michelfelder EC. Initial fetal cardiovascular profile score predicts recipient twin outcome in twin-twin transfusion syndrome. *J Am Soc Echocardiogr* 2008; **21**: 1105–1108.
15. Eidem BW, Edwards JM, Cetta F. Quantitative assessment of fetal ventricular function: establishing normal values of the

myocardial performance index in the fetus. *Echocardiography* 2001; **18**: 9–13.
16. Hernandez-Andrade E, López-Tenorio J, Figueroa-Diesel H, Sanin-Blair J, Carreras E, Cabero L, Gratacos E. A modified myocardial performance (Tei) index based on the use of valve clicks improves reproducibility of fetal left cardiac function assessment. *Ultrasound Obstet Gynecol* 2005; **26**: 227–232.
17. Ichizuka K, Matsuoka R, Hasegawa J, Shirato N, Jimbo M, Otsuki K, Sekizawa A, Farina A, Okai T. The Tei index for evaluation of fetal myocardial performance in sick fetuses. *Early Hum Dev* 2005; **81**: 273–279.
18. Raboisson MJ, Bourdages M, Fouron JC. Measuring left ventricular myocardial performance index in fetuses. *Am J Cardiol* 2003; **91**: 919–921.
19. Tsutsumi T, Ishii M, Eto G, Hota M, Kato H. Serial evaluation for myocardial performance in fetuses and neonates using a new Doppler index. *Pediatr Int* 1999; **41**: 722–727.
20. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, Tajik AJ, Seward JB. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function a study in normals and dilated cardiomyopathy. *J Cardiol* 1995; **26**: 357–366.
21. Favre R, Koch A, Weingertner AS, Sananes N, Trieu NT, Kohler M, Guerra F, Nisand I. Vascular pattern in monochorionic placentas with spontaneous TAPS and TTTS with residual anastomoses after laser: a case–control study. *Prenat Diagn* 2013; **33**: 979–982.
22. Lopriore E, Holtkamp N, Sueters M, Middeldorp JM, Walther FJ, Oepkes D. Acute peripartum twin-twin transfusion syndrome: Incidence, risk factors, placental characteristics and neonatal outcome. *J Obstet Gynaecol Res* 2014; **40**: 18–24.
23. Lopriore E, Middeldorp JM, Oepkes D, Klumper FJ, Walther FJ, Vandenbussche FP. Residual anastomoses after fetoscopic laser surgery in twin-to-twin transfusion syndrome: frequency, associated risks and outcome. *Placenta* 2007; **28**: 204–208.
24. Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. *Am J Obstet Gynecol* 2009; **201**: 66.e1–4.
25. Van Mieghem T, Gucciardo L, Lewi P, Lewi L, Van Schoubroeck D, Devlieger R, De Catte L, Verhaeghe J, Deprest J. Validation of the fetal myocardial performance index in the second and third trimesters of gestation. *Ultrasound Obstet Gynecol* 2009; **33**: 58–63.
26. Ghawi H, Gendi S, Mallula K, Zghouzi M, Faza N, Awad S. Fetal left and right ventricle myocardial performance index: defining normal values for the second and third trimesters single tertiary center experience. *Pediatr Cardiol* 2013; **34**: 1808–1815.
27. Van Mieghem T, Klaritsch P, Doné E, Gucciardo L, Lewi P, Verhaeghe J, Lewi L, Deprest J. Assessment of fetal cardiac function before and after therapy for twin-to-twin transfusion syndrome. *Am J Obstet Gynecol* 2009; **200**: 400.e1–7.
28. Bebbington MW, Tiblad E, Huesler-Charles M, Wilson RD, Mann SE, Johnson MP. Outcomes in a cohort of patients with Stage I twin-to-twin transfusion syndrome. *Ultrasound Obstet Gynecol* 2010; **36**: 48–51.
29. Stirnemann JJ, Nasr B, Proulx F, Essaoui M, Ville Y. Evaluation of the CHOP cardiovascular score as a prognostic predictor of outcome in twin–twin transfusion syndrome after laser coagulation of placental vessels in a prospective cohort. *Ultrasound Obstet Gynecol* 2010; **36**: 52–57.
30. Papanna R, Mann LK, Molina S, Johnson A, Moise KJ. Changes in the recipient fetal Tei index in the peri-operative period after laser photocoagulation of placental anastomoses for twin-twin transfusion syndrome. *Prenat Diagn* 2011; **31**: 176–180.
31. Habli M, O'Brien T, Nowack E, Khoury S, Barton JR, Sibai B. Peripartum cardiomyopathy: prognostic factors for long-term maternal outcome. *Am J Obstet Gynecol* 2008; **199**: 415.e1–5.
32. Stirnemann JJ, Mougeot M, Proulx F, Nasr B, Essaoui M, Fouron JC, Ville Y. Profiling fetal cardiac function in twin–twin transfusion syndrome. *Ultrasound Obstet Gynecol* 2010; **35**: 19–27.
33. Friedman D, Buyon J, Kim M, Glickstein JS. Fetal cardiac function assessed by Doppler myocardial performance index (Tei Index). *Ultrasound Obstet Gynecol* 2003; **21**: 33–36.
34. Acharya G, Pavlovic M, Ewing L, Nollmann D, Leshko J, Huhta JC. Comparison between pulsed-wave Doppler- and tissue Doppler-derived Tei indices in fetuses with and without congenital heart disease. *Ultrasound Obstet Gynecol* 2008; **31**: 406–411.
35. Habli M, Michelfelder E, Livingston J, Harmon J, Lim FY, Polzin W, Crombleholme T. Acute effects of selective fetoscopic laser photocoagulation on recipient cardiac function in twin-twin transfusion syndrome. *Am J Obstet Gynecol* 2008; **199**: 412.e1–6.
36. Gratacós E, Van Schoubroeck D, Carreras E, Devlieger R, Roma E, Cabero L, Deprest J. Impact of laser coagulation in severe twin–twin transfusion syndrome on fetal Doppler indices and venous blood flow volume. *Ultrasound Obstet Gynecol* 2002; **20**: 125–130.

DOI: 10.1002/pd.4393 PRENATAL **DIAGNOSIS**

ORIGINAL ARTICLE

Predictive value of cardiovascular parameters in stages 1 and 2 of twin-to-twin transfusion syndrome

Elodie Gapp-Born[1]*, Nicolas Sananes[1,2], Fernando Guerra[1], Monique Kohler[1], Anne Sophie Weingertner[1], Gabrielle Fritz[1], Brigitte Viville[1], Bruno Langer[1], Erik Sauleau[3], Israël Nisand[1] and Romain Favre[1]

[1]Department of Obstetrics and Gynaecology, Strasbourg University Teaching Hospital, Strasbourg, France
[2]Biomatériaux et Bioingénierie, INSERM, UMR-S 1121, Strasbourg, France
[3]Department of Biostatistics, Strasbourg University Teaching Hospital, Strasbourg, France
*Correspondence to: Elodie Gapp-Born. E-mail: elodie.gapp@chru-strasbourg.fr

ABSTRACT

Objective The Quintero staging of twin-to-twin transfusion syndrome (TTTS) does not include a comprehensive cardiovascular assessment. The aim of this study is to assess the predictive value of the myocardial performance index (MPI) and the Children's Hospital of Philadelphia (CHOP) score on recipient survival in Quintero stages 1 and 2 TTTS.

Methods The cohort study was based on prospectively collected data between May 2008 and February 2013 in a population of stages 1 and 2 TTTS. Comparisons between groups were carried out using Student's *t*-test and χ^2-test. A stepwise ascending multivariate logistic regression model was then built.

Results A total of 73 pregnancies in stages 1 and 2 of Quintero's classification were treated with laser. Rates of recipient fetal losses were higher when MPI was above 0.43 ms (71.4% vs 28.6%, $p = 0.022$). Rate of CHOP score above 5 was higher in the fetal loss group (28.6% vs 5.1%, $p = 0.022$). After adjustment for Quintero stages 1 or 2, the risk of recipient loss rate is higher according to CHOP score [OR 7.6; 95% confidence interval (CI) 1.3–43.5] or MPI value (OR 3.7; 95% CI 1.0–13.9).

Conclusion The CHOP score and MPI are correlated with the recipient survival in stages 1 and 2 TTTS. © 2014 John Wiley & Sons, Ltd.

Funding sources: None
Conflicts of interest: None declared

INTRODUCTION

The twin-twin transfusion syndrome (TTTS) complicates 10% of monochorionic diamniotic twin pregnancies.[1–3] It accounts for 12% of mortality and 17% of perinatal morbidity in all twin pregnancies.[3–5] Untreated, severe forms are associated with a survival rate of less than 20%.[6,7] Fetoscopy-guided laser photocoagulation of placental anastomoses significantly improves survival and is currently the reference treatment.[8] Nevertheless, this is not a procedure to be undertaken lightly because it can itself give rise to maternal morbidity[8,9] and fetal morbidity and mortality.[8,9] It is therefore essential to have reliable prognostic factors, in order to be able to identify among cases of TTTS, those pregnancies that might benefit from a laser treatment.

Quintero's classification is the most widely used system for categorizing TTTS.[10] Notwithstanding, it now appears to have significant shortcomings, and its prognostic value is contentious,[8,11] especially because it does not integrate early-stage cardiovascular parameters. Fetal cardiovascular modifications in the early stages of the disease have been described, in particular in the recipient fetus.[11–15] The recipient may develop a specific cardiomyopathy because of the increased post-load and transplacental anastomotic passage of angiotensin (produced by the donor), giving rise to poorer myocardial compliance.[15,16]

Other factors for TTTS that take account of fetal cardiac function have been proposed.[14,17–22] The Children's Hospital of Philadelphia (CHOP) cardiovascular score comprises 12 parameters based on umbilical Doppler of the donor, venous Doppler, and cardiac function analysis of the recipient. The CHOP score is expressed as a score ranging from 0 to 20: the higher the score, the worse the cardiac function. The Tei index or myocardial performance index (MPI) evaluates global, systolic, and diastolic myocardial function.[23] It has the advantage of being independent of the gestational age,[18,24,25] ventricular geometry and fetal heart rate.[16,23] The MPI value increases when ventricular dysfunction is present.[20] Its major utility is to allow straightforward and non-invasive assessment of right heart function. This is crucial,[14,20] because the right ventricle is responsible for the bulk of perfusion during fetal

life.[20] Studies evaluating the prognostic value of the CHOP score and MPI in TTTS are scarce. To the best of our knowledge, none has looked in depth to their relevance in the lower stages of Quintero's classification.

The principal objective of our study was to evaluate the prognostic value of the CHOP cardiovascular score and Tei index for the risk of recipient fetal loss in the stages 1 and 2 of Quintero's classification. The secondary objective was to study the prognostic value of these tools for global survival of both fetuses.

METHODS

This was a single-center cohort study carried out in monochorionic diamniotic twin pregnancies complicated by TTTS at an early stage (Quintero 1 or 2) and treated by laser at the Centre Médico-Chirurgical and Obstétrical (CMCO) between July 2008 and October 2012. This is a center of reference for the diagnosis and the treatment for TTTS in the east of France. All the data were prospectively collected as part of routine care and then retrospectively analyzed to test our hypothesis.

Clinical and ultrasonographic data were collated using the computerized medical dossier system (DIAMM®, Micro6®).

Each mother underwent detailed obstetrical ultrasonography along with biometric, morphological, and Doppler studies in using either Voluson E8 or Voluson 130 ultrasound systems.

The diagnosis of TTTS was defined by the presence of oligohydramnios sequence in a monochorionic diamniotic twin gestation with like-sex twins, a single placental mass and a thin intertwine membrane. The Quintero stage was established in all cases.[10] First stage is defined as an oligamnios in the donor twin (deepest vertical amniotic fluid pocket $\leq$2 cm) and a polyhydramnios in the recipient twin [deepest vertical amniotic fluid pocket >8 cm before 20 weeks' gestation (WG) or >10 cm after 20 WG], the latter being the criteria used in Europe and the bladder of the donor fetus being still visible. The second stage is defined by the absence of the donor bladder. It should be noted that laser was used for Quintero stage 1 TTTS only when there was maternal discomfort from the polyhydramnios (uterine contractions, abdominal pain, and breathing discomfort). Cardiac assessment comprised measurement of the Tei index (for our analysis, we were interested in the Tei index values for the right heart only) and the CHOP cardiovascular score. The technique for measuring the Tei index was described by Hernandez-Andrade *et al.*[19] The Doppler was placed in such a way that tricuspid and pulmonary valve movements were obtained as distinct images. The isovolumetric contraction time (ICT) was obtained by measuring the time lapse between the end of the tricuspid valve closing click and the beginning of the pulmonary valve opening click, ejection time (ET) between the beginning of the pulmonary valve opening click and the end of its closing click, and the isovolumetric relaxation time (IRT) between the end of the pulmonary valve closing click and the beginning of the tricuspid valve opening click. The MPI was then calculated by applying the formula (ICT + IRT)/ET. Estimation of the CHOP cardiovascular score was based on the sum of the 12 cardiovascular parameters defined by Rychik[14] in 2007. For all analyses, the thresholds applied for both factors are those found in the literature. Accordingly, for the CHOP cardiovascular score, we retained the threshold of 5[26] and for the MPI that of 0.43 ms[19,27]: above these thresholds, the value was considered abnormal.

All patients included underwent laser photocoagulation. The surgery was performed by two experienced operators with 1.3 or 2.0 mm fetoscopes (the outer diameter of the sheath was 10 French) according to gestational age, under local anesthesia and antibiotic coverage. First, the method used was the selective coagulation. Since 2010, patients were randomized to photocoagulation technique: either the selective technique described by Quintero *et al.*[28] or the Solomon technique described by Lopriore *et al.*[29]

An amnioreduction was performed after the procedure to obtain a normal quantity of amniotic fluid in the recipient twin (residual pocket <4 cm). Usually, the patients left the hospital the day after surgery and were subjected to weekly then bi-monthly ultrasound monitoring.

After delivery, each placental mass was freshly recuperated and forwarded without preparation by the maternity units in which the infants issuing from these pregnancies were born. Amniotic membranes were peeled from the surface of the placental plate, the umbilical cords sectioned beneath the clamps, the placenta rinsed, and excess blood squeezed out along the length of the umbilical cords. The umbilical arteries and veins were catheterized. For prime visualization of the anastomoses, veins were injected with eosin and arteries with methylene blue until all the peripheral branches were optimally rendered. The cord was then clamped in order to maintain optimal filling of the vessels. The identification of residual anastomoses was processed by visual inspection, and then, a digital picture was taken using a Sony DSC-W220 digital camera (12.1 megapixels), and the image tool software (for Window v.3.0) was used to measure the diameter of anastomoses on the surface of the chorionic plate. This evaluation was performed by a single operator who knew the outcome of the pregnancies because only placentas with double survival and double intrauterine death were analyzed. The principal assessment criterion was defined as the loss of the recipient fetus. Fetal loss is defined by the death of the recipient twin occurring during pregnancy or at early neonatal period (<7 days); the donor fetus is alive or not. The secondary endpoints were the loss of one or both twins, and the study of the correlation between the CHOP score and the right ventricular (RV) Tei index.

Statistical analysis was performed using SPSS software (version 16, SPSS, Inc, Chicago, IL, USA). All analyses were carried out with a type 1 or α risk set at 5%.

Normally distributed continuous variables are displayed as mean and standard deviation, and categorical variables are presented as ratios and percentages. Comparisons for normally distributed continuous variables were made using Student's *t*-tests and for categorical variables using χ^2-tests. Univariate analysis was performed in order to identify variables that might explain the outcome. A multivariate logistic regression model was then constructed after manual selection of variables. Odds ratio (OR) and their associated confidence intervals (CIs) were computed for each predictor.

Finally, we used a non-parametric analysis to study the relationship between both cardiovascular factors studied.

This study was approved by the French Data Protection Authority (CNIL) under number 1672926 v.0.

RESULTS

The study involved 73 pregnancies complicated by early-stage TTTS: 29 in Quintero stage 1 and 44 in Quintero stage 2. Laser treatment took place at a median gestational age of 19.2 WG (+/−2.7). The median gestational age at delivery was 33 WG (+/−5.4). In our cohort, 14 recipient fetuses died, two in Quintero stage 1 and 12 in Quintero stage 2. Among these recipient fetuses, seven died in the 24 h following the laser surgery; there were two additional intrauterine fetal deaths of undetermined origin later on (one 31 days after the laser, and for the other, this information is missing). Four died in the context of premature rupture of the membranes and/or chorioamnionitis between 6 and 54 days after laser. There was one early neonatal death after spontaneous preterm birth at 28 WG (90 days after the laser) (Figure 1). With regard to the donor fetuses, we observed eight losses in Quintero stage 1 group and 13 losses in Quintero stage 2 group. In ten pregnancies (13.7%), both fetuses died, and 15 (20.5%) suffered single fetus losses: the donor twin died in 11 (15.1%) cases and the recipient fetuses in four cases (5.5%) (Figure 1).

The characteristics of the cohort based on the recipient fetus outcome (loss/living) are shown in Table 1. Both populations were comparable in terms of maternal age, parity, gestational age at laser treatment, placental localization, and the presence or absence of residual placental anastomoses.

Among the 73 pregnancies, 44 (60.3%) were treated with the selective technique and 29 (39.7%) with the Solomon technique. The selective coagulation is associated with a higher rate of fetal loss but without statistically significant difference: 57.1% versus 42.9%, $p = 0.59$, Table 1.

On univariate analysis, it emerged that there was a significantly higher recipient mortality rate in Quintero stage 2 TTTS compared with stage 1 fetuses: 85.7% versus 14.3% ($p = 0.028$). Similarly, the risk of loss of this twin was significantly higher when the MPI value was superior to 0.43: 71.4% versus 28.6% ($p = 0.022$). The proportion of CHOP cardiovascular score above 5 is higher in the fetal loss group than in living recipient fetuses group: 28.6% versus 5.1% ($p = 0.022$), Table 1.

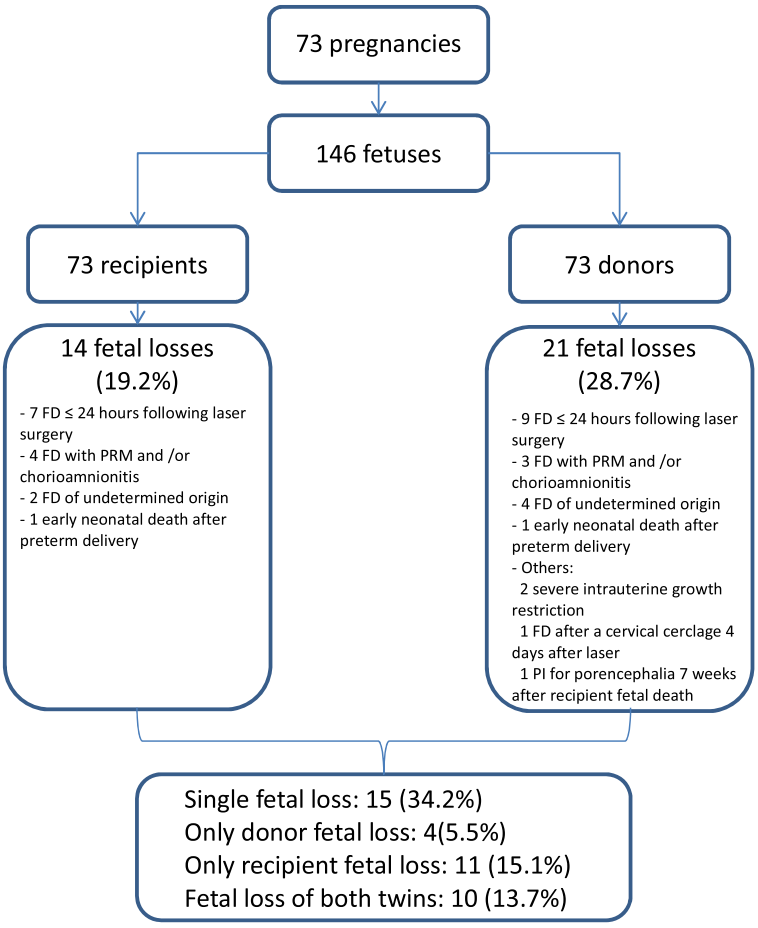


Figure 1 Flow chart of patients studied. FD, fetal death; PRM, premature rupture of membranes; PI, pregnancy interruption

Table 1 **Univariate comparison between the group of living recipient fetuses and recipient fetal losses**

Variables	Living recipient fetuses $n=59$	Recipient fetal loss $n=14$	p-value
Mean maternal age (±SD)	30.07 (+/−5.58)	29.86 (+/−4.57)	0.896
Mean gestational age on laser (±SD)	20 (+/−2.67)	19.52 (+/−3.11)	0.559
Posterior placental localization: *n* (%)	40 (67.8%)	7 (50%)	0.173
Anterior placental localization: *n* (%)	19 (32.2%)	7 (50%)	
Quintero stage 1: *n* (%)	27 (45.8%)	2 (14.3%)	0.028
Quintero stage 2: *n* (%)	32 (54.2%)	12 (85.7%)	
Right MPI ≤0.43: *n* (%)	37 (62.7%)	4 (28.6%)	0.022
Right MPI >0.43: *n* (%)	22 (37.3%)	10 (71.4%)	
CHOP score ≤5: n (%)	56 (94.9%)	10 (71.4%)	0.022
CHOP score >5: n (%)	3 (5.1%)	4 (28.6%)	
None residual anastomoses: *n* (%)	21 (60%)	4 (57.1%)	0.603
Residual anastomoses ≥1: *n* (%)	14 (40%)	3 (42.9%)	
None residual AV anastomoses: *n* (%)	25 (71.4%)	5 (71.4%)	0.66
Residual AV anastomoses ≥1: *n* (%)	10 (28.6%)	2 (28.6%)	
Selective technique of laser coagulation	36 (61%)	8 (57.1%)	0.59
Solomon technique of laser coagulation	23 (39%)	6 (42.9%)	

SD, standard deviation; MPI, myocardial performance index; CHOP, Children's Hospital of Philadelphia; AV, arterio-venous anastomoses.

On multivariate analysis, after adjustment for the Quintero stage, the risk of recipient loss was significantly higher when the CHOP score was above 5: OR = 7.58 (95% CI 1.32–43.48), $p=0.023$ (Table 2). This also applied when the MPI value was superior than 0.43: OR = 3.75 (1.01–13.89), $p=0.048$ (Table 2).

With regard to analysis in relation to the global outcome of pregnancy (loss of one or both twins/two living twins), on univariate analysis, there was no significant difference in global survival, either in relation to the Quintero stage or CHOP score. The rate of loss of at least one twin was higher when the MPI value was above 0.43: 46.9% versus 24.4%, $p=0.039$. On multivariate analysis, after adjustment for the Quintero stage, the risk of loss of at least one twin was higher when the MPI value was above 0.43: OR = 2.73 (95% CI 1.01–7.41), $p=0.047$.

Moreover, we found a significant positive correlation between the CHOP score and the RV Tei index in the two first stages of Quintero's classification with a coefficient of correlation of 0.25, $p=0.035$.

DISCUSSION

We found that the CHOP score and MPI value are predictors of recipient fetus loss in lower-stage TTTS, after adjustment for the Quintero stage. Similarly, an increased MPI is associated with a higher risk of loss of at least one of the twins. This shows that the Quintero classification is insufficient for predicting outcome of recipient survival in stages 1 and 2.

The Quintero classification is the most widely used TTTS staging system, but its prognostic value is currently subject to debate,[11,30,31] especially because it does not incorporate

Table 2 **Prediction of recipient fetal loss using several univariate and multivariate models**

Models predictive of recipient fetal loss		OR	95% CI		p-value
			Inf.	Sup.	
Univariate models	MPI	4.20	1.18	14.93	0.027
	CHOP	7.46	1.45	38.46	0.016
	Quintero	5.05	1.04	24.39	0.045
Multivariate models	CHOP	7.58	1.32	43.48	0.023
	Quintero	5.13	0.99	26.31	0.052
	MPI	3.75	1.01	13.89	0.048
	Quintero	4.47	0.89	22.22	0.069
	MPI	2.70	0.68	10.75	0.158
	CHOP	5.10	0.79	32.26	0.086
	Quintero	4.44	0.85	23.26	0.077

OR, odds ratio; CI, confidence interval; MPI, myocardial performance index; CHOP, Children's Hospital of Philadelphia; Inf., inferior; Sup., superior.
MPI: ≥0.43 versus <0.43; CHOP: >5 versus ≤5; Quintero: stage 2 versus stage 1.

cardiovascular parameters.[17,32] The prognosis of stages 3 and 4 TTTS is poor and the utility of a laser treatment beyond doubt. In the early stages, the prognosis is dependent on cardiovascular modifications, which should be taken into account in order to enhance the Quintero classification. Fetal ultrasonography would appear to be of interest, particularly in stage 1 when the utility of laser treatment is controversial.

The cohort size in our series was fairly substantial given the prevalence of TTTS, but it did not allow, for reasons of statistical power, for a separate analysis of stages 1 and 2 of the Quintero classification. A degree of selection bias may be present in our population because solely pregnancies treated by laser were analyzed (some stage 1 pregnancies were not included); however, that does not appear to us to call into question the validity of our results. Ultrasonography along with CHOP cardiovascular score and TEI index measurements were conducted by the same operator, avoiding inter-observer variations. For our ultrasonographer, the mean time taken to measure the 12 parameters of the CHOP cardiovascular score was 15 min and the MPI 1 was 2 min.

Studies with the CHOP cardiovascular score are relatively scarce.[14,26,33] Our study has shown a significant increase in the risk of recipient fetal loss with a sevenfold higher risk when the CHOP score is above 5. To our knowledge, only a single study[26] has tested the prognostic value of this score in a population treated by laser but failed to find a significant link between the fetal survival rate (global and recipient fetus) and CHOP cardiovascular score. A study into exclusively Quintero classification stage 1 pregnancies did not find a correlation between a high CHOP score and an increased risk of disease progression or greater need to perform amnioreduction[33] nor gestational age at delivery. Neonatal survival was not analyzed. The CHOP cardiovascular score, by means of the 11 parameters that it measures in the recipient fetus, would probably allow analysis of the chronology of fetal cardiac damage. However, it is a score that is difficult to establish because it demands both an experienced ultrasonographer and a considerable working time: its total completion time has been estimated at 60 min.[14] This time can be reduced to 30–45 min with training and experience.[14] Routinely, obtaining the CHOP cardiovascular score raises the problem of the duration of ultrasonography. It is therefore necessary, before making it a feature of current practice, that its application and utility in the management of TTTS be justified.

Studies evaluating the utility of MPI in TTTS are also fairly scanty. To our knowledge, our cohort is the largest to date. We found that when the MPI value is above 0.43, the risk of recipient fetal loss is increased threefold. The studies found in the literature have principally examined modifications in the cardiac function of the recipient fetus[27,34,35] but without assessing the prognostic value of the Tei index on the obstetrical outcome.[14,20,36] Others have investigated the obstetrical outcome but have not found a genuine correlation.[36] The majority of these studies concur in respect of various findings. The Tei index is a new Doppler index evaluating systolic and diastolic cardiac function.[23] It is technically straightforward to obtain and has a high degree of reproducibility.[19] It has a key advantage: it allows ready measurement of right fetal heart function, a measurement that hitherto has been difficult to obtain.[14,20] The value of the MPI is significantly higher in cases of TTTS than in its absence and in the recipient fetus compared with the donor fetus.[12,20]

Most studies have evaluated global fetal cardiac function based on the MPI of right and left hearts[12,15,27,35]; only a few authors have investigated the Tei index of the left ventricle alone.[25,37] In our study, we sought to evaluate the MPI of the right heart in the recipient fetus. Indeed, the literature has clearly shown the importance of studying right heart function in the fetus.[14,20] In 43.8% of the recipient fetuses in our cohort, the right MPI was abnormal (>0.43), reflecting the high prevalence of early vascular repercussions as early as Quintero stages 1 and 2, as has already been observed.[35] The mean MPI value in our cohort was 0.43 ms, which is less than that reported in the literature[34,14,20] probably because the gestational age was close to 20 weeks, earlier than that found in the literature.[14,20,25,34,35,38] The Tei index reveals fetuses with cardiac dysfunction. These fetuses are more vulnerable, so it seems necessary to introduce a factor in the lower stages of Quintero's classification that takes account of this information. MPI appears to be a good one.

The focus of this article was the recipient twin loss. It would be useful to have also a test to predict the outcome of the donor, especially in our series where the donor loss rate is higher than the recipient loss rate in both stages 1 and 2. However, we have not analyzed the prediction of donor fetal loss on the basis of cardiovascular parameters because there is no rational pathophysiology that underlies this hypothesis. Moreover, in the CHOP score, only one item of 12 concerns the donor twin.

In our study, 60.3% of the pregnancies were treated by selective laser surgery, and only 39.7% were treated by Solomon technique. This difference can be explained by the fact that before 2010, the selective technique was preferentially used. Since 2010, patients were part of a randomized trial (selective technique vs Solomon technique), which was recently concluded.[39] In this study, there is no statistically significant difference between these two techniques ($p = 0.59$).

Concerning the evaluation of the placenta after birth, it makes sense to study only placenta with double survival and double intrauterine death. Indeed, when the laser is complicated by the loss of one of the twins early after the procedure, the placental architecture is altered given the necrotic degradation and maceration. In our study, we analyzed 42 placentas out of 58 cases (72.4%). We found residual anastomoses in 17 (40.5%) cases and residual arterio-venous anastomoses in 12 (28.5%) cases. These results are in line with the results found in other studies. Lewi *et al.*[40] found between 21% and 100% of residual anastomoses according to the outcome, and Lopriore *et al.*[41] found up to 33% of residual anastomoses.

Evaluation of the placenta after delivery does probably not reflect the physiology because the pressures used to fill the vessels are certainly higher than physiological pressure. Moreover, it is possible that anastomoses have not been visualized during the laser surgery either because they had a too small diameter or because they were still not present. The laser technique is imperfect. An alternative laser surgery technique is to use a technique in which the entire vascular equator is coagulated: the Solomon technique (vs selective coagulation technique).

The correlation we found between CHOP score and RV Tei index is probably related to the physiological relationship between both. Indeed, the CHOP score includes a simple visual assessment of Tei index by evaluation of tricuspid inflow patterns. As normal double peak inflow shifts into a single peak, the time between tricuspid valve closure and valve opening lengthens, thereby contributing to a higher Tei index value.

CONCLUSION

The Quintero classification is insufficient for determining the prognosis of recipient fetuses in TTTS. In stages 1 and 2, the incorporation of cardiovascular parameters predicted recipient fetal loss.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- The Quintero's classification for twin-to-twin transfusion syndrome (TTTS) does not integrate cardiovascular parameters. Studies evaluating the prognostic value of the Children's Hospital of Philadelphia (CHOP) score and the myocardial performance index (MPI) in TTTS, especially in lower stages, are scarce.

WHAT DOES THIS STUDY ADD?

- The CHOP score and MPI are strongly correlated with recipient survival and can predict fetal loss. They may be used in order to improve evaluation of stages 1 and 2 of TTTS.

REFERENCES

1. Feldstein VA, Filly RA. Complications of monochorionic twins. Radiol Clin North Am 2003;41(4):709–27.
2. Trevett T, Johnson A. Monochorionic twin pregnancies. Clin Perinatol 2005;32(2):475–94, viii.
3. Harkness UF, Crombleholme TM. Twin-twin transfusion syndrome: where do we go from here? Semin Perinatol 2005;29(5):296–304.
4. Sebire NJ, Snijders RJ, Hughes K, *et al.* The hidden mortality of monochorionic twin pregnancies. Br J Obstet Gynaecol 1997;104(10): 1203–7.
5. Steinberg LH, Hurley VA, Desmedt E, Beischer NA. Acute polyhydramnios in twin pregnancies. Aust N Z J Obstet Gynaecol 1990;30(3):196–200.
6. Salomon LJ, Ville Y. Twin-to-twin transfusion syndrome: diagnosis and treatment. Bull Acad Natl Med 2008;192(8):1575–86; discussion 86–7.
7. Ville Y, Van Peborgh P, Gagnon A, *et al.* Surgical treatment of twin-to-twin transfusion syndrome: coagulation of anastomoses with a Nd:YAG laser, under endosonographic control. Forty four cases. J Gynecol Obstet Biol Reprod (Paris) 1997;26(2):175–81.
8. Senat MV, Deprest J, Boulvain M, *et al.* Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. N Engl J Med 2004;351(2):136–44.
9. Yamamoto M, El Murr L, Robyr R, *et al.* Incidence and impact of perioperative complications in 175 fetoscopy-guided laser coagulations of chorionic plate anastomoses in fetofetal transfusion syndrome before 26 weeks of gestation. Am J Obstet Gynecol 2005;193(3 Pt 2):1110–6.
10. Quintero RA, Morales WJ, Allen MH, *et al.* Staging of twin-twin transfusion syndrome. J Perinatol 1999;19(8 Pt 1):550–5.
11. Taylor MJ, Govender L, Jolly M, *et al.* Validation of the Quintero staging system for twin-twin transfusion syndrome. Obstet Gynecol 2002;100(6): 1257–65.
12. Raboisson MJ, Fouron JC, Lamoureux J, *et al.* Early intertwin differences in myocardial performance during the twin-to-twin transfusion syndrome. Circulation 2004;110(19):3043–8.
13. Barrea C, Alkazaleh F, Ryan G, *et al.* Prenatal cardiovascular manifestations in the twin-to-twin transfusion syndrome recipients and the impact of therapeutic amnioreduction. Am J Obstet Gynecol 2005;192(3):892–902.
14. Rychik J, Tian Z, Bebbington M, *et al.* The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease. Am J Obstet Gynecol 2007;197(4):392 e1–8.
15. Michelfelder E, Gottliebson W, Border W, *et al.* Early manifestations and spectrum of recipient twin cardiomyopathy in twin-twin transfusion syndrome: relation to Quintero stage. Ultrasound Obstet Gynecol 2007;30(7):965–71.
16. Tei C, Dujardin KS, Hodge DO, *et al.* Doppler index combining systolic and diastolic myocardial performance: clinical value in cardiac amyloidosis. J Am Coll Cardiol 1996;28(3):658–64.
17. Shah AD, Border WL, Crombleholme TM, Michelfelder EC. Initial fetal cardiovascular profile score predicts recipient twin outcome in twin-twin transfusion syndrome. J Am Soc Echocardiogr 2008;21(10):1105–8.
18. Eidem BW, Edwards JM, Cetta F. Quantitative assessment of fetal ventricular function: establishing normal values of the myocardial performance index in the fetus. Echocardiography 2001;18(1):9–13.
19. Hernandez-Andrade E, Lopez-Tenorio J, Figueroa-Diesel H, *et al.* A modified myocardial performance (Tei) index based on the use of valve clicks improves reproducibility of fetal left cardiac function assessment. Ultrasound Obstet Gynecol 2005;26(3):227–32.
20. Ichizuka K, Matsuoka R, Hasegawa J, *et al.* The Tei index for evaluation of fetal myocardial performance in sick fetuses. Early Hum Dev 2005;81(3):273–9.
21. Raboisson MJ, Bourdages M, Fouron JC. Measuring left ventricular myocardial performance index in fetuses. Am J Cardiol 2003;91(7): 919–21.
22. Tsutsumi T, Ishii M, Eto G, *et al.* Serial evaluation for myocardial performance in fetuses and neonates using a new Doppler index. Pediatr Int 1999;41(6):722–7.
23. Tei C, Ling LH, Hodge DO, *et al.* New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. J Cardiol 1995;26(6):357–66.
24. Falkensammer CB, Paul J, Huhta JC. Fetal congestive heart failure: correlation of Tei-index and cardiovascular-score. J Perinat Med 2001;29(5): 390–8.
25. Friedman D, Buyon J, Kim M, Glickstein JS. Fetal cardiac function assessed by Doppler myocardial performance index (Tei index). Ultrasound Obstet Gynecol 2003;21(1):33–6.
26. Stirnemann JJ, Nasr B, Proulx F, *et al.* Evaluation of the CHOP cardiovascular score as a prognostic predictor of outcome in twin-twin transfusion syndrome after laser coagulation of placental vessels in a prospective cohort. Ultrasound Obstet Gynecol 2010;36(1):52–7.
27. Van Mieghem T, Klaritsch P, Done E, *et al.* Assessment of fetal cardiac function before and after therapy for twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2009;200(4):400 e1–7.
28. Quintero RA, Comas C, Bornick PW, *et al.* Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2000;16(3):230–6.

29. Lopriore E, Slaghekke F, Middeldorp JM, *et al.* Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;201(1):66 e1–4.
30. Quintero RA, Dickinson JE, Morales WJ, *et al.* Stage-based treatment of twin-twin transfusion syndrome. Am J Obstet Gynecol 2003;188(5):1333–40.
31. Huber A, Diehl W, Bregenzer T, *et al.* Stage-related outcome in twin-twin transfusion syndrome treated by fetoscopic laser coagulation. Obstet Gynecol 2006;108(2):333–7.
32. Barrea C, Hornberger LK, Alkazaleh F, *et al.* Impact of selective laser ablation of placental anastomoses on the cardiovascular pathology of the recipient twin in severe twin-twin transfusion syndrome. Am J Obstet Gynecol 2006;195(5):1388–95.
33. Bebbington MW, Tiblad E, Huesler-Charles M, *et al.* Outcomes in a cohort of patients with stage I twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2010;36(1):48–51.
34. Papanna R, Mann LK, Molina S, *et al.* Changes in the recipient fetal Tei index in the peri-operative period after laser photocoagulation of placental anastomoses for twin-twin transfusion syndrome. Prenat Diagn 2011;31(2):176–80.
35. Habli M, O'Brien T, Nowack E, *et al.* Peripartum cardiomyopathy: prognostic factors for long-term maternal outcome. Am J Obstet Gynecol 2008;199(4):415 e1–5.
36. Stirnemann JJ, Mougeot M, Proulx F, *et al.* Profiling fetal cardiac function in twin-twin transfusion syndrome. Ultrasound Obstet Gynecol 2010;35(1):19–27.
37. Van Mieghem T, Gucciardo L, Lewi P, *et al.* Validation of the fetal myocardial performance index in the second and third trimesters of gestation. Ultrasound Obstet Gynecol 2009;33 (1):58–63.
38. Acharya G, Pavlovic M, Ewing L, *et al.* Comparison between pulsed-wave Doppler- and tissue Doppler-derived Tei indices in fetuses with and without congenital heart disease. Ultrasound Obstet Gynecol 2008;31(4):406–11.
39. Slaghekke F, Lopriore E, Lewi L, *et al.* Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014, DOI: 10.1016/S0140-6736(13)62419-8.
40. Lewi L, Jani J, Cannie M, *et al.* Intertwin anastomoses in monochorionic placentas after fetoscopic laser coagulation for twin-to-twin transfusion syndrome: is there more than meets the eye? Am J Obstet Gynecol 2006;194(3):790–5.
41. Lopriore E, Slaghekke F, Middeldorp JM, *et al.* Accurate and simple evaluation of vascular anastomoses in monochorionic placenta using colored dye. J Vis Exp 2011;(55):e3208:1–7.

Ultrasound Obstet Gynecol 2014; **44**: 299–303
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.13375

Fetoscopic laser coagulation for twin–twin transfusion syndrome before 17 weeks' gestation: laser data, complications and neonatal outcome

L. LECOINTRE*, N. SANANES*†‡, A. S. WEINGERTNER*, M. KOHLER*, F. GUERRA*, G. FRITZ†, B. VIVILLE†, B. LANGER†, I. NISAND* and R. FAVRE*

**Fetal Medicine, CMCO-HUS, Schiltigheim/Strasbourg, France; †Department of Gynaecology & Obstetrics, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ‡INSERM, UMR-S 1121, 'Biomatériaux et Bioingénierie', Strasbourg, France*

KEYWORDS: fetoscopic laser therapy; monochorionic twins; TTTS

ABSTRACT

Objective *To compare laser data, complications and neonatal outcome in pregnancies that undergo 'early' (≤ 17 weeks' gestation) fetoscopic laser ablation of placental vascular anastomoses for twin–twin transfusion syndrome (TTTS) with those from 'conventional' cases treated after 17 weeks.*

Methods *This was a cohort study of data collected prospectively between January 2004 and December 2012. We included monochorionic diamniotic twin pregnancies complicated by TTTS and treated by fetoscopic laser coagulation. Pregnancies were grouped according to laser treatment ≤ 17 gestational weeks or > 17 weeks and obstetric and neonatal outcomes were compared between groups.*

Results *A total of 178 pregnancies with TTTS underwent laser therapy: 40 at or before 17 weeks and 138 after 17 weeks. There was no statistically significant difference between these two groups with respect to the rate of preterm prelabor rupture of membranes (PPROM), gestational age at PPROM and rate of PPROM occurring in the 7 days following fetoscopic laser coagulation. In the early group, the interval between performing fetoscopic laser coagulation and the time of delivery was significantly longer (104 days* vs *74 days,* P = *0.0002) and the delivery rate within 7 days of laser treatment was significantly lower (2.5%* vs *15.9%,* P = *0.026). There was no significant difference between the two groups with regard to the rates of pregnancy without live birth (15.4%* vs *15.4%,* P = *0.993), with one live birth (84.6%* vs *84.6%,* P = *0.993) and with two live births (64.1%* vs *58.1%,* P = *0.500).*

Conclusion *In the event of early TTTS, fetoscopic laser coagulation is technically feasible before 17 gestational weeks and obstetric and neonatal outcomes are comparable with those in cases of laser treatment performed after 17 weeks.* *Copyright © 2014 ISUOG. Published by John Wiley & Sons Ltd.*

INTRODUCTION

Twin–twin transfusion syndrome (TTTS) is a specific complication of multiple monochorionic pregnancies; its prevalence is thought to be between 10 and 15%[1,2]. TTTS is staged following the five-stage classification system proposed by Quintero *et al.*[3]. The global prognosis of TTTS is poor in the absence of treatment; in a retrospective study of 136 cases managed expectantly up to 28 weeks' gestation, the global survival rate was 27%, with severe neurological complications in 25% of the survivors[4]. Fetoscopic laser coagulation of vascular anastomoses has become established as the reference treatment for TTTS. The Eurofoetus multicenter randomized clinical trial[5] demonstrated that it is a more effective first-line treatment than is serial amnioreduction for severe TTTS diagnosed before 26 weeks. Also, a significant benefit was conferred on those undergoing laser coagulation, with respect to rate of survival of at least one twin (76% *vs* 56%; $P < 0.05$) as well as gestational age at delivery and short- and long-term neurological morbidity[6].

In the Eurofoetus trial, the lower cut-off age for inclusion was 16 gestational weeks[5]. Part of the rationale for this gestational age limit was related to the fact that before 16 weeks, amniochorionic fusion may not yet have occurred and vessels are very small in diameter and thus

Correspondence to: Dr L. Lecointre, Département de Gynécologie-Obstétrique, Hôpital de Hautepierre, 1 avenue Molière, 67098 Strasbourg Cedex, France (e-mail: liselecointre@hotmail.fr)

Accepted: 14 March 2014

not necessarily visible. However, improvements in surgical technique and material used (better-adapted fiberoptics), the growing experience of operators and the improving postoperative prognosis raise the question as to just how relevant this threshold now is.

Rossi and D'Addario[7], in a systematic review of literature on umbilical cord occlusion for selective feticide in monochorionic twins, showed that the survival rate was improved when surgery was performed after 18 weeks. Moreover, Stirnemann *et al.*[8], in a nomogram for perioperative prognostic risk-assessment for percutaneous fetoscopic laser coagulation in TTTS, showed that 18 + 6 weeks is a prognostic cut-off for the risk of twin loss. However, Baud *et al.*[9] recently published pre- and postnatal characteristics of newborns showing that those which had undergone early fetoscopic laser coagulation before 17 weeks had similar outcomes to those undergoing later procedures.

The objective of our study was to compare fetuses undergoing laser coagulation before and after 17 gestational weeks, with regard to laser data, complications and neonatal outcome.

SUBJECTS AND METHODS

This was a cohort study on data collected prospectively between January 2004 and December 2012. Included were monochorionic diamniotic twin pregnancies complicated by TTTS and treated by fetoscopic laser coagulation at the Schiltigheim medicosurgical and obstetric center (CMCO), part of Strasbourg University Hospitals, France. This study was approved by the French data protection authority (CNIL) under number 1689910.

Chorionicity was diagnosed on ultrasound examination in the first trimester by absence of the lambda sign. TTTS was diagnosed on ultrasound by the association of oligohydramnios (deepest vertical pocket < 2 cm) in the donor and polyhydramnios (deepest vertical pocket > 8 cm if the pregnancy was < 20 gestational weeks or > 10 cm if it was > 20 weeks) in the recipient cotwin[10,11]. The severity of TTTS was graded using the Quintero classification[3]. In our practice, fetoscopic laser coagulation is proposed routinely for all 'severe' stages (Stages II–IV) and for Stage I in the event of massive polyhydramnios of the recipient and/or poor maternal tolerance of this polyhydramnios.

Fetoscopic laser coagulation was performed under maternal sedation with 100 mg Atarax® (Hydroxyzine) if required, according to the patient's psychological condition, the evening before surgery, and administration of 5 mg Hypnovel® (Midazolam) and 100 mg Atarax *per os* in all cases, 2 h before the procedure. We also applied local anesthesia to the skin and uterine wall (1% Xylocaine® (Lidocaïne)). The entire operation was conducted under ultrasound guidance. The procedure was carried out using a Storz 1.3–2.0-mm diameter fetoscope with a right-angled or 30° inclined optical device passing through a right-angled or curved sheath depending on the placental location. Depending on the size of the fetoscope, 8–12-French-gauge trocars were used. Coagulation was achieved using a 400-μm laser fiber with a maximum power of 40 W, which was fed through the fetoscope sheath. At the beginning of the procedure, vascular anastomoses on the placental surface were mapped, after identification of the interamniotic membrane, and then coagulated. Repeat cartography at the end of the procedure allowed us to check that all the anastomoses were coagulated and none had become patent again. Two types of photocoagulation techniques could be used: either a pure selective technique, as described by Quintero *et al.*[12], or the Solomon technique, described by the Leiden team[13], in which the entire vascular equator is coagulated. Antibiotic prophylaxis with 1 g Clamoxyl® (Amoxicilline) intravenously was administered intraoperatively. At the end of the procedure, amniodrainage was conducted in order to reduce the largest cisternal pocket in the recipient to 5 cm or less. Tocolysis was not done routinely. Parenteral treatment with antispasmodic was administered in the event of pelvic pain. Follow-up checks of TTTS status, fetal vitality and hemodynamic criteria were carried out by ultrasound including Doppler 24 and 48 h postoperatively. If progress was favorable, follow-up at 1 week was scheduled, with conventional follow-up thereafter every 2 weeks. Delivery was arranged for around 35 weeks.

Our study population was divided into two groups on the basis of gestational age at the time of fetoscopic laser coagulation. The 'early laser' group included all pregnancies with a gestational age ≤ 17 weeks at laser coagulation, and the 'conventional laser' group all those aged over 17 weeks.

The Food and Drug Administration in the USA prohibits the use of fetoscopic techniques prior to 16 weeks. For this reason, we felt it important to provide separately details of the cases treated at or before 16 weeks.

Clinical features of the patients, TTTS classification, fetoscopic laser coagulation data, complications and neonatal outcome were obtained prospectively from our hospital database. Most patients delivered in the treatment center. For patients who delivered elsewhere, an accurate delivery report was obtained.

Descriptive data are presented as mean and SD for continuous variables and as numbers and percentages for categorical variables. Univariate analysis consisted of comparing both groups by means of Student's *t*-tests for continuous variables and chi-square tests for categorical variables. $P < 0.05$ was considered statistically significant. SAS 9.3 statistical software (SAS Institute Inc., Cary, NC, USA) was used for all data analyses.

RESULTS

Between January 2004 and December 2012, there were 178 cases of TTTS treated by fetoscopic surgery with laser coagulation of placental vascular anastomoses. Forty (22.5%) TTTS cases were in the early-laser group (coagulation at or before 17 weeks) and 138 (77.5%) were in the conventional-laser group (coagulation after 17 weeks). Univariate analysis of maternal and obstetric

Table 1 Maternal and obstetric characteristics, laser data, obstetric outcome and live births in 178 monochorionic diamniotic twin pregnancies with twin–twin transfusion syndrome (TTTS) treated by fetoscopic laser coagulation at ≤ 17 ('early') or > 17 ('conventional') gestational weeks

Variable	*Early (n = 40)*	*Conventional (n = 138)*	P
Maternal and obstetric characteristics			
Maternal age (years)	32.0 ± 5.9	29.4 ± 5.3	0.170
Nulliparous	12 (30.0)	56 (40.6)	0.225
TTTS stage			0.004
I	1 (2.5)	33 (23.9)	
II	21 (52.5)	61 (44.2)	
III	18 (45.0)	37 (26.8)	
IV	0 (0)	7 (5.1)	
Fetoscopic laser coagulation data			
Gestational age at procedure (days)	114 ± 6	147 ± 18	< 0.0001
Placenta			0.231
Anterior	14 (35.0)	63 (45.7)	
Posterior	26 (65.0)	75 (54.3)	
Cervical length (mm)	41.9 ± 9.2	38.3 ± 11.4	0.043
Cerclage	0 (0)	3 (2.2)	0.347
Procedure time (min)	24.4 ± 9.7	28.9 ± 10.8	0.014
Endoscope			< 0.0001
1.3 mm	22 (55.0)	5 (3.6)	
2 mm	18 (45.0)	133 (96.4)	
Approach			0.473
Central	22 (55.0)	67 (48.6)	
Lateral	18 (45.0)	71 (51.4)	
Number of vessels coagulated	8.3 ± 3.4	10.2 ± 4.7	0.017
Technique			0.432
Conventional	32 (80.0)	102 (73.9)	
Solomon	8 (20.0)	36 (26.1)	
Obstetric outcome			
PPROM	7 (18.4)	24 (17.9)	0.940
Gestational age at PPROM (days)	219 ± 21	199 ± 35	0.087
Laser–PPROM interval (days)	103 ± 20	57 ± 32	0.0005
PPROM < 7 days after laser	0 (0)	2 (11.1)	0.358
PPROM < 32 gestational weeks	3 (42.9)	13 (65.0)	0.305
Gestational age at delivery (days)	218 ± 45	221 ± 37	0.709
Laser–delivery interval (days)	104 ± 43	74 ± 41	0.0002
Delivery < 7 days after laser	1 (2.5)	22 (15.9)	0.026
Delivery < 32 gestational weeks	14 (35.9)	59 (44.7)	0.329
Delivery route			0.383
Cesarean	24 (61.5)	73 (53.7)	
Vaginal	15 (38.5)	63 (46.3)	
Donor birth weight (g)	1309 ± 761	1219 ± 807	0.523
Recipient birth weight (g)	1376 ± 816	1556 ± 858	0.236
TAPS	1 (2.5)	6 (4.4)	0.597
Live births			
None	6 (15.4)	21 (15.4)	0.993
One	8 (20.5)	36 (26.5)	0.736
At least one	33 (84.6)	115 (84.6)	0.993
Two	25 (64.1)	79 (58.1)	0.500
Donor	30 (76.9)	93 (68.4)	0.304
Recipient	28 (71.8)	101 (74.3)	0.757

Data are given as mean ± SD or *n* (%). PPROM, preterm prelabor rupture of membranes; TAPS, twin anemia–polycythemia sequence.

characteristics, of fetoscopic laser coagulation data and of obstetric outcomes and live birth is displayed in Table 1.

In the early-laser group, the gestational age at the time of the procedure was significantly lower than that in the conventional-laser group: 16 + 2 *vs* 21 weeks ($P < 0.0001$). The procedure time was also significantly shorter (24.4 *vs* 28.9 min, $P = 0.014$), the number of vessels coagulated significantly fewer (8.3 *vs* 10.2, $P = 0.017$) and the 1.3-mm endoscope used significantly more often (55% *vs* 3.6%, $P < 0.0001$). TTTS was also significantly more advanced in terms of the Quintero classification ($P = 0.004$).

We did not observe any significant difference with regard to the rate of preterm prelabor rupture of membranes (PPROM) before 32 weeks between the early-laser group and the conventional-laser group (42.9% *vs* 65%,

Table 2 Outcomes of the subgroup of monochorionic diamniotic twin pregnancies with twin–twin transfusion syndrome (TTTS) that were treated by fetoscopic laser coagulation prior to 16 weeks' gestation

Case	GA at laser therapy (weeks)	TTTS stage	Placental position	Duration of surgery (min)	PPROM	GA at delivery (weeks)	Adverse outcome	
							Recipient	Donor
1	13.6	III	Posterior	20	No	16.3	IUFD	IUFD
2	15.1	II	Posterior	17	No	28	—	—
3	15.2	II	Posterior	6	Yes	21	IUFD	IUFD
4	15.4	III	Anterior	21	No	36.3	—	—
5	15.5	III	Posterior	15	No	17.5	IUFD	IUFD
6	16	III	Posterior	20	No	32	—	—
7	16	III	Posterior	25	No	35	IUFD	—
8	16	III	Anterior	28	No	19	IUFD	IUFD
9	16	II	Anterior	11	No	38.2	IUFD	—
10	16	II	Posterior	37	No	37.2	—	—
11	16	III	Anterior	22	No	35	—	—

GA, gestational age; IUFD, intrauterine fetal death; NND, neonatal death; PPROM, preterm prelabor rupture of membranes.

$P = 0.305$). Moreover, there was also no statistically significant difference between these two groups with respect to PPROM rate, gestational age at PPROM and PPROM rate in the 7 days following fetoscopic laser coagulation. This may be explained by the fact that we tended to use small-diameter endoscopes (1.3 mm) for procedures carried out before 17 weeks. However, among the early-laser group, there was no significant difference in the rate of PPROM according to the diameter of endoscope used (1.3 or 2 mm): 23% *vs* 12%, $P = 0.34$.

Concerning the delivery rate before 32 weeks and gestational age at delivery, there was no significant difference between the early-laser group and the conventional-laser group (35.9% *vs* 44.7%, $P = 0.329$ and 218 days *vs* 221 days, $P = 0.709$). However, in the early-laser group, the interval between the fetoscopic laser coagulation procedure and delivery was significantly longer (104 days *vs* 74 days, $P = 0.0002$) and the delivery rate within 7 days of the procedure was significantly lower (2.5% *vs* 15.9%, $P = 0.026$).

We did not find any significant difference between the two groups with regard to the rate of pregnancies without live birth (15.4% *vs* 15.4%, $P = 0.993$), with at least one live birth (84.6% *vs* 84.6%, $P = 0.993$) or with two live births (64.1% *vs* 58.1%, $P = 0.500$).

Table 2 reports pregnancies that were treated with laser therapy at ≤ 16 gestational weeks. Among these 11 cases, four (36.4%) were Stage II and seven (63.6%) were Stage III. Only one (9%) case was complicated by PPROM within 7 days of laser therapy. Of the 22 fetuses, 12 (54.5%) were liveborn.

DISCUSSION

Our experience shows that 'early' fetoscopic laser coagulation in the treatment of TTTS is feasible before 17 gestational weeks. We did not observe any significant difference with respect to obstetric and neonatal outcomes compared with laser coagulation after 17 weeks.

The Food and Drug Administration recommends that fetoscopic laser coagulation be applied solely for treating severe TTTS between 16 and 26 gestational weeks[14,15]. In 2009, the French National College of Gynecologists and Obstetricians reported that treatment by fetoscopic laser has been shown to be the best first-line treatment for TTTS before 26 weeks, but that its use before 17 weeks had not been studied[16,17]. The study by Baud *et al.*[9] in 2013 was the first to evaluate the feasibility of using laser before 17 weeks in the treatment of twin pregnancies complicated by severe TTTS. It should be noted that we evaluated not the utility of performing this procedure but its feasibility and its consequences in terms of maternal and fetal outcomes.

The large cohort size of our series, with 40 pregnancies undergoing early laser treatment, is the key feature of our study. Fetoscopic laser coagulation was performed in the event of advanced-stage TTTS or Stage I with major polyhydramnios and/or poor maternal tolerance of this polyhydramnios. In the group receiving laser treatment before 17 weeks, TTTS stages were significantly more severe.

We did not analyze data relating to fetoscopic laser coagulation after 26 weeks, which was applicable to only four cases. However, we did not exclude them from statistical analysis since, according to Baud *et al.*[9], the prognosis in this group is no different from that of the rest of the population. In addition, in our experience, the conditions of access to the chorionic surface are not modified at this time, and we have never had any difficulty in coagulating vessels of large diameter.

PPROM is the most feared complication when performing fetoscopic laser coagulation before 17 weeks, because the amnion and chorion may not yet have fused at this age. In our study, the PPROM rate was comparable in pregnancies with laser coagulation performed before and in those in which it was performed after 17 weeks: 18.4 *vs* 17.9% ($P = 0.94$). Habli *et al.*[18] found a comparable rate of PPROM (17.8%) but did not specify the lowest gestational age at which laser was performed. In the study by Baud *et al.*[9], the PPROM rate was linked inversely to the gestational age at which fetoscopic laser coagulation was performed (38% before 16 weeks; 19% between 16 and 17 weeks and 6% after 17 weeks), reflecting the degree of amnion–chorion fusion, although these results

should be seen in the light of the very small sample sizes in each of their three groups.

We perform amnioinfusion only in very rare (1.1%) cases, whereas Baud *et al.*'s team carried out amnioinfusion routinely. Although amnioinfusion may facilitate visualization of the placenta, it is not impossible that iatrogenic distension of the uterine cavity increases the risk of membrane rupture. However, in our work, as in that of Baud *et al.*, there was no increase in the number of premature deliveries nor any difference in terms of live-birth rate in pregnancies which underwent early laser coagulation compared with the group treated by laser after 17 gestational weeks.

In conclusion, in the event of early TTTS, fetoscopic laser coagulation is technically feasible before 17 gestational weeks. In these cases, obstetric outcome and live-birth rate were comparable with those of cases in which laser coagulation was performed after 17 weeks. We found no significant difference between the two groups with respect to the rate of PPROM, gestational age at PPROM, rate of PPROM within 7 days of fetoscopic laser coagulation, rate of delivery before 32 weeks and gestational age at delivery, and no difference with regard to rates of pregnancy with no live births, with at least one live birth or with two live births.

REFERENCES

1. Lewi L, Gucciardo L, Van Mieghem T, de Koninck P, Beck V, Medek H, Van Schoubroeck D, Devlieger R, De Catte L, Deprest J. Monochorionic diamniotic twin pregnancies: natural history and risk stratification. *Fetal Diagn Ther* 2010; **27**: 121–133.
2. Fisk NM, Duncombe GJ, Sullivan MH. The basic and clinical science of twin–twin transfusion syndrome. *Placenta* 2009; **30**: 379–390.
3. Quintero RA, Morales WJ, Allen MH, Bornick PW, Johnson PK, Kruger M. Staging of twin–twin transfusion syndrome. *J Perinatol* 1999; **19**: 550–555.
4. Berghella V, Kaufmann M. Natural history of twin–twin transfusion syndrome. *J Reprod Med* 2001; **46**: 480–484.
5. Senat MV, Deprest J, Boulvain M, Paupe A, Winer N, Ville Y. Endoscopic laser surgery versus serial amnioreduction for severe twin-to-twin transfusion syndrome. *N Engl J Med* 2004; **351**: 136–144.
6. Salomon LJ, Örtqvist L, Aegerter P, Bussieres L, Staracci S, Stirnemann J, Essaoui M, Bernard JP, Ville Y. Long-term developmental follow-up of infants who participated in a randomized clinical trial of amniocentesis vs laser photocoagulation for the treatment of twin-to-twin transfusion syndrome. *Am J Obstet Gynecol* 2010; **203**: 444.e1–7.
7. Rossi AC, D'Addario V. Umbilical cord occlusion for selective feticide in complicated monochorionic twins: a systematic review of literature. *Am J Obstet Gynecol* 2009: **200**: 123–129.
8. Stirnemann JJ, Nasr B, Essaoui M, Bussieres L, Ville Y. A nomogram for perioperative prognostic risk-assessment in twin–twin transfusion syndrome. *Prenat Diagn* 2013; **33**: 103–108.
9. Baud D, Windrim R, Keunen J, Kelly EN, Shah P, van Mieghem T, Seaward PG, Ryan G. Fetoscopic laser therapy for twin–twin transfusion syndrome before 17 and after 26 weeks' gestation. *Am J Obstet Gynecol* 2013; **208**: 197.e1–7.
10. Van Mieghem T, Baud D, Devlieger R, Lewi L, Ryan G, De Catte L, Deprest J. Minimally invasive fetal therapy. *Best Pract Res Clin Obstet Gynaecol* 2012; **26**: 711–725.
11. Gratacos E, Deprest J. Current experience with fetoscopy and the Eurofoetus registry for fetoscopic procedures. *Eur J Obstet Gynecol Reprod Biol* 2000; **92**: 151–159.
12. Quintero R, Comas C, Bornick PW, Allen MH, Kruger M. Selective versus non-selective laser coagulation of placental vessels in twin-to-twin transfusion syndrome. *Ultrasound Obstet Gynecol* 2000; **16**: 230–236.
13. Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP. Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. *Am J Obstet Gynecol* 2009; **201**: 66.e1–4.
14. US Food and Drug Administration (2010). Humanitarian device exemption overview. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/PremarketSubmissions/HumanitarianDeviceExemption/default.htm [Accessed 12 December 2012].
15. US Food and Drug Administration (2009). Karl Storz rigid TTTS fetoscopy instrument set–H040005. http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm078275.htm [Accessed 12 December 2012].
16. Stirnemann J, Essaoui M, Ville Y. Extrait des mises à jour en Gynécologie et Obstétrique. Tome XXXIII. CNGOF (Collège National des Gynécologues et Obstétriciens Français): Paris, 2009.
17. Quarello E, Molho M, Ville Y. Incidence mechanisms, and patterns of fœtal cerebral lesions in twin-to-twin transfusion syndrome. *J Matern Fetal Neonatal Med* 2007; **20**: 589–597.
18. Habli M, Bombrys A, Lewis D, Lim FY, Polzin W, Maxwell R, Crombleholme T. Incidence of complications in twin–twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. *Am J Obstet Gynecol* 2009; **201**: 417.e1–7.

DOI: 10.1002/pd.4545 PRENATAL DIAGNOSIS

ORIGINAL ARTICLE

Evaluation of prenatal and postnatal diagnostic criteria for twin anemia-polycythemia sequence

Marine Veujoz[1,2]*, Nicolas Sananès[1,2,3], François Severac[2,4], Nicolas Meyer[2,4], Anne-Sophie Weingertner[1,2], Monique Kohler[1,2], Fernando Guerra[1,2], Adrien Gaudineau[1,2], Israël Nisand[1,2] and Romain Favre[1,2]

[1]Department of Obstetrics and Gynecology, Strasbourg University Teaching Hospital, Strasbourg, France
[2]Federation of Translational Medicine, Strasbourg University Teaching Hospital, Strasbourg, France
[3]INSERM, UMR-S 1121, 'Biomatériaux et Bioingénierie', Strasbourg, France
[4]Department of Public Health, Strasbourg University Teaching Hospital, Strasbourg, France
*Correspondence to: Marine Veujoz. E-mail: veujozm@hotmail.com

ABSTRACT

Objective The aim of this study is to analyze the relevance of the prenatal and postnatal diagnostic parameters of twin anemia-polycythemia sequence (TAPS).

Methods Diagnostic data of all cases of TAPS followed in our institution between 2006 and 2013 were reviewed. Statistical analyses were conducted using Bayesian methods.

Results Twenty cases of TAPS were included. We found a relationship between the hemoglobin level and the middle cerebral artery peak systolic velocity (coefficient −0.25 [−0.34, −0.15], Pr(coef < 0) = 99.99%). Sensitivity and specificity of the prenatal diagnosis were 71% and 50%, respectively, regarding the correspondence with postnatal diagnosis. There was no correlation between the number [odds ratio (OR) = 0.89 [0.72, 1.10], Pr(OR > 1) = 14.8%)], the mean diameter (OR = 0.98 [0.32, 3.06], Pr(OR > 1) = 48.9%), or the total diameter (OR = 0.79 [0.36, 1.53], Pr(OR > 1) = 26.3%) of arteriovenous anastomoses and the severity of TAPS.

Conclusion Middle cerebral artery peak systolic velocity is a reliable tool for estimating the hemoglobin level in cases of TAPS. The correspondence between prenatal and postnatal diagnosis is imperfect. Further studies are required to evaluate opportunity of widening postnatal diagnostic criteria. © 2014 John Wiley & Sons, Ltd.

Funding sources: None
Conflicts of interest: None declared

INTRODUCTION

The twin anemia-polycythemia sequence (TAPS) is a rare complication of monochorionic diamniotic twin pregnancies first described by Lopriore in 2007,[1] in which anemia in one fetus or newborn is associated with polycythemia in the twin. It may occur spontaneously (incidence estimated at 1–5%)[2–5] or following a laser procedure for twin–twin transfusion syndrome (TTTS) (incidence estimated at 2–13%).[4–7] The diagnosis may be made prenatally or postnatally by means of the criteria proposed in 2010 by Slaghekke.[8] During the prenatal period, it is based on measurement of the middle cerebral artery peak systolic velocity (MCA-PSV) in both fetuses. In the postnatal period, it is inferred from a discrepancy in the hemoglobin (Hb) and reticulocyte levels, as well as from a specific angioarchitecture marked by the presence of minute arteriovenous (AV) anastomoses and by slender and infrequent superficial anastomoses.[9]

In view of the recent description of the pathology and its low incidence, cohorts in the literature are few and far between.[2–4,6,8,10–23] In particular, there are scanty data on the relevance of prenatal or postnatal diagnostic criteria. The diagnostic value of the MCA-PSV has been studied in cases of immune[24] and nonimmune anemia[25–28] but not with TAPS, notably to evaluate polycythemia. To the best of our knowledge, the degree of fit between prenatal and postnatal diagnoses and the correlation between placental angioarchitecture and disease severity have never been assessed. The principal objective of our study was to analyze the relevance of the prenatal and postnatal diagnostic parameters of TAPS. Its secondary objective was to compare these factors between spontaneous and postlaser TAPS.

METHODS

We conducted a prospective single-center cohort study enrolling all monochorionic diamniotic twin pregnancies complicated by TAPS diagnosed prenatally or postnatally that were managed at the gynecology-obstetrics hub of the

Strasbourg University Teaching Hospitals between December 2006 and August 2013.

The selected diagnostic criteria for TAPS were those drawn up by Slaghekke.[8] In the prenatal period, they associate the absence of the twin oligo-polyhydramnios sequence with measurement of a MCA-PSV greater than 1.5 multiples of median (MoM) in one fetus (anemic, donor) and less than 1 MoM in the other fetus (polycythemic, recipient). The MCA-PSV was measured using the technique described by Mari[24] using Voluson 730 or E8 General Electric® devices. We used nomograms established by the same team to calculate MoM out of MCA-PSV.[29] In the postnatal period, the diagnosis is based on an intertwin Hb difference of more than 8 g/dL combined with a reticulocyte ratio of more than 1.7 or the finding of inframillimetric anastomoses. Cases that showed discrepant Hb levels at birth of more than 8 g/dL but did not meet the other postnatal criteria were also enrolled in the study if there was a strong clinical impression including no evidence for an acute peripartum TTTS (characterized by normal reticulocyte count for the anemic twin and constant presence of superficial anastomoses).[30] The severity of TAPS was defined using Slaghekke's classification.[8]

Follow-up of each monochorial diamniotic pregnancy consisted in an ultrasound every 2 weeks, to search signs of complications such as TTTS and TAPS. In case of diagnosis of TAPS before 32 weeks of gestation (WG), *in utero* treatment was proposed in TAPS stage 3 or 4 or in case of stage 1 or 2 with rapid aggravation. We opted for fetoscopy with laser coagulation of the placental anastomoses. If the technical conditions were not favorable (anterior lying placenta that was difficult to access), *in utero* transfusion was performed. After 32 WG, therapeutic options were expectant management or delivery. Laser photocoagulation (for TTTS or TAPS) was carried out using a pure selective technique as described by Quintero[31] or the Solomon technique in which the entire vascular equator is coagulated.[32,33] Follow-up checks were carried out 24 and 48 h postoperatively, at 1 week and then every 2 weeks.

Placentas were kept without preparation before analysis. The umbilical vessels were catheterized for injection of coloration dye and then clamped to maintain optimal filling of the vessels. Photographs of both surfaces of the placenta were taken using a Sony-DSC-W220 digital apparatus (12.1 megapixels). Images obtained were processed using the Image Tool Windows software version 3-0 in order to determine the number and diameter of the anastomoses [AV, venovenous, arterioarterial (AA), and venoarterial (VA)]. The venous branch was measured for AV and VA anastomoses.

Demographic data, prenatal and postnatal diagnostic findings, prenatal care, and obstetrical and neonatal outcomes were collated prospectively. Cases of TAPS were classified as spontaneous or iatrogenic TAPS depending on their mode of onset.

In order to study the correlation between measurement of the MCA-PSV and Hb level, we made use of the MCA-PSV values measured prior to possible *in utero* transfusion or in the 48-h period before delivery. Hb assay was carried out during each *in utero* transfusion procedure and at birth.

To analyze the relationship between the number of AV anastomoses and the disease stage, we factored in the number of anastomoses coagulated during the procedure for patients treated by laser or the number of anastomoses visualized on postnatal placental analysis and the prelaser stage or the postnatal stage for patients not treated with laser.

Continuous variables are expressed as mean and standard deviation. Categorical variables are expressed with numbers and percentages. Statistical analysis were conducted using Bayesian methods[34,35] with R 3.0.0 software and all required packages in their latest updates at the time of analysis and WinBUGS software (estimation using Markov chain Monte Carlo with Gibbs sampling). After a burn-in of 5000 updates, 100 000 iterations were performed, and convergence was checked using trace plots of the sample values for each iteration. Beta distributions (Be(a,b)) were used to estimate proportions, while means were estimated using Normal distributions (N (m, standard deviation)). When available, results from previous studies were used to provide informative Bayesian priors. In the absence of prior information for estimation of a proportion, a noninformative (Be(1,1)) prior was adopted. Each result is presented as a difference with a 95% credibility interval and a probability of exceeding 0 (or 1 for an odds ratio (OR)). We remind that Bayesian analysis does not use the (frequentist) *p*-value and that the probability of exceeding 0 or 1 must not be confused with a *p*-value. Priors are detailed in the Section on Results.

This study received the authorization of the French Commission Nationale de l'Informatique et des Libertés (CNIL) and is registered as no. 1728548.

RESULTS

Our population is described in Figure 1. In all, 20 patients developed TAPS during the inclusion period, that is, a global incidence calculated at 4.6% (433 patients followed for monochorionic diamniotic twin pregnancy during the inclusion period). TAPS occurred spontaneously in half of the cases, that is, an incidence of spontaneous TAPS calculated at 3.7% (272 patients not treated by laser for TTTS) and an incidence of iatrogenic TAPS calculated at 6.2% (161 patients treated by laser for TTTS, 110 using the selective technique and 51 using Solomon's technique). When laser was used for TTTS, the incidence of TAPS was 8.2% for the selective technique (nine cases) and 2% for the Solomon technique (one case).

The diagnosis was made prenatally in 17 cases (85%) at a mean gestational age of 174 +/−41 days of gestation, with mean MCA-PSV at 1.8 MoM (+/−0.3) for anemic fetuses and 0.7 MoM (+/−0.1) for polycythemic fetuses. *In utero* management included laser treatment in five spontaneous TAPS and two iatrogenic TAPS (seven cases, 41.1%), *in utero* transfusion in two iatrogenic cases (11.8%), and expectant management or fetal extraction in eight cases (47.1%). Maternal and prenatal data are displayed in Table 1.

Three cases complicated by *in utero* fetal death of one or both twins were excluded from the postnatal analysis: double fetal demise complicating a stage 4 TAPS at 14 WG +1 day and demise of the anemic fetus in two cases of spontaneous TAPS within the day after laser therapy at 19 and 20 WG (prelaser

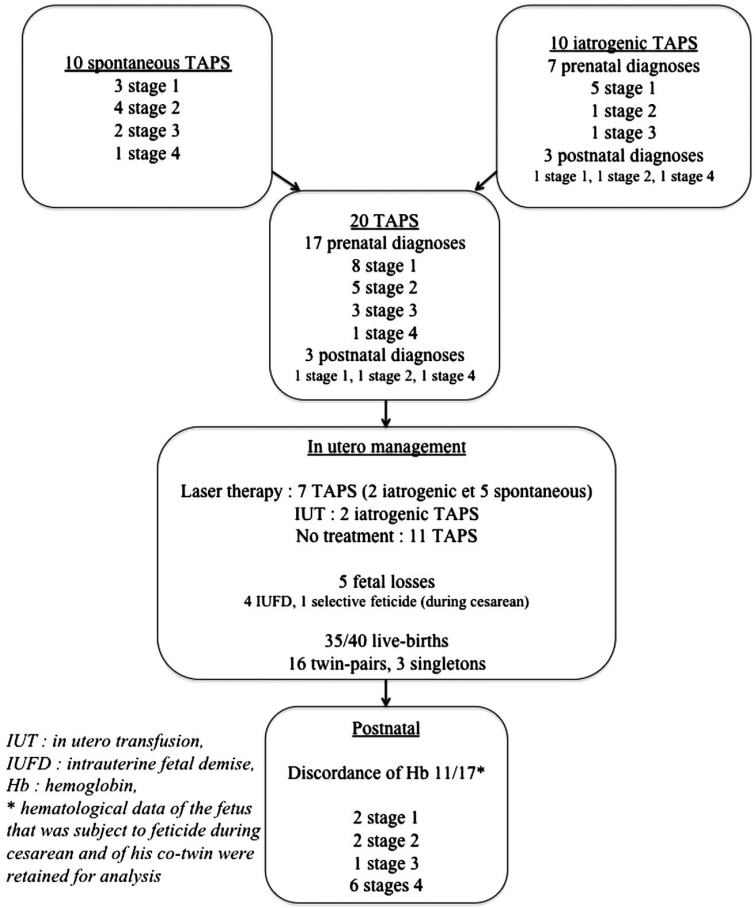


Figure 1 Description of population

Table 1 Maternal ($n = 20$) and prenatal diagnostic ($n = 17$) data

	Total	Spontaneous TAPS	Iatrogenic TAPS
Age (mean/SD)	29.3 (+/−4)	28.6 (+/−4)	30 (+/−4)
Nulliparity (*n*, %)	9/20 (45%)	2/10 (20%)	7/10 (70%)
BMI (mean, SD)	23.4 (+/−5.1)	20.6 (+/−2.7)	26.3 (+/−5.4)
GA at diagnosis (days) (mean, +/−SD) ($n = 17$)	174 (+/−41)	173 (+/−51)	176 (+/−21)
Prenatal stage 1 (*n*, %)	8/17 (47%)	3/10 (30%)	5/7 (71.4%)
Prenatal stage 2 (*n*, %)	5/17 (29.4%)	4/10 (40%)	1/7 (14.3%)
Prenatal stage 3 (*n*, %)	3/17 (17.7%)	2/10 (20%)	1/7 (14.3%)
Prenatal stage 4 (*n*, %)	1/17 (5.9%)	1/10 (10%)	0
Anemic MCA-PSV at diagnosis (MoM) (mean, +/−SD)	1.8 (+/−0.3)	1.9 (+/−0.3)	1.7 (+/−0.2)
Polycythemic MCA-PSV at diagnosis (MoM) (mean, +/−SD)	0.7 (+/−0.1)	0.7 (+/−0.1)	0.8 (+/−0.1)

TAPS, twin anemia-polycythemia sequence; SD, standard deviation; BMI, body mass index; GA, gestational age; MCA-PSV, middle cerebral artery peak systolic velocity; MoM, multiples of median.

PSV of anemic fetuses: 1.73 and 2.38 MoM). One anemic fetus was subject to feticide during cesarean because of ischemic cerebral lesions. Intracordonal injection of potassium chloride and barbituric was realized before extraction of the infant subject to feticide as this is authorized by French legislation. Birth weight and hematological data of this infant and of its cotwin were retained for analysis. In all, 19 polycythemic or formerly polycythemic infants and 16 anemic or formerly anemic infants were live births. Neonatal data are presented in Table 2 and therefore concern 17 twin pairs. On average,

Table 2 **Neonatal data (*n* = 17/*N* = 34)**

	Total	Spontaneous TAPS (*n* = 7)	Iatrogenic TAPS(*n* = 10)
GA at delivery (days) (mean, +/−SD)	225 (+/−13)	231 (+/−8)	221 (+/−15)
Anemic BW (g) (mean, +/−SD)	1370 (+/−384)	1498 (+/−368)	1281 (+/−388)
Polycythemic BW (g) (mean, +/−SD)	1628 (+/−386)	1828 (+/−360)	1487 (+/−354)
No TAPS	6/17 (35.3%)	4/7 (57.1%)	2/10 (20%)
Postnatal stage 1 (*n*, %)	2/17 (11.8%)	0	2/10 (20%)
Postnatal stage 2 (*n*, %)	2/17 (11.8%)	1/7 (14.3%)	1/10 (10%)
Postnatal stage 3 (*n*, %)	1/17 (5.8%)	0	1/10 (10%)
Postnatal stage 4 (*n*, %)	6/17 (35.3%)	2 (28.6%)	4 (40%)
Anemic Hb (g/dL) (mean, +/−SD)	9.2 (+/−4.8)	11.3 (+/−5.8)	7.7 (+/−3.5)
Polycythemic Hb (g/dL) (mean, +/−SD)	19.5 (+/−3.8)	18.8 (+/−3.6)	20 (+/−4)
Reticulocyte ratio (mean, +/−SD)	1.30 (+/−0.57)	1.4 (+/−0.62)	1.24 (+/−0.55)

TAPS, twin anemia-polycythemia sequence; *n*, number of cases; *N*, number of children; SD, standard deviation; GA, gestational age; BW, birth weight.

patients delivered at 225 +/−13 days, with a mean Hb level of 9.2 +/−4.8 g/dL for the 'anemic' group and 19.5 +/−3.8 g/dL for the 'polycythemic' group.

Nine cases did not present postnatal TAPS, because of resolution after laser treatment (four cases), resolution after *in utero* transfusion (one case), one 'false positive' case (spontaneous resolution?), and *in utero* death of one or both fetuses (three cases).

Of the 11 cases presenting postnatal TAPS, eight cases were diagnosed prenatally and three cases only postnatally. Four of these cases did not strictly meet the postnatal criteria as set out by the Leiden team but were nevertheless considered as being TAPS because of a strong clinical presumption reinforced by the lack of evidence of acute peripartum transfusion (Table 3). In these four cases, there was a discrepancy in the Hb levels of more than 8 g/dL. Delivery took place by Cesarean section in all cases, and superficial anastomosis (AA or venovenous) was absent in two cases, which does not suggest an acute peripartum transfusion syndrome. Furthermore, the two-toned placental appearance, which is typical of TAPS,[12] was found in 100% of the placentas analyzed, with the presence of at least 1 AV anastomosis per placenta. In two cases (cases 1 and 2), prior prenatal TAPS diagnosis indicated a chronic disease. Another patient (case 3) was diagnosed with TTTS at 20 WG, with consecutive laser therapy. Last MCA-PSV measurement was performed 2 weeks before delivery and was normal. At birth, Hb discordance was 17 g/dL, and we found two juxtamillimetric anastomoses (one AV and one VA). Last patient (case 4) was diagnosed with TTTS at 18 WG with consecutive difficult and incomplete laser therapy. Subsequent follow-up for MCA-PSV was into normal ranges. At birth, Hb discordance was 9.1 g/dL, and reticulocyte ratio was 1.62.

The Hb level was linearly linked to the MCA-PSV value (23 measurements made in 18 fetuses, anemic or polycythemic) (Figure 2). Mixed model Bayesian linear regression found a coefficient associated with the MCA-PSV variable that was calculated to be −0.25 [−0.34, −0.15], $Pr(coef < 0) = 99.99\%$. The correlation established on the basis of our data was as follows: $Hb = 25.67 - 0.2474 \times MCA\text{-}PSV$.

Regarding the correspondence between prenatal and postnatal diagnosis, data were analyzed from nine patients (MCA-PSV measured less than 48 h before delivery). Sensitivity and specificity of the prenatal diagnosis of TAPS were, respectively, 71% and 50%. False negative and false positive rates were, respectively, 66.7% (two cases) and 16.7% (one case) with a positive predictive value of 83%, a negative predictive value of 33%, a positive likelihood ratio of 1.42, and a negative likelihood ratio of 0.58.

Among the 20 cases of TAPS, we found at least one AV anastomosis in 16 cases, either during laser treatment (seven cases) or during postnatal examination of the placenta (nine cases). The other four cases corresponded to double intra

Table 3 **Features of the four atypical cases**

Case no.	Prenatal diagnosis	Delivery	Delta Hb (g/dL)	Reticulocyte ratio	Placental architecture	Placental aspect
1	YES	Cesarean	17.4	0.19	/	/
2	YES	Cesarean	10.9	1.2	1 AV = 1.19 mm	Bicolor
3	NO	Cesarean	17.3	0.86	1 AV = 1.31 mm	Bicolor
					1 VA = 1.31 mm	
4	NO	Cesarean	9.10	1.62	3 AV = 7.29 mm	Bicolor
					2 VA = 4.24 mm	
					2 AA = 4.05 mm	

Hb, hemoglobin; AV, arteriovenous; VA, venoarterial; AA, arterioarterial.

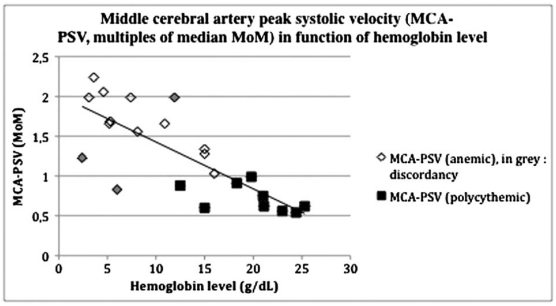


Figure 2 Middle cerebral artery peak systolic [(MCA-PSV, multiples of median (MoM)] in function of hemoglobin level

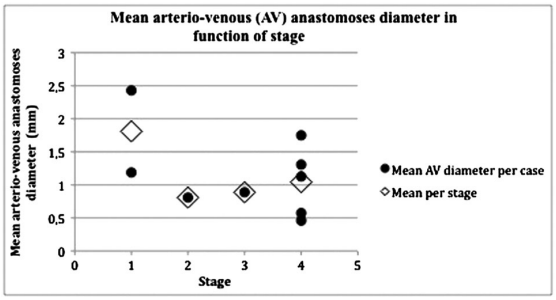


Figure 4 Mean arteriovenous (AV) anastomoses diameter in function of stage

uterine fetal demise at 14 WG +1 day at a time when it was impossible to analyze the placenta, and three cases without AV anastomosis at placental examination. Among the seven cases treated by laser, postnatal placental analysis was impossible in three cases (maceration due to fetal demise or to fixation error). No residual anastomosis was found in the four remaining cases. The number of AV anastomoses in relation to the grade of TAPS severity is illustrated in Figure 3. There was no correlation between the number of AV anastomoses and the severity of TAPS (OR = 0.89 [0.72, 1.10], Pr(OR > 1) = 14.8%). Analysis of the relationship between the diameter of the AV anastomoses and the disease stage was feasible in nine placentas. The mean diameter of the AV anastomoses for each stage is reported in Figure 4. Statistical analysis did not reveal any correlation between the severity of TAPS and the mean (OR = 0.98 [0.32, 3.06], Pr(OR > 1) = 48.9%) or total AV diameter (OR = 0.79 [0.36, 1.53], Pr(OR > 1) = 26.3%). It should be noted that two placentas presented one or two AA anastomoses, and four placentas had one or several VA anastomoses, that is, incidences of 22.2% and 44.4%, respectively.

The mean interval between a laser procedure for TTTS and diagnosis of an iatrogenic TAPS was 17 +/−21 days. Spontaneous TAPS was diagnosed at 173 +/−51 days on average, as compared with 176 +/−21 days for iatrogenic TAPS: the difference estimated by the statistical model was 0.84 days [−32.48, 33.46], Pr(diff > 0) = 52.6% (prior: spontaneous TAPS N (182, 32),[3] iatrogenic TAPS N (182, 32)[21]). In the prenatal period, 30% of cases of spontaneous TAPS were stage 1, as compared with 71.4% for iatrogenic TAPS (estimated difference = 33.7% [−8, 69], Pr(OR > 1) = 94.7%) (prior: Be (1,1) for both groups).

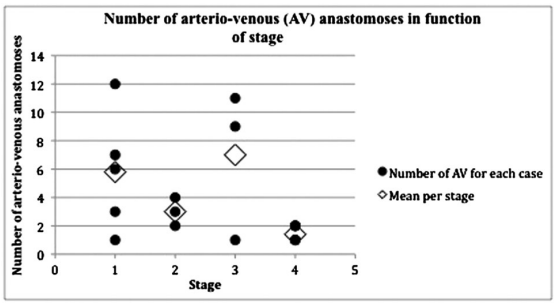


Figure 3 Number of arteriovenous (AV) anastomoses in function of stage

DISCUSSION

As far as we know, our study is the first to evaluate the diagnostic criteria for TAPS since their publication in 2010 by Slaghekke *et al.*[8] We found a significant link between MCA-PSV and Hb level for fetuses presenting TAPS. Our data show that the correspondence between the prenatal and postnatal diagnoses is imperfect. Lastly, we did not find a correlation between the placental angioarchitecture and the severity of TAPS. The size of our cohort was limited but relatively large in view of the incidence of TAPS.

In our cohort, the global incidence TAPS was 4.6%, with an incidence of 3.7% for spontaneous TAPS and 6.2% for iatrogenic TAPS. In the literature, there is a global incidence between 2% and 7.2%.[4,5] There are further data concerning the incidence of TAPS depending on its mode of onset.[2–7] Figures vary from team to team, with different diagnostic criteria (prenatal or postnatal diagnosis) depending for example, on when the study was published (cut-off point chosen for MCA-PSV in the polycythemic fetus at 1 or 0.8 MoM, delta Hb > 8 g/dL or anemic Hb < 11 g/dL and polycythemic Hb > 20 g/dL). Note should be made of the recruitment bias in our series (as in the others), because our cohorts stem from reference centers.

In 2006, Robyr *et al.* demonstrated a correlation between MCA-PSV and the Hb level in a subgroup of twins presenting a marked Hb level discrepancy after laser photocoagulation for TTTS (which corresponded in fact to iatrogenic TAPS, except that the term had yet to be devised).[6] We also found a strong link between MCA-PSV and Hb level in our cohort consisting of cases of both iatrogenic and spontaneous TAPS. This relation exists in case of anemia and in case of polycythemia. The existence of a correlation between a reduced MCA-PSV and polycythemia in newborn at term has already been shown by Weissman.[36] Our results also confirmed a link between these two parameters, the MCA-PSV having been measured *in utero* in our study and in the preterm. The MCA-PSV appears therefore to be a reliable parameter for estimating the blood Hb level in the event of TAPS. Nevertheless, our results suggest that the prenatal diagnostic criterion remains imperfect in view of the respective false

positive and false negative levels of 16.7% and 66.7%, respectively. Indeed, we could identify discordance between MCA-PSV and Hb level in three cases, two anemic fetus having normal MCA-PSV and low Hb level and, on the contrary, one anemic fetus having high PSV and normal Hb level (Figure 2). With regard to the single false positive case, MCA-PSV measurements suggested a stage 1 spontaneous TAPS in one patient – not previously monitored in our center – at 31 WG +1 day, which led to fetal extraction after corticosteroid therapy for pulmonary maturation. At birth, there was no discrepancy in Hb levels, and placental analysis failed to detect any anastomosis. In this case, MCA-PSV values were measured on a single occasion only (48 h before delivery), and we therefore had no previous data on earlier kinetics. The lack of anastomosis could suggest that an anastomotic thrombosis had given rise to an evolving spontaneous resolution, as previously described by Lopriore.[37] A poor measuring technique – extrinsic cause of false positives and false negatives – could also be incriminated. Both false negative cases were iatrogenic TAPS in which the MCA-PSV values returned to normal (measures at 29 and 33 WG +4 days) either after a second laser procedure or spontaneously. At birth, the clinical pictures corresponded to stage 4 TAPS. Analysis of the films revealed imperfect MCA-PSV measurements in three out of the four fetuses concerned. This underlines the importance of measuring technique quality,[38] because difficulties may be heightened in the third trimester, especially in a twin pregnancy. It should be noted that the nomograms we used to convert PSV into MoM are those proposed by Mari, which were established for singleton pregnancies.[29] This could have an influence on sensitivity and specificity of MCA-PSV MoM in our series. That being said, Klaritsch showed that reference ranges of singleton can be used between 18 and 37 WG in monochorionic pregnancies, whereas before 18 WG, MCA-PSV values are lower in singletons.[39] In our cohort, only two cases were diagnosed before 18 WG, and therefore, we assume that this issue has limited effect on study validity. To avoid this problem, use of MCA-PSV ratio or difference could be an interesting alternative to specific cut-off. Further studies are necessary to confirm this hypothesis. Accurate diagnosis is crucial as it enables to establish proper therapeutic project. Prenatal therapeutic options are the followings: expectative management, *in utero* transfusion of the anemic fetus possibly combined with partial exchange transfusion in the recipient, laser coagulation of the placental anastomoses, selective termination of pregnancy, or fetal extraction.[6,8,10–13,15,18,21,23]

As far as we know, no study has examined the correlation between angioarchitecture and severity of TAPS. On the basis of our data, the number and mean or total diameter of AV anastomoses do not appear to be correlated to the disease stage. This may be due to the difficulty of integrating other factors into the analysis such as VA or AA anastomoses, which could have a protective function by equilibrating or counterbalancing the flow from the donor to the recipient fetus, as it has been shown in case of TTTS.[40] In our cohort, the presence of this type of anastomosis did not appear however to be associated with the earliest stages. It should be noted that we did not use placental angiography. This technique, that has been evaluated by Lewi,[41] provides a more accurate placental analysis and could have improve quality of our study.

In the event of an isolated discrepancy between the Hb levels at birth, the main differential diagnosis to be distinguished from TAPS is the acute peripartum TTTS. Of low incidence (2.5%), this syndrome is characterized by the presence of superficial anastomoses in 100% of cases.[30] Moreover, because it is an acute phenomenon, the reticulocyte level is not increased in the anemic infant. In our cohort, four cases were strongly suggestive of TAPS but did not strictly meet Slaghekke's postnatal diagnostic criteria because the reticulocyte ratio was less than 1.7 (although very close to the cut-off level in one case) and the anastomoses were slightly greater than 1 mm (except for one case where the angioarchitecture was highly atypical).[8] It would therefore appear that the postnatal diagnostic criteria for TAPS are perhaps too restrictive and that there are grounds for widening them. With regard to the reticulocyte ratio, it is probable that a compensatory phenomenon such as hyperreticulocytosis would not occur in the event of a TAPS developing just a short time before delivery. Concerning the millimeter threshold for the anastomoses, the placental angioarchitecture may sometimes be more complex, with partial compensation afforded by the existence of different types of anastomosis. Study of other criteria such as macroscopic placental appearance (bicolor aspect) could be part of the postnatal diagnostic procedure, at least in case of atypical presentation. Mode of delivery could also be a diagnostic tool, but data are few and discordant. According to cases reported by Lopriore,[30] acute peripartum TTTS occurs solely with normal vaginal deliveries. Mabuchi found on the contrary that it could also occur after cesarean section. Nevertheless, in the latter study, reticulocyte ratio was the only criteria used to distinguish TAPS from acute peripartum TTTS, without regard for placental architecture.[42]

In our study, the incidence of iatrogenic TAPS was 6.2%. In the literature, the incidence is thought to be between 2% and 13%.[4–7] The heterogeneity of results reflects not only differences in terms of diagnostic criteria but also differences linked to the laser procedure such as the operator's experience and the laser photocoagulation technique. In our cohort, and on a wider scale in the 'Solomon trial' cohort in which our center participated, the incidence of TAPS was, respectively, 8.2% and 16% for the selective technique versus 2% and 3% for the Solomon technique.[32] Lopriore observed in 2008 that spontaneous TAPS appeared after 30 WG, whereas postlaser TAPS, following on from TTTS – a second trimester disorder – occurred earlier.[19] In our cohort, however, there was no difference in terms of gestational age at diagnosis between the two groups. It should be noted that in half of our cases, spontaneous TAPS was diagnosed before 24 WG, although the mean gestational age was heavily skewed by one case that occurred at 12 WG. This is in alignment with the more recent data in the literature[3,8,16,20,22,23] and is probably linked to a more routinized detection of TAPS in reference centers.

The stage at diagnosis appeared less advanced in patients presenting iatrogenic TAPS. It would however be incorrect to conclude that postlaser TAPS is less severe than spontaneous TAPS on account of a follow-up bias. As it happens, patients

who undergo laser treatment for TTTS benefit from closer monitoring and screening for specific complications, especially TAPS, which may explain why the diagnosis is made at a potentially earlier stage.

CONCLUSION

In conclusion, the MCA-PSV is a reliable tool for estimating the Hb level in cases of TAPS, both for anemia and polycythemia, on condition that measurement is carried out with due respect for quality criteria. The postnatal diagnostic criteria seem to be too restrictive, in particular concerning the reticulocyte ratio and angioarchitecture. Further studies are required to evaluate these diagnostic criteria.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Twin anemia-polycythemia sequence (TAPS) is a rare complication of monochorionic pregnancies. Diagnostic is based on peak systolic velocity measured in the middle cerebral artery, hematological data at birth, and placental angioarchitecture.

WHAT DOES THIS STUDY ADD?

- Middle cerebral artery peak systolic velocity is reliable to estimate Hb level in case of TAPS for both anemic and polycythemic twins. Correspondence between prenatal and postnatal diagnosis is imperfect. We did not find a link between number or diameter of arteriovenous anastomoses and severity of TAPS.

REFERENCES

1. Lopriore E, Middeldorp JM, Oepkes D, *et al.* Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28(1):47–51.
2. Lewi L, Jani J, Blickstein I, *et al.* The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199(5):514.e1–8.
3. Gucciardo L, Lewi L, Vaast P, *et al.* Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30(5):438–42.
4. De Villiers SF, Slaghekke F, Middeldorp JM, *et al.* Placental characteristics in monochorionic twins with spontaneous versus post-laser twin anemia-polycythemia sequence. Placenta 2013;34(5):456–9.
5. Nakayama S, Ishii K, Kawaguchi H, *et al.* Perinatal outcome of monochorionic diamniotic twin pregnancies managed from early gestation at a single center. J Obstet Gynaecol Res 2012;38(4):692–7.
6. Robyr R, Lewi L, Salomon LJ, *et al.* Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194(3):796–803.
7. Habli M, Bombrys A, Lewis D, *et al.* Incidence of complications in twin–twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201(4):417.e1–7.
8. Slaghekke F, Kist WJ, Oepkes D, *et al.* Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27(4):181–90.
9. Zhao DP, de Villiers SF, Slaghekke F, *et al.* Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta 2013;34(7):589–93.
10. Groussolles M, Sartor A, Connan L, Vayssière C. Evolution of middle cerebral artery peak systolic velocity after a successful laser procedure for iatrogenic twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2012;39(3):354–6.
11. Fratelli N, Prefumo F, Zambolo C, *et al.* Conservative management in a case of iatrogenic twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2012;39(5):597–8.
12. Weingertner AS, Kohler A, Kohler M, *et al.* Clinical and placental characteristics in four new cases of twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2010;35(4):490–4.
13. Herway C, Johnson A, Moise K, Moise KJ. Fetal intraperitoneal transfusion for iatrogenic twin anemia-polycythemia sequence after laser therapy. Ultrasound Obstet Gynecol 2009;33(5):592–4.
14. Favre R, Koch A, Weingertner A-S, *et al.* Vascular pattern in monochorionic placentas with spontaneous TAPS and TTTS with residual anastomoses after laser: a case control study. Prenat Diagn 2013;33 (10):979–82.
15. Sainz JA, Romero C, García-Mejido J, *et al.* Analysis of middle cerebral artery peak systolic velocity in monochorionic twin pregnancies as a method for identifying spontaneous twin anaemia-polycythaemia sequence. J Matern Fetal Neonatal Med 2013;1–3.
16. Luminoso D, Figueira CO, Marins M, Peralta CFA. Fetal brain lesion associated with spontaneous twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2013;42(6):721–2.
17. Verbeek L, Slaghekke F, Hulzebos CV, *et al.* Hypoalbuminemia in donors with twin anemia-polycythemia sequence: a matched case-control study. Fetal Diagn Ther 2013;33(4):241–5.
18. Ishii K, Hayashi S, Mabuchi A, *et al.* Therapy by laser equatorial placental dichorionization for early-onset spontaneous twin anemia-polycythemia sequence. Fetal Diagn Ther 2014;35(1):65–8.
19. Lopriore E, Deprest J, Slaghekke F, *et al.* Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstet Gynecol 2008;112(4):753–8.
20. Lopriore E, Slaghekke F, Kersbergen KJ, *et al.* Severe cerebral injury in a recipient with twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2013;41 (6):702–6.
21. Genova L, Slaghekke F, Klumper FJ, *et al.* Management of twin anemia-polycythemia sequence using intrauterine blood transfusion for the donor and partial exchange transfusion for the recipient. Fetal Diagn Ther 2013;34(2):121–6.
22. Assaf SA, Benirschke K, Chmait RH. Spontaneous twin anemia-polycythemia sequence complicated by recipient placental vascular thrombosis and hydrops fetalis. J Matern Fetal Neonatal Med 2011;24 (3):549–52.
23. Diehl W, Glosemeyer P, Tavares De Sousa M, *et al.* Twin anemia-polycythemia sequence in a case of monoamniotic twins: twin anemia-polycythemia sequence in monoamniotic twins. Ultrasound Obstet Gynecol 2013;42(1):108–11.
24. Mari G, Deter RL, Carpenter RL, *et al.* Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. N Engl J Med 2000;342(1):9–14.
25. Cosmi E, Mari G, Chiaie LD, *et al.* Noninvasive diagnosis by Doppler ultrasonography of fetal anemia resulting from parvovirus infection. Am J Obstet Gynecol 2002;187(5):1290–3.
26. Senat M., Loizeau S, Couderc S, *et al.* The value of middle cerebral artery peak systolic velocity in the diagnosis of fetal anemia after intrauterine death of one monochorionic twin. Am J Obstet Gynecol 2003;189(5):1320–4.
27. Hernandez-Andrade E, Scheier M, Dezerega V, *et al.* Fetal middle cerebral artery peak systolic velocity in the investigation of non-immune hydrops. Ultrasound Obstet Gynecol 2004;23(5):442–5.
28. Sueters M, Arabin B, Oepkes D. Doppler sonography for predicting fetal anemia caused by massive fetomaternal hemorrhage. Ultrasound Obstet Gynecol 2003;22(2):186–9.
29. Mari G, Adrignolo A, Abuhamad AZ, *et al.* Diagnosis of fetal anemia with Doppler ultrasound in the pregnancy complicated by maternal blood group immunization. Ultrasound Obstet Gynecol 1995;5(6):400–5.
30. Lopriore E, Holtkamp N, Sueters M, *et al.* Acute peripartum twin–twin transfusion syndrome: incidence, risk factors, placental characteristics and neonatal outcome. J Obstet Gynaecol Res 2014;40(1):18–24.
31. Quintero RA, Comas C, Bornick PW, *et al.* Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2000;16(3):230–6.

32. Slaghekke F, Lopriore E, Lewi L, *et al.* Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383(9935):2144–51.
33. Lopriore E, Slaghekke F, Middeldorp JM, *et al.* Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;201(1):66.e1–4.
34. Ntzoufras I. *Bayesian Modeling Using WinBUGS.* Wiley: Hokboken, NJ, 2009.
35. Freedman L. Bayesian statistical methods. BMJ 1996;313(7057):569–70.
36. Weissman A, Olanovski I, Weiner Z, Blazer S. Doppler middle cerebral artery peak systolic velocity for diagnosis of neonatal anemia. J Ultrasound Med 2012;31(9):1381–5.
37. Lopriore E, Hecher K, Vandenbussche FPHA, *et al.* Fetoscopic laser treatment of twin-to-twin transfusion syndrome followed by severe twin anemia-polycythemia sequence with spontaneous resolution. Am J Obstet Gynecol 2008;198(2):e4–7.
38. Mari G, Abuhamad AZ, Cosmi E, *et al.* Middle cerebral artery peak systolic velocity technique and variability. J Ultrasound Med 2005;24 (4):425–30.
39. Klaritsch P, Deprest J, Van Mieghem T, *et al.* Reference ranges for middle cerebral artery peak systolic velocity in monochorionic diamniotic twins: a longitudinal study. Ultrasound Obstet Gynecol 2009;34(2):149–54.
40. Umur A, van Gemert MJC, Nikkels PGJ, Ross MG. Monochorionic twins and twin–twin transfusion syndrome: the protective role of arterio-arterial anastomoses. Placenta 2002;23(2-3):201–9.
41. Lewi L, Cannie M, Blickstein I, *et al.* Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. Am J Obstet Gynecol 2007;197(6):587.e1–8.
42. Mabuchi A, Ishii K, Yamamoto R, *et al.* Clinical characteristics of monochorionic twins with large hemoglobin level discordance at birth. Ultrasound Obstet Gynecol 2014;44(3):311–5.

Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2015;38:170–178
DOI: 10.1159/000380822

Received: October 22, 2014
Accepted: February 9, 2015
Published online: March 17, 2015

Evaluation of the Utility of in utero Treatment of Twin Anemia-Polycythemia Sequence

Nicolas Sananès[a, c] Marine Veujoz[a] Francois Severac[b] Maël Barthoulot[b] Nicolas Meyer[b] Anne-Sophie Weingertner[a] Monique Kohler[a] Fernando Guerra[a] Adrien Gaudineau[a] Israël Nisand[a] Romain Favre[a]

Departments of [a]Obstetrics and Gynecology and [b]Public Health, Strasbourg University Teaching Hospitals, and [c]INSERM, UMR-S 1121, 'Biomatériaux et Bioingénierie', Strasbourg, France

Key Words
Twin anemia-polycythemia sequence · Twin pregnancy · Monochorionic diamniotic twin pregnancy · Laser coagulation · Fetal transfusion

Abstract
Objective: The aim of this study is to evaluate the interest in the in utero treatment of twin anemia-polycythemia sequence (TAPS). ***Methods:*** The obstetrical and neonatal data on all cases of TAPS followed up in our institution between 2006 and 2013 were reviewed. Statistical analyses were conducted using Bayesian methods. ***Results:*** Twenty cases of TAPS were included. Laser therapy or intrauterine transfusion (IUT) was performed on the donor twin in 9 cases. Eleven cases were included in the 'nontreated' group (managed expectantly or diagnosed at birth). The gestational age at diagnosis was lower in the group with treated TAPS [difference (diff) = –22.20 days (–57.13, 14.28), probability (Pr) (diff >0) = 10.6%]. The rate of preterm premature rupture of membranes was higher in the group with treated TAPS [diff = 22.5% (–14, 57), Pr (diff >0) = 89%], but overall mortality was similar. The interval between diagnosis and delivery was longer [diff = 44.37 days (9.41, 77.90), Pr (diff >0) = 99.2%], the TAPS resolution rate was higher [diff = 49.9% (12, 81), Pr (diff >0) = 99.4%], and the neonatal transfusion rate was lower [diff = –30.5% (–60, 0), Pr (diff >0) = 2.6%] in the treated group. ***Conclusion:*** In utero treatment for TAPS is associated with a higher resolution rate of TAPS and a longer time between diagnosis and birth, but overall mortality is the same as with expectant management.

Introduction

Twin anemia-polycythemia sequence (TAPS) is a complication of monochorionic diamniotic twin pregnancies first described in 2007 by Lopriore et al. [1]. Its pathology is characterized by an intertwin difference in hemoglobin levels. It may be diagnosed either in the antenatal or the postnatal period and presents a typical angioarchitecture (slender and rather scanty arteriovenous anastomoses) [2]. It is a heterogeneous disorder, both in terms of its mode of onset [either after laser treatment of twin-twin transfusion syndrome (TTTS) or spontaneously] and its severity. There is no consensus about its antenatal management, and different therapeutic alternatives have been described: simple surveillance, intrauterine transfusion (IUT) of the anemic fetus possibly combined with partial

1015–3837/15/0383–0170$39.50/0

E-Mail karger@karger.com
www.karger.com/fdt

Nicolas Sananès
Service de gynécologie obstétrique
Hôpitaux universitaires de Strasbourg
FR–67091 Strasbourg Cedex (France)
E-Mail nicolas.sananes@chru-strasbourg.fr

Table 1. Pre- and postnatal classification of TAPS [2]

	Antenatal stage: findings at Doppler ultrasound examination	Postnatal stage: intertwin Hb difference, g/dl
Stage 1	MCA-PSV donor >1.5 MoM and MCA-PSV recipient <1 MoM without other signs of fetal compromise	>8.0
Stage 2	MCA-PSV donor >1.7 MoM and MCA-PSV recipient <0.8 MoM without other signs of fetal compromise	>11.0
Stage 3	As stage 1 or 2, with cardiac compromise of the donor, defined as critically abnormal flow	>14.0
Stage 4	Hydrops of donor	>17.0
Stage 5	Intrauterine demise of one or both fetuses preceded by TAPS	>20.0

MoM = Multiple(s) of the median; Hb = hemoglobin.

exchange transfusion in the recipient, laser coagulation of the placental anastomoses, selective termination of pregnancy or fetal extraction [2–11]. On account of the low incidence of TAPS (1–5% of monochorionic diamniotic twin pregnancies for spontaneous TAPS [12–15] and 2–13% for iatrogenic TAPS [4, 14–16]), therapeutic options have been presented only in case reports or small-size descriptive cohorts. The objective of our study was to examine obstetrical and neonatal outcomes depending on whether or not an antenatal treatment (IUT or laser) had been provided in a series of TAPS.

Materials and Methods

We carried out a single-center prospective cohort study, with enrollment of all monochorionic diamniotic twin pregnancies complicated by TAPS diagnosed ante- or postnatally and managed at the gynecology and obstetrics hub of the Strasbourg University Teaching Hospitals between December 2006 and August 2013.

The diagnostic criteria for TAPS selected were those drawn up by Slaghekke et al. [2]. In the antenatal period, they associate the absence of twin oligo-polyhydramnios sequence with measurement of a middle cerebral artery peak systolic velocity (MCA-PSV) >1.5 multiples of the median in one fetus (anemic, donor) and <1 multiple of the median in the other fetus (polycythemic, recipient). The MCA-PSV was measured using the technique described by Mari et al. [17] by means of a Voluson 730 or E8 General Electric® device. In the postnatal period, the diagnosis is based on an intertwin hemoglobin difference of >8 g/dl combined with a reticulocyte ratio of >1.7 or the finding of inframillimetric anastomoses. Cases that showed discrepant hemoglobin levels at birth of >8 g/dl but did not meet the other postnatal criteria were also enrolled in the study if there was a strong clinical impression and no evidence of an acute peripartum TTTS [18]. The severity of TAPS was defined using the classification by Slaghekke et al. [2] (table 1).

The follow-up of each monochorionic diamniotic pregnancy consisted of an ultrasound every 2 weeks. In the case of a diagnosis of TAPS before 32 weeks of gestation (WG), in utero treatment was proposed for TAPS stage 3 or 4 or for TAPS stage 1 or 2 with rapid aggravation. As soon as it had been decided to treat a case antenatally, we opted for fetoscopy with laser coagulation of the placental anastomoses, because this is the only causative treatment. For late cases, and if the technical conditions were not favorable (anterior-lying placenta with anticipated incorrect access of the vascular equator and, consequently, risk of incomplete surgery), IUT was performed. After 32 WG, the therapeutic options were expectant management or delivery. Each case was presented at the weekly meetings of the pluridisciplinary prenatal diagnosis center (CPDPN) to validate the in utero management. Two physicians (R.F. and A.-S.W.) performed IUT and laser surgery; they are both well trained in fetoscopic procedures.

The laser photocoagulation technique has already been described in a previous paper [19]. Amnioinfusion was always performed to facilitate access to the vascular equator. The preferred photocoagulation technique was complete coagulation of the vascular equator (Solomon technique) [20, 21]. In the case of a technical difficulty, the selective technique was adopted [22]. Follow-up checks of fetal vitality and TAPS status were carried out 24 and 48 h postoperatively, at 1 week and, superseded by conventional checkups, every 2 weeks. For IUT, the sedation and anesthesia protocol was similar to that followed for laser photocoagulation. The procedure was always carried out with a pediatrician being present. Access was by direct transabdominal needling, with puncture of the umbilical vein at the cord insertion. A 20-gauge needle was used for initial sampling of blood (5–10 ml) to obtain the hemoglobin level. Fetal immobilization was obtained by curarization (Nimbex® at a dose of 0.15 mg/kg estimated fetal weight). Transfusion was then carried out using leukocyte-free, radiated, CMV-negative, 0 rhesus-negative red cell concentrates compatible with the maternal blood group, the volume being calculated on the basis of the gestational age and initial hemoglobin level. MCA-PSV was checked the day after the procedure, at 7 days and every week to 2 weeks thereafter.

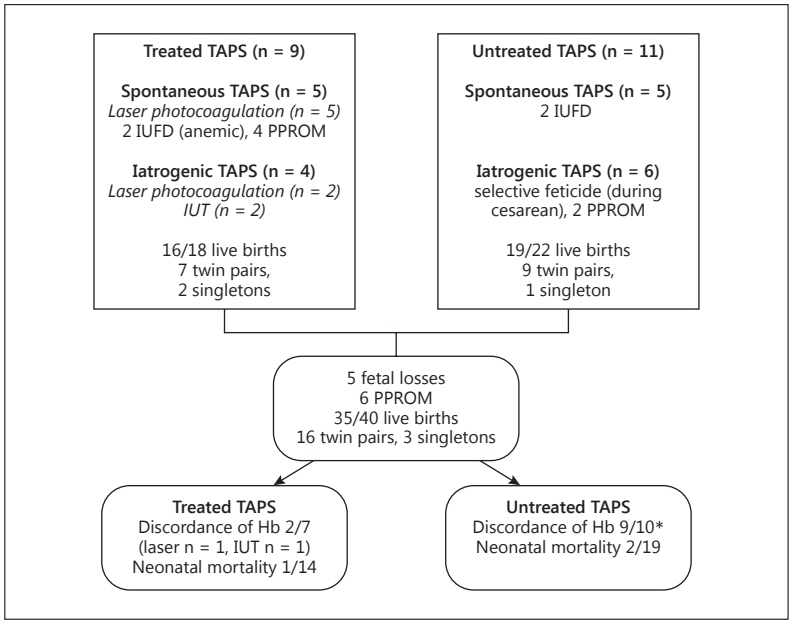


Fig. 1. Description of the study population. IUFD = Intrauterine fetal demise; Hb = hemoglobin. * The hematological data on the fetus that was subject to feticide during cesarean section and those on his co-twin were retained for analysis.

After delivery, photographs of both surfaces of the placenta were taken using a Sony-DSC-W220 digital apparatus (12.1 megapixels) after injection of a coloration dye. The placental weight of each child and the number of arteriovenous anastomoses were gathered.

Data on demographics and antenatal care as well as obstetrical and neonatal outcomes were collected prospectively. The term 'overall mortality' was used to describe the sum of in utero mortality and neonatal mortality (within 28 days of birth). Unequal sharing was defined by a ratio of the weights of the two hemiplacentas of >1.5. A difference of >20% in weight between the two neonates signaled a birth weight discordance [23]. The data were analyzed and compared between 'treated' TAPS and 'nontreated' TAPS.

Continuous variables are expressed as means (μ) and standard deviations (σ or SD). Categorical variables are expressed as numbers and percentages. Statistical analyses were conducted using Bayesian methods [24, 25] with R 3.0.0 software and all the required packages in their latest updates at the time of analysis as well as WinBUGS software (estimation using Markov chain Monte Carlo with Gibbs sampling). After a burn-in of 5,000 updates, 100,000 iterations were performed and convergence was checked using trace plots of the sample values for each iteration. Beta distributions [Be(a,b)] were used to estimate proportions, while means were estimated using normal distribution [$N(\mu, \sigma)$]. If available, results from previous studies were used to provide informative Bayesian priors. Studies in which some of our cases had already been reported on could not be used as priors to avoid the duplication of data [6, 26]. In the absence of prior information for the estimation of a proportion, a noninformative [Be(1,1)] prior was adopted. For the estimation of a mean, the prior was derived from the data (empirical Bayes methods) [27]. The same prior was used for both groups in order to minimize the difference of means. Each result is presented as a difference (diff) [(result of treated group) – (result of nontreated group)] with a 95% credibility interval and a probability (Pr) of exceeding 0 (or 1 for an OR). The priors are presented in supplementary table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000380822). This study received the authorization of the French Commission Nationale de l'Informatique et des Libertés (CNIL) and is registered as No. 1728548.

Results

Our patient population is described in figure 1. In all, 20 patients developed TAPS during the inclusion period, i.e. a global incidence of 4.6% was calculated (433 patients followed up for monochorionic diamniotic twin pregnancy during the inclusion period); 10 had spontaneous TAPS and 10 iatrogenic TAPS after laser. The diagnosis was made in the antenatal period in 17 cases (85%) at a mean gestational age of 24.9 ± 5.9 WG. In utero management included laser treatment in 7 cases (41.1%; 5 photocoagulations using the Solomon technique and 2 selective photocoagulations), IUT in 2 cases (11.8%), expectant management in 5 cases (29.4%), fetal extraction after corticosteroid therapy for pulmonary maturation in 2 cases and as an emergency in 1 case (17.6%; abnormal fetal heart rhythm in the anemic fetus). The 3 cases diagnosed in the postnatal period were included in the nontreated

Table 2. Maternal and diagnostic data for comparisons between the treated and the nontreated group

	Laser (n = 7)	IUT (n = 2)	Treated TAPS (n = 9)	Nontreated TAPS (n = 11)	Statistical analysis, diff (95% CI), Pr (diff >0) or OR (95% CI), Pr (OR >1)
Age, years	31.1±2.5	28±2.8	30.4±2.8	28.4±4.7	2.07 (–1.46, 5.61), 88%
Parity ≥1	6 (85.7)	2 (100)	8 (88.8)	3 (27.3)	51.1% (16, 79), 99.7%
BMI	21.9±5.2	24±5.7	22.3±5.1	24.4±5.1	–2.04 (–6.79, 2.76), 18.5%
GA at diagnosis, WG	22.3±3.6	26±1.3	23.1±3.6	27±7.3	–3.2 (–8.2, 2), 10.6%
Spontaneous TAPS	5 (71.4)	0 (0)	5 (55.6)	5 (45.4)	8.6% (–31, 46), 67.0%
Iatrogenic TAPS	2 (28.6)	2 (100)	4 (44.4)	6 (54.6)	–8.6% (–46, 31), 33.3%
Stage at diagnosis – prenatal					
1	3 (42.8)	1 (50)	4 (44.4)	4 (36.3)	
2	2 (28.6)	1 (50)	3 (33.3)	2 (18.2)	OR = 0.77 (0.24, 2.41),
3	2 (28.6)	0 (0)	2 (22.2)	1 (9.1)	32.6% (prenatal stage)
4	0 (0)	0 (0)	0 (0)	1 (9.1)	
Stage at diagnosis – postnatal					
1	–		–	1 (9.1)	–
2	–		–	1 (9.1)	–
3	–		–	0 (0)	–
4	–		–	1 (9.1)	–

Values denote means ± SD or n (%) unless specified otherwise. BMI = Body mass index; GA = gestational age; 95% CI = 95% credibility interval.

group. Of these 3 cases, 2 did not strictly meet all the diagnostic criteria. They were included because the clinical presumption of the diagnosis was strong. Indeed, there was a discrepancy in the hemoglobin levels of >8 g/dl, the two-toned placental appearance – which is typical of TAPS [6] – was present, and we found at least 1 arteriovenous anastomosis per placenta. Besides, delivery took place by cesarean section, which does not suggest an indication of acute peripartum transfusion syndrome. Ultimately, 9 cases of TAPS were included in the treated group and 11 in the nontreated group.

The maternal and diagnostic features of TAPS are displayed in table 2. The obstetrical and neonatal data for the entire cohort are reported in table 3. Table 4 concerns the subgroup of pregnancies with two live fetuses: 3 cases of pregnancy complicated by intrauterine fetal demise of one or both twins were excluded from the analysis, whereas the weight and hematological data on a twin pair of which the anemic fetus was subject to feticide during cesarean section were retained for the analysis. The gestational age at the time of diagnosis, in the event of antenatal diagnosis, was 23.1 ± 3.6 WG in treated TAPS versus 27 ± 7.3 WG in nontreated TAPS, with an estimated difference of –3.2 WG (–8.2, 2) [Pr (diff >0) = 10.6%]. The two groups were comparable with regard to the diagnostic stage in the antenatal period as well as the proportion of spontaneous and iatrogenic TAPS. The rate of preterm premature rupture of the membranes (PPROM) was 44.4% in the treated TAPS group versus 18.2% in the nontreated TAPS group, with an estimated difference of 22.5% (–14, 57) [Pr (diff >0) = 89.0%]. The mean gestational age at delivery (or miscarriage) was 33.4 ± 3 WG in the event of treatment versus 30.4 ± 5.7 WG in the absence of treatment, with an estimated difference of 2.8 WG (–1.1, 6.5) [Pr (diff >0) = 92.4%]. The interval between diagnosis and delivery (or miscarriage) was longer for treated TAPS (10.3 ± 5.7 vs. 2.9 ± 4.1 WG for the other group), with an estimated difference of 6.3 WG (1.3, 11.1) [Pr (diff >0) = 99.2%]. At birth, the rate of TAPS resolution was greater in the event of treatment (71.4 vs. 10% in the absence of treatment), with an estimated difference of 49.9% (12, 81) [Pr (diff >0) = 99.4%, OR = 12.65 (1.7, 155)]. In the treated group, the intertwin hemoglobin difference was 6.2 ± 8.3 g/dl (vs. 13.3 ± 5.6 g/dl in the other group), with an estimated difference of –7.17 g/dl (–14.78, 0.46) [Pr (diff >0) = 3.2%]. Transfusion of the anemic infant during hospitalization was necessary in 89% of the cases in the nontreated TAPS group (vs. 57% in the treated TAPS group), with an estimated difference of –30.5% (–60, 0) [Pr (diff >0) = 2.6%, OR = 5.17 (0.99, 32.34)]. Overall mortality was 17% in the treated and 23% in the nontreated TAPS

Table 3. Obstetrical data for comparisons between the treated and the nontreated group

	Laser (n = 7)	IUT (n = 2)	Treated TAPS (n = 9)	Nontreated TAPS (n = 11)	Statistical analysis, diff (95% CI), Pr (diff >0)
PPROM	4 (57.1)	0 (0)	4 (44.4)	2 (18.2)	22.5% (–14, 57), 89.0%
GA at birth, WG	33.9±3.4	32.1±1	33.4±3	30.4±5.7	2.8 (–1.1, 6.5), 92.4%
Time between diagnosis and birth, WG	11.6±6.4	6.1±0.3	10.3±5.7	2.9±4.1[a]	6.3 (1.3, 11.1), 99.2%
Live births					
Anemic	5/7 (71.4)	2/2 (100)	7/9 (78)	9/11 (82)	–7.3% (–28, 14), 23.9%
Polycythemic	7/7 (100)	2/2 (100)	9/9 (100)	10/11 (91)	–2.0% (–18, 15), 38.5%
Total	12/14 (85.7)	4/4 (100)	16/18 (89)	19/22 (86)	–6.6% (–19, 7), 15.1%
Overall mortality					
Anemic	3/7 (42.9)	0/2 (0)	3/9 (33)	3/11 (27)	13.1% (–11, 36), 86.1%
Polycythemic	0/7 (0)	0/2 (0)	0/9 (0)	2/11 (18)	–2.3% (–21, 15), 40.1%
Total	3/14 (21.4)	0/4 (0)	3/18 (17)	5/22 (23)	0 (–13, 13), 49.6%
Both children alive (day 28)	4/7 (57.1)	4/4 (100)	6/9 (67)	8/11 (73)	–6.6% (–28, 15), 27.1%
At least one child alive (day 28)	7/7 (100)	4/4 (100)	9/9 (100)	9/11 (82)	6.8% (–4, 20), 89.7%

Values denote means ± SD or n (%) unless specified otherwise. GA = Gestational age; 95% CI = 95% credibility interval.
[a] n = 8; cases diagnosed postnatally excluded.

Table 4. Obstetrical and neonatal data (pregnancies with fetal loss excluded)

	Laser (n = 5/N = 10)	IUT (n = 2/N = 4)	Treated TAPS (n = 7/N = 14)	Nontreated TAPS (n = 10/N = 19[a])	Statistical analysis, diff (95% CI), Pr (diff >0)
GA at birth, WG	32.3±2	32.1±1	32.3±1.7	32±2.1	0.3 (–1.7, 2.1), 61.3%
Time between diagnosis and birth, WG	9±5.3	6.1±0.3	8.1±4.6	3±4.9[b]	4.4 (–0.5, 9), 96.3%
Child born after cesarean section	7/10 (10)	2/4 (50)	9/14 (64.3)	19/19 (100)	–3.3% (–21, 15), 35.2%
Birth weight, g					
Anemic	1,529±464	1,288±187	1,460±404	1,307±378	123.65 (–232.7, 471.5), 76.4%
Polycythemic	1,686±302	1,525±233	1,640±276	1,619±462	15.7 (–320.8, 345.3), 54.4%
Birth weight discordance	2/5 (40)	0/2 (0)	2/7 (29)	6/10 (60)	–21.6% (–54, 13), 10.8%
Unequal sharing	0/3 (0)	1/2 (50)	1/5 (20)	6/10 (60)	–29.6% (–67, 13), 8.9%
Hb, g/dl					
Anemic	12.8±6.4	10.2±6.9	12±6	7.2±2.5	4.75 (–0.54, 9.97), 96.4%
Polycythemic	17.3±3	19.7±6.6	18±3.8	20.5±3.6	–2.56 (–6.48, 1.33), 9%
Hb discordance >8 g/dl	1/5 (20)	1/2 (50)	2/7 (28.6)	9/10 (90)	–49.9% (–81, –12), 0.6%
Hb delta, g/dl	4.8±7.1	9.6±13.5	6.2±8.3	13.3±5.6	–7.17 (–14.78, 0.46), 3.2%
Reticulocyte ratio	0.8±0.4	1.1±0.5	0.9±0.4	1.6±0.5	–0.7 (–0.46, 0.3), 33.3%
Postnatal stage					
No TAPS	4/5 (80)	1/2 (50)	5 (71.4)	1 (10)	49.9% (12, 81), 99.4%
1	0 (0)	0 (0)	0 (0)	2 (20)	–
2	0 (0)	0 (0)	0 (0)	2 (20)	–
3	0 (0)	0 (0)	0 (0)	1 (10)	–
4	1 (20)	1 (50)	2 (28.6)	4 (40)	–
Blood transfusion – anemic	2/5 (40)	2/2 (50)	4/7 (57.1)	8/9 (88.9)	–30.5% (–60, 0), 2.6%
Exchange transfusion – polycythemic	0/5 (0)	1/2 (50)	1/7 (14.3)	4/10 (40.0)	–29.7% (–57, 1), 2.8%

Values denote means ± SD or n (%) unless specified otherwise. n = Number of cases; N = number of newborns; GA = gestational age; Hb = hemoglobin; 95% CI = 95% credibility interval.
[a] The hematological data and weight of the fetus that was subject to feticide during cesarean section and those of his co-twin were retained for analysis.
[b] n = 7; cases diagnosed postnatally excluded.

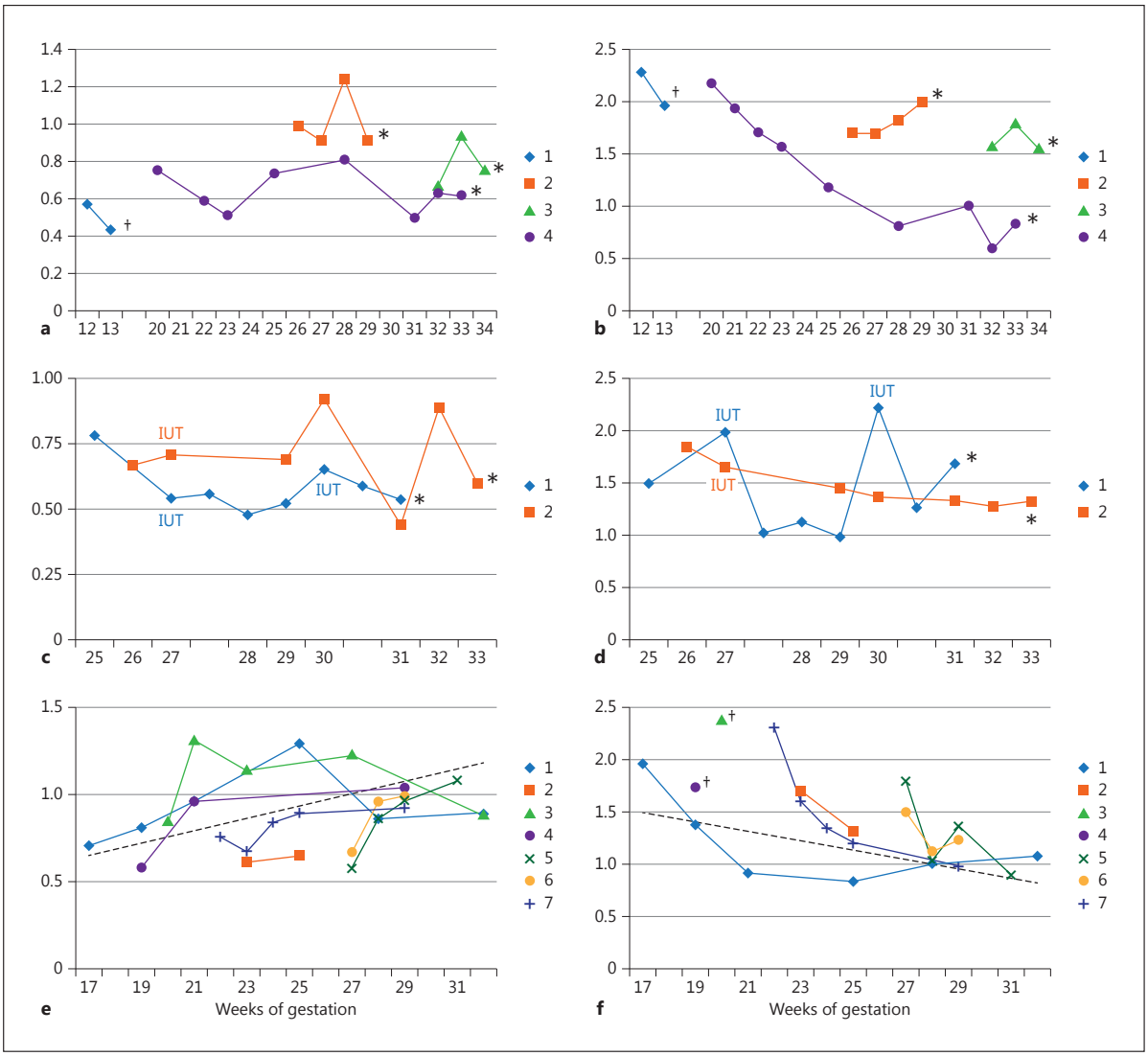


Fig. 2. Evolution of MCA-PSV. **a** Polycythemic twins in expectant management. **b** Anemic twins in expectant management. **c** Polycythemic twins treated with IUT. **d** Anemic twins treated with IUT. **e** Polycythemic twins after laser. **f** Anemic twins after laser. † Intrauterine fetal demise. * Delivery.

group. A nil estimated difference was retrieved after integration of priors [i.e. the priors pejorated our result; estimated difference = 0% (–13, 13), Pr (diff >0) = 50.4%].

The changes in MCA-PSV in the polycythemic and anemic fetuses in relation to each therapeutic option (expectant management, IUT or laser) are shown in figure 2. In the expectant management group, the data concern 4 of the 5 cases (a single measurement was made for the fifth case prior to delivery). For the cases treated with laser, the first measurement corresponds to the MCA-PSV before laser treatment. Three patients were referred back to their gynecologist for ongoing surveillance (cases No. 2, 4 and 7). For the other 4 patients, delivery took place at most 2 weeks after the last measurement.

Of the 7 cases of TAPS treated with laser, 3 placentas were not exploitable. In 2 cases, maceration occurred due to in utero death of one of the two fetuses. An analysis of the third placenta was impossible because of a fixation error; unfortunately, this was the single case in which there was a difference in hemoglobin level at birth, reflecting a failure of laser photocoagulation. Of the 13 cases of TAPS not treated with laser, 12 placentas could be analyzed (1 case was excluded because of early in utero death of both fetuses). Comparative analysis revealed an absence of residual anastomoses in the 4 cases treated with laser, whereas arteriovenous anastomoses were detected in 75% of the cases of nontreated TAPS [estimated difference = 54.6% (12, 84), Pr (>0) = 99.3%, OR = 18.48 (1.65, 665.37)].

Discussion

Our study suggests that antenatal treatment resolves most cases of TAPS and prolongs the interval between diagnosis of TAPS and delivery at the expense of a rise in PPROM. Overall, there was no difference in terms of overall mortality.

As far as we know, there are limited data in the literature that would enable a rigorous evaluation of the utility of antenatal therapy of TAPS; this is due to the rarity of this sequence and the lack of randomized studies. A multicenter study (including some of our data) on the feasibility of laser for treating TAPS has recently been published [26]. Rather than opting for a specific treatment, this study examined the utility of a therapeutic strategy for treating TAPS with laser or IUT depending on local conditions. Indeed, although laser has the advantage of being an etiological treatment, technical conditions are such that it is not always feasible; IUT would then appear to be an alternative, even if it is only a purely symptomatic treatment.

Our study did not show any benefits of antenatal treatment with regard to fetal and neonatal mortality. Nevertheless, we found that in the treated TAPS group, the rate of TAPS resolution was higher, the interval between diagnosis and delivery was longer, and the gestational age at delivery was more advanced. In addition, the intertwin hemoglobin difference at birth was less marked, and recourse to postnatal hematological treatments was less frequent. There also was birth weight discordance in more than half of the nontreated TAPS cases, whereas this affected only 29% of the treated TAPS cases. Zhao et al. [28], too, found a more marked tendency towards discrepant birth weights in the event of TAPS versus uncomplicated monochorionic diamniotic twin pregnancies. Correction of anemia therefore appears to be associated with improved growth of the anemic fetus. Nevertheless, birth weight discordance could also be a consequence of unequal sharing, which was more frequent in nontreated TAPS. We did not study the neurological development of these infants, but it should be noted that neurological lesions have been reported in both anemic [5] and polycythemic fetuses [29, 30].

Performing an in utero procedure was associated with an increased risk of PPROM: this reflects the iatrogenicity associated with any invasive therapeutic act. Nevertheless, the gestational age at delivery was higher if antenatal treatment was provided. It is noteworthy that none of the 4 patients in our series who underwent two invasive procedures (laser for TTTS and laser or transfusion for TAPS) had PPROM.

The laser procedure is the only etiological treatment, as shown by the graphs which display modifications in MCA-PSV and by placental analysis. In a recent study by Slaghekke et al. [26] on the effect of laser photocoagulation on TAPS (n = 8) as compared to no treatment (n = 27) or IUT (n = 17), the survival rate after laser was 94% (vs. 83 and 84% with expectant management and IUT, respectively; $p = 0.30$), with a prolonged diagnosis-delivery interval (11 vs. 5 and 8 WG; $p < 0.01$) and no severe hematological complications (vs. 72 and 52%; $p < 0.01$). It should be noted that placental photocoagulation for TAPS is more complicated than for TTTS due to the absence of polyhydramnios, a less tense uterine wall, more opaque amniotic fluid as well as slender and often marginal anastomoses [6, 28]. In the particular case of iatrogenic TAPS after laser treatment, the rationale for repeating the laser procedure should be carefully considered; if the anastomoses were not visualized during the first laser treatment for TTTS, it is unlikely that they will be identified any better during a second procedure. Changing the access route may be helpful, since it may be easier to visualize persisting anastomoses from a different viewpoint. In any event, if a laser treatment is performed, the Solomon technique should be preferred insofar as it is feasible [21].

IUT is a symptomatic treatment, and it sometimes needs to be repeated; this was the case with one of our patients. Study of the MCA-PSV changes illustrates that TAPS recurs at some point following the procedure. However, one of our cases presented a less typical progress, with resolution of TAPS following IUT and no relapse. A similar case was described by Robyr et al. [4]. Absence of anastomosis on placental analysis may sug-

gest an anastomotic thrombosis, as has been suggested by Lopriore et al. [31]. In any event, IUT should be preferred in cases of TAPS occurring at a later stage, in order to avoid (if possible) having to repeat this procedure. In our cohort, it is noteworthy that the MCA-PSV readings in the recipient fetuses did not show any significant post-procedural modifications, although increased polycythemia is a complication commonly described with IUT and it may give rise to complications [4], especially of a neurological type [32]. Some authors have described partial exchange transfusion of the polycythemic fetus to be associated with IUT of the anemic twin, with the advantage of reducing the potential risk of complications due to polycythemia. However, this procedure is symptomatic and requires double needling [5]. Expectant management is part of the armamentarium for selected cases [8]. A very early case of TAPS, i.e. inaccessible to any in utero therapy, ought to be monitored before any decision is made in order to perform the appropriate procedure at the appropriate time. In our cohort, the course was unfavorable, with double intrauterine fetal demise at 14 WG, underlining the considerable prognostic variability of this disorder. A slowly progressing stage 1 or 2 TAPS, especially if diagnosed after 30 WG, may also benefit from closer monitoring at least twice monthly or even weekly, as has recently been proposed by our team [33], with antenatal corticosteroid therapy and fetal extraction at what seems to be the right time [7, 34]. Finally, the decision to proceed with fetal extraction should be discussed after 32 WG whenever there is recurrence or worsening of TAPS. Selective feticide or termination of pregnancy can be considered as a therapeutic option for the more severe cases.

The observational design of our study introduced a potential bias into our analysis, since TAPS cases receiving antenatal treatment are not necessarily comparable with TAPS cases for which expectant management has been selected. There was no difference between the groups with regard to grade of severity, but TAPS cases undergoing in utero treatment were those occurring earliest in pregnancy. It would therefore appear that our study minimized the utility of an antenatal treatment strategy, since those cases treated were the most severe. The size of our cohort was limited, but nonetheless relatively large in view of the incidence of TAPS. Small-size samples generally provide little information. Our Bayesian approach, integrating some previously published data into the priors, would therefore appear to be of particular relevance.

In conclusion, antenatal treatment appears to be an option for selected cases of TAPS even if there is no evidence that it improves overall (fetal and neonatal) mortality rates. Laser photocoagulation of the placenta is the sole etiological treatment, but IUT may be considered if there are technical difficulties. Studies are needed to investigate the neurodevelopmental effects of in utero intervention.

References

1 Lopriore E, Middeldorp JM, Oepkes D, Kanhai HH, Walther FJ, Vandenbussche FP: Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47–51.

2 Slaghekke F, Kist WJ, Oepkes D, Pasman SA, Middeldorp JM, Klumper FJ, et al: Twin anemia-polycythemia sequence: diagnostic criteria, classification, perinatal management and outcome. Fetal Diagn Ther 2010;27:181–190.

3 Herway C, Johnson A, Moise K, Moise KJ Jr: Fetal intraperitoneal transfusion for iatrogenic twin anemia-polycythemia sequence after laser therapy. Ultrasound Obstet Gynecol 2009;33:592–594.

4 Robyr R, Lewi L, Salomon LJ, Yamamoto M, Bernard J-P, Deprest J, et al: Prevalence and management of late fetal complications following successful selective laser coagulation of chorionic plate anastomoses in twin-to-twin transfusion syndrome. Am J Obstet Gynecol 2006;194:796–803.

5 Genova L, Slaghekke F, Klumper FJ, Middeldorp JM, Steggerda SJ, Oepkes D, et al: Management of twin anemia-polycythemia sequence using intrauterine blood transfusion for the donor and partial exchange transfusion for the recipient. Fetal Diagn Ther 2013;34:121–126.

6 Weingertner AS, Kohler A, Kohler M, Boufet N, Hunsinger MC, Mager C, et al: Clinical and placental characteristics in four new cases of twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2010;35:490–494.

7 Groussolles M, Sartor A, Connan L, Vayssière C: Evolution of middle cerebral artery peak systolic velocity after a successful laser procedure for iatrogenic twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2012;39:354–356.

8 Fratelli N, Prefumo F, Zambolo C, Zanardini C, Fichera A, Frusca T: Conservative management in a case of iatrogenic twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2012;39:597–598.

9 Sainz JA, Romero C, García-Mejido J, Soto F, Turmo E: Analysis of middle cerebral artery peak systolic velocity in monochorionic twin pregnancies as a method for identifying spontaneous twin anaemia-polycythaemia sequence. J Matern Fetal Neonatal Med 2013;27:1174–1176.

10 Ishii K, Hayashi S, Mabuchi A, Taguchi T, Yamamoto R, Murata M, et al: Therapy by laser equatorial placental dichorionization for early-onset spontaneous twin anemia-polycythemia sequence. Fetal Diagn Ther 2014;35:65–68.

11 Diehl W, Glosemeyer P, Tavares De Sousa M, Hollwitz B, Ortmeyer G, Hecher K: Twin anemia-polycythemia sequence in a case of monoamniotic twins. Ultrasound Obstet Gynecol 2013;42:108–111.

12 Lewi L, Jani J, Blickstein I, Huber A, Gucciardo L, Van Mieghem T, et al: The outcome of monochorionic diamniotic twin gestations in the era of invasive fetal therapy: a prospective cohort study. Am J Obstet Gynecol 2008;199:514.e1–e8.

13 Gucciardo L, Lewi L, Vaast P, Debska M, De Catte L, Van Mieghem T, et al: Twin anemia polycythemia sequence from a prenatal perspective. Prenat Diagn 2010;30:438–442.
14 De Villiers SF, Slaghekke F, Middeldorp JM, Walther FJ, Oepkes D, Lopriore E: Placental characteristics in monochorionic twins with spontaneous versus post-laser twin anemia-polycythemia sequence. Placenta 2013;34:456–459.
15 Nakayama S, Ishii K, Kawaguchi H, Hayashi S, Hidaka N, Murakoshi T, et al: Perinatal outcome of monochorionic diamniotic twin pregnancies managed from early gestation at a single center. J Obstet Gynaecol Res 2012;38:692–697.
16 Habli M, Bombrys A, Lewis D, Lim F-Y, Polzin W, Maxwell R, et al: Incidence of complications in twin-twin transfusion syndrome after selective fetoscopic laser photocoagulation: a single-center experience. Am J Obstet Gynecol 2009;201:417.e1–e7.
17 Mari G, Deter RL, Carpenter RL, Rahman F, Zimmerman R, Moise KJ Jr, et al: Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. N Engl J Med 2000;342:9–14.
18 Lopriore E, Holtkamp N, Sueters M, Middeldorp JM, Walther FJ, Oepkes D: Acute peripartum twin-twin transfusion syndrome: incidence, risk factors, placental characteristics and neonatal outcome. J Obstet Gynaecol Res 2014;40:18–24.
19 Lecointre L, Sananès N, Weingertner AS, Kohler M, Guerra F, Fritz G, et al: Fetoscopic laser coagulation for twin-twin transfusion syndrome before 17 weeks' gestation: laser data, complications and neonatal outcome. Ultrasound Obstet Gynecol 2014;44:299–303.
20 Lopriore E, Slaghekke F, Middeldorp JM, Klumper FJ, Oepkes D, Vandenbussche FP: Residual anastomoses in twin-to-twin transfusion syndrome treated with selective fetoscopic laser surgery: localization, size, and consequences. Am J Obstet Gynecol 2009;201:66.e1–e4.
21 Slaghekke F, Lopriore E, Lewi L, Middeldorp JM, van Zwet EW, Weingertner AS, et al: Fetoscopic laser coagulation of the vascular equator versus selective coagulation for twin-to-twin transfusion syndrome: an open-label randomised controlled trial. Lancet 2014;383:2144–2151.
22 Quintero RA, Comas C, Bornick PW, Allen MH, Kruger M: Selective versus non-selective laser photocoagulation of placental vessels in twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2000;16:230–236.
23 Fick AL, Feldstein VA, Norton ME, Wassel Fyr C, Caughey AB, Machin GA: Unequal placental sharing and birth weight discordance in monochorionic diamniotic twins. Am J Obstet Gynecol 2006;195:178–183.
24 Ntzoufras I: Bayesian Modeling Using WinBUGS. Wiley, 2009.
25 Freedman L: Bayesian statistical methods. BMJ 1996;313:569–570.
26 Slaghekke F, Favre R, Peeters SHP, Middeldorp JM, Weingertner AS, van Zwet EW, et al: Laser surgery as a management option for twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2014;44:304–310.
27 Carlin BP, Louis TA: Bayes and Empirical Bayes Methods for Data Analysis, ed 2. Boca Raton, Chapman and Hall/CRC, 2000.
28 Zhao DP, de Villiers SF, Slaghekke F, Walther FJ, Middeldorp JM, Oepkes D, et al: Prevalence, size, number and localization of vascular anastomoses in monochorionic placentas. Placenta 2013;34:589–593.
29 Luminoso D, Figueira CO, Marins M, Peralta CFA: Fetal brain lesion associated with spontaneous twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2013;42:721–722.
30 Lopriore E, Slaghekke F, Kersbergen KJ, de Vries LS, Drogtrop AP, Middeldorp JM, et al: Severe cerebral injury in a recipient with twin anemia-polycythemia sequence. Ultrasound Obstet Gynecol 2013;41:702–706.
31 Lopriore E, Hecher K, Vandenbussche FP, van den Wijngaard JPHM, Klumper FJ, Oepkes D: Fetoscopic laser treatment of twin-to-twin transfusion syndrome followed by severe twin anemia-polycythemia sequence with spontaneous resolution. Am J Obstet Gynecol 2008;198:e4–e7.
32 Slaghekke F, van Klink JMM, Koopman HM, Middeldorp JM, Oepkes D, Lopriore E: Neurodevelopmental outcome in twin anemia polycythemia sequence after laser surgery for twin-twin transfusion syndrome. Ultrasound Obstet Gynecol 2014;44:316–321.
33 Favre R, Koch A, Weingertner AS, Sananès N, Trieu NT, Kohler M, et al: Vascular pattern in monochorionic placentas with spontaneous TAPS and TTTS with residual anastomoses after laser: a case-control study. Prenat Diagn 2013;33:979–982.
34 Lopriore E, Deprest J, Slaghekke F, Oepkes D, Middeldorp JM, Vandenbussche FP, et al: Placental characteristics in monochorionic twins with and without twin anemia-polycythemia sequence. Obstet Gynecol 2008;112:753–758.

G Model
JOGOH-103; No. of Pages 6
ARTICLE IN PRESS

J Gynecol Obstet Hum Reprod xxx (2017) xxx–xxx

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com

Original Article

Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series

A. Koch [a,*], R. Favre [a], B. Viville [a], G. Fritz [a], M. Kohler [a], F. Guerra [a], L. Lecointre [a], A. Gaudineau [a], B. Langer [a], A.-S. Weingertner [a], N. Sananès [a,b]

[a] Department of obstetrics and gynecology, Strasbourg University Hospital, 67091 Strasbourg cedex, France
[b] Inserm, UMR-S 1121, "Biomatériaux et Bioingénierie", 67000 Strasbourg, France

ARTICLE INFO

Article history:
Received 4 May 2017
Received in revised form 7 September 2017
Accepted 19 September 2017
Available online xxx

Keywords:
Selective intra-uterine growth restriction
Monochorionic diamniotic pregnancies
Umbilical Doppler
Laser photocoagulation
Bichorionization

ABSTRACT

Introduction. – The objective was to report on a consecutive series of monochorionic diamniotic pregnancies complicated by selective Intra-Uterine Growth Restriction (sIUGR) and to describe perinatal outcomes based on whether or not there were umbilical Doppler findings, and specifically to study those pregnancies treated by laser.
Material and methods. – This was a retrospective cohort study enrolling monochorionic diamniotic pregnancies presenting isolated sIUGR after 16 weeks' gestation (WG).
Results. – Of the 25 cases of sIUGR, 16 were type I and 9 type II or III. Types II and III occurred earlier than type I (22.3 versus 24.3 WG), were more severe (discordance of 37% versus 23%), and delivered earlier (31.3 versus 33.9 WG). Survival was 12/18 (66.7%) for types II or III versus 32/32 (100%) for type I. Five laser photocoagulation procedures were attempted and allowed the survival of both twins in 2 cases. Overall survival after laser was 6/10 (60%).
Discussion. – Isolated sIUGR is associated with high perinatal morbidity and mortality. Laser photocoagulation treatment is feasible and may enable survival of both twins in some cases, but may be technically difficult.

Introduction

Monochorionic diamniotic twin pregnancies present specific complications linked to placental architecture and vascular anastomoses: Twin-Twin Transfusion Syndrome (TTTS), Twin Anemia-Polycythemia Sequence (TAPS), and selective Intra-Uterine Growth Restriction (sIUGR). This last entity is thought to occur in 10 to 15% of monochorionic pregnancies [1,2]. It is defined as growth below the 10th percentile in one of the twins, which is usually associated with a more than 25% difference in estimated fetal weights. Several hypotheses have been advanced to account for this intertwin growth discrepancy. The principal cause of growth restriction is the unequally shared placenta, the intertwin weight discrepancy being all the more flagrant when there is a major difference in placental sharing. Velamentous cord insertion is an indirect marker of unequal placental sharing and is more often found in cases of sIUGR [3]. Lastly, it should be noted that if there is unequal placental sharing it is common to find wider arteriovenous anastomoses and a generally far denser network of feto-fetal anastomoses [4]. These anastomoses are undoubtedly what enable the twin with the smaller placental share to survive.

Selective growth restriction in monochorionic pregnancies leads to varying degrees of morbidity and mortality depending on the severity of sIUGR, the intertwin growth differential and Doppler anomalies [5–8]. Nevertheless, sIUGR remains an entity, which is not widely understood, either in terms of its clinical presentation, or its natural course and complications. Moreover, there is no consensus about appropriate management, whether by attempted laser photocoagulation, selective termination of pregnancy or based on a "wait-and-see" policy [9–12]. Our hypothesis is that laser photocoagulation may enable the survival of both twins.

The objective of this paper was to report on a consecutive series of monochorionic diamniotic pregnancies complicated by sIUGR and to describe perinatal outcomes based on whether or not there

Abbreviations: sIUGR, selective intra-uterine growth restriction; WG, weeks'gestation; TTTS, twin-twin transfusion syndrome; TAPS, twin anemia-polycythemia sequence; IUFD, intra-uterine fetal death; EFW, estimated fetal weight.

* Corresponding author. Service de gynécologie-obstétrique, hôpitaux universitaires de Strasbourg, 1, place de l'Hôpital, 67091 Strasbourg cedex, France.
E-mail address: antoine.koch@chru-strasbourg.fr (A. Koch).

http://dx.doi.org/10.1016/j.jogoh.2017.09.004
2468-7847/

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

were umbilical Doppler findings, and specifically to study those pregnancies treated by laser.

Material and methods

Study type

This was a retrospective cohort study of monochorionic diamniotic pregnancies presenting sIUGR after 16 weeks' gestation (WG), followed up from January 2008 to December 2015 at the University Hospitals of Strasbourg which consists of the Schiltigheim Medico-Surgical and Obstetric Center (level 2 maternity unit) and the Hôpital de Hautepierre (level 3 maternity unit), the latter being the regional prenatal diagnostic referral center. Exclusion criteria were the finding of TTTS, TAPS, chromosomal and/or constitutional anomalies and Intra-Uterine Fetal Death (IUFD) at the time of diagnosis. The study was registered with the French data protection agency, CNIL, registration number 1940490.

Definitions

Selective IUGR was defined as an Estimated Fetal Weight (EFW) below the 10th percentile for one of the twins based on the standard curves of the French National College of Gynaecologists and Obstetricians (CNGOF) [13,14]. Ultrasound estimation of fetal weight was calculated using Hadlock's formula [15]. Selective IUGR was categorized into 3 types based on umbilical artery Doppler investigation of the fetus presenting sIUGR in line with Gratacòs's classification [13]: type I (normal Doppler), type II (persistent absent or reversed end-diastolic flow) and type III (intermittent absent or reversed end-diastolic flow). The growth discrepancy was calculated using the formula: (EFW of larger fetus – W of fetus with sIUGR)/EFW of larger fetus.

TTTS was defined as the presence of oligohydramnios with a deepest vertical pocket less than 2 cm in the donor, and polyhydramnios with a deepest vertical pocket greater than 8 cm before 20 WG and greater than 10 cm after 20 WG in the recipient [16].

TAPS corresponded to the presence of one anemic twin defined by a cerebral Peak Systolic Velocity (PSV) greater than 1.5 multiples of median (MoM) and the other polycythemic twin defined by a cerebral PSV less than 1 MoM [17,18], occurring spontaneously or following TTTS laser treatment [19].

Early neonatal death was defined by the occurrence of neonatal death within the first seven days of life.

Management

Chorionicity was diagnosed by an absent lambda sign on first trimester echography. Each monochorionic diamniotic pregnancy underwent repeat ultrasound monitoring every two weeks. Echography was performed by a specialized physician skilled in the use of Voluson 730, E8 and E10 devices (General Electric®, USA). Patients were in a slight left lateral decubitus position. Ultrasound investigation routinely included biometric measurements, assessment of the amount of amniotic fluid (measurement of the deepest vertical pocket), and Doppler evaluation of the umbilical, cerebral and aortic arteries. Localization of cord insertions was routinely determined. Pulsed Doppler examination of both umbilical arteries was performed in a free loop of the umbilical cord near placental insertion. The angle of insonation was zero or as near to zero as possible. Lastly, a complete morphological examination was carried out including especially an analysis of the cerebral structures and cardiac hemodynamics.

Laser photocoagulation treatment was offered in cases of sIUGR associated with umbilical Doppler anomalies (type II and III) and if technical conditions were favorable especially with regard to the placental localization. Cord coagulation of the sIUGR twin was only considered in the event of imminent demise. Patients were counselled by a multidisciplinary team and gave their informed consent. The local therapy board approved both the fetal interventions and the clinical study. Fetoscopic laser coagulation was performed under maternal sedation with Hypnovel® [midazolam] 5 mg and Atarax® [hydroxyzine] 100 mg orally one hour before the procedure. Local anesthesia of the skin and uterine wall was obtained using 1 % Xylocaine® [lidocaine] at the start of the session. The procedure was conducted using a Storz® 1.3 to 2 mm diameter fetoscope (Karl Storz Endoscopy®, Germany) with a right-angled or 30°-inclined lens passing through a right-angled or curved sheath depending on the placental localization. Depending on the fetoscope size, 8 to 12 French gauge trocars were also used. The trocar was inserted into the amniotic cavity of the larger twin. In order to facilitate the procedure, amnioinfusion was routinely conducted. Coagulation was achieved using a 400 μm laser diode fiber, with a maximum power of 40 Watts, which was extended through the fetoscope sheath. Coagulation was performed along the entire length of the membrane insertion. The entire procedure took place under ultrasound guidance. A prophylactic antibiotic treatment with 1 g Clamoxyl® [amoxicillin] was administered intravenously during the procedure. Routine tocolysis was not applied. A parenteral treatment with an antispasmodic was administered if the patient experienced pelvic pain. Fetal vital signs and hemodynamic criteria were checked by Doppler investigation 24 and 48 hours following the procedure. After the surgery, the patients were subjected to weekly and then bi-monthly ultrasound monitoring.

The choice of delivery method was based on the individual case and depended principally on the gestational age of the pregnancy, fetal presentations, cervical status and whether the situation was an emergency. If there was no spontaneous onset of labor at 36 WG, either induction or immediate cesarean was performed. Birth-weights were expressed as a percentile based on the French national growth curves [20].

Data analysis

All variables were collected prospectively and entered in the Diamm® electronic medical record system (Micro6® society, France). These variables included maternal biometric data, obstetrical characteristics, ultrasound findings and perinatal outcome.

Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed as numbers and percentages.

Results

Study population

A total of 72 patients were followed during the study period. Of this total, 43 were excluded owing to TTTS, two because of TAPS, one because of fetal malformation and another because of IUFD at the time of diagnosis (Fig. 1). In the end, 25 pregnancies presenting sIUGR met the criteria for analysis.

Of these cases, 16 were type I and 9 types II or III. Maternal characteristics were similar for both groups with respect to maternal age, gravidity, parity and body mass index. Of the nine type II or III pregnancies, five underwent laser treatment.

Ultrasound data

The ultrasound data for cases of sIUGR with normal and abnormal umbilical Doppler findings are displayed in Table 1. Of

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

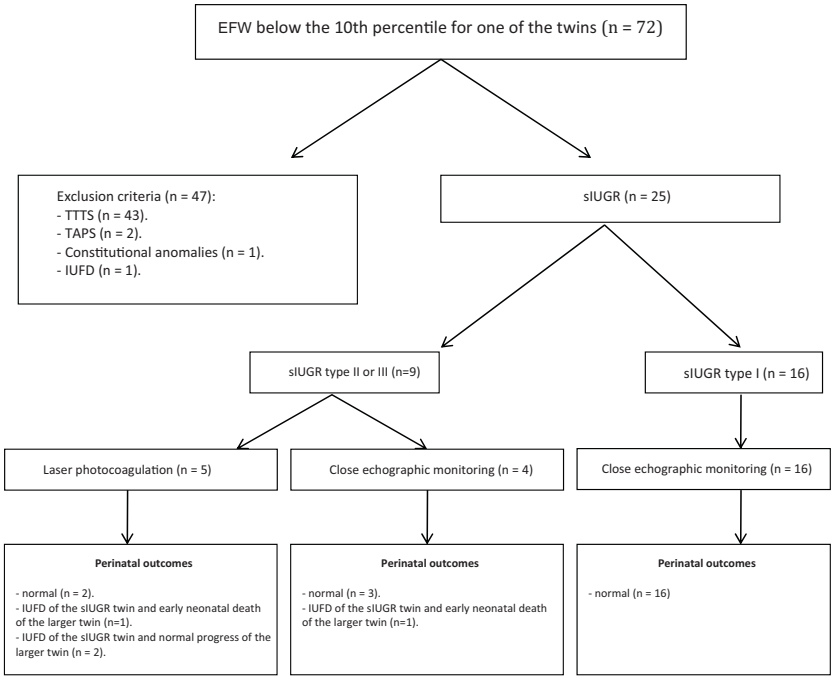


Fig. 1. Flow chart. EFW: estimated fetal weight; TTTS: twin-twin transfusion syndrome; TAPS: twin anemia-polycythemia sequence; IUFD: intrauterine fetal death; sIUGR: selective intrauterine growth restriction.

the 25 cases of sIUGR, 16 had a normal umbilical Doppler on diagnosis and nine exhibited Doppler anomalies. Of those with Doppler anomalies, two corresponded to type II and seven type III. Cases of sIUGR with abnormal umbilical Dopplers (types II and III) occurred on average earlier than those with normal umbilical Dopplers (type I), at 22.3 WG and 24.3 WG respectively. The estimated fetal weight for larger twins was comparable irrespective of the type of sIUGR, although EFW for fetuses presenting sIUGR was more severely restricted in type II or III cases than in type I. The intertwin EFW difference was also more marked in type II or III cases than in type I, at 37% and 23% respectively.

Cord insertion in larger fetuses was comparable in both groups, being mostly central and never velamentous. Umbilical Doppler was nearly always normal. Cord insertion in fetuses presenting sIUGR was in contrast hardly ever central, being mostly lateralized in type I and velamentous in types II or III.

Table 1
Ultrasound findings according to the Doppler type.

	Type I (n = 16)	Type II or III (n = 9)
Gestational age at diagnosis (WG)	24.3 ± 5,7	22.3 ± 3,1
EFW of the larger twin (g)	850 ± 694	545 ± 280
Percentile of the larger twin	45	47
EFW of the sIUGR twin (g)	659 ± 560	356 ± 222
Percentile of the sIUGR twin	4	1.5
Weight difference (%)	23	37
Cord insertion of the larger twin		
Central	9/14 (64%)	7/9 (78%)
Lateralized	5/14 (36%)	2/9 (22%)
Velamentous	0 (0%)	0 (0%)
Cord insertion of the sIUGR twin		
Central	1/12 (8%)	0 (0%)
Lateralized	8/12 (67%)	4/9 (44%)
Velamentous	3/12 (25%)	5/9 (56%)

WG: weeks' gestation; EFW: estimated fetal weight. Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed as numbers and percentages.

Perinatal data

Comparative perinatal data for sIUGR cases with normal umbilical Doppler versus abnormal Doppler findings are shown in Table 2. Patients with type II or III sIUGR delivered at an early gestational age than patients with type I: 31.3 WG versus 33.9 WG respectively. Birth-weights for twins were comparable, but the weight difference was more marked in patients with an abnormal Doppler (46% versus 29%). Morbidity and mortality were also more severe in types II or III, with 33% fetal loss. With regard to fetal gender, there were twice as many female as male fetuses irrespective of the type of sIUGR.

Management of type II or III sIUGR

Ultrasound and perinatal data for the five cases of type II or III sIUGR which underwent laser photocoagulation are displayed in Table 3. The procedure was carried out at the latest two weeks following diagnosis. These pregnancies presented a severe weight discrepancy of more than 30% and a very low percentile for the sIUGR twin. In one case, fetoscopy did not enable laser photocoagulation to be performed in view of the fact that it was technically impossible to gain access to the vascular equator. The pregnancy was subsequently complicated by IUFD of the sIUGR twin and early neonatal death of the larger twin owing to neurological and renal sequelae. Bichorionization was conducted by laser photocoagulation in the other four cases and was assessed as satisfactory. In two cases umbilical Doppler findings had reverted to normal. The other two cases culminated in IUFD.

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

Table 2
Perinatal outcomes according to the Doppler types.

	Type I ($n=16$)	Type II or III ($n=9$)
Gestational age at delivery (WG)	33.9 ± 2.9	31.2 ± 2.6
Onset of labor		
Spontaneous	2/16 (12.5%)	5/9 (56%)
Induction	2/16 (12.5%)	1/9 (11%)
Cesarean	12/16 (75%)	3/9 (33%)
Mode of delivery		
Vaginal birth	2/16 (12.5%)	5/9 (56%)
Cesarean during labor	2/16 (12.5%)	1/9 (11%)
Emergency cesarean	12/16 (75%)	3/9 (33%)
Weight of the larger twin (g)	2057 ± 593	1550 ± 455
Percentile of the larger twin	48	53
Weight of the sIUGR twin (g)	1465 ± 459	806 ± 408
Percentile of the sIUGR twin	5	7
Weight difference (%)	29	46
Outcomes of the larger twin		
Normal	16/16 (100%)	7/9 (78%)
Early neonatal death	0 (0%)	2/9 (22%)
IUFD	0 (0%)	0 (0%)
Outcomes of the sIUGR twin		
Normal	16/16 (100%)	5/9 (56%)
Early neonatal death	0 (0%)	0 (0%)
IUFD	0 (0%)	4/9 (44%)
Fetal gender		
Male	5/16 (31%)	3/9 (33%)
Female	11/16 (69%)	6/9 (67%)

WG: weeks' gestation; EFW: estimated fetal weight; IUFD: intra-uterine fetal death. Continuous variables were expressed as mean and standard deviation. Categorical variables were expressed as numbers and percentages.

Delivery took place on average 9 weeks and 2 days after laser photocoagulation. An increasing weight discrepancy was noted. In these four cases, the larger twin exhibited normal progress.

The other four cases of type II/III sIUGR did not undergo laser photocoagulation because of technical difficulties. There was one type II and three types III, diagnosed on average at 22,3 WG. Significantly, three of the four patients presented an anterior placenta. The fourth patient developed very early type III sIUGR (17 WG) and received close ultrasound monitoring. Since growth patterns were retained in both fetuses and Doppler findings were stable, laser photocoagulation was ultimately not performed. Patients delivered on average at 31 WG. Two living twins were born in three pregnancies. The fourth was complicated by IUFD of the sIUGR twin and early neonatal death of the larger twin owing to prematurity and multi-organ failure.

Discussion

Our study shows that isolated sIUGR is associated with high perinatal morality. The prognosis appears to be all the more unfavorable when the age on diagnosis is more premature, severe sIUGR and Doppler anomalies are present (these 3 parameters are often found in association). Laser photocoagulation treatment is feasible and may enable survival of both twins in some cases, but may be technically difficult or even impossible.

This is one of the rare studies on isolated sIUGR, with detailed ultrasound and perinatal data. The number of patients in our series is limited and couldn't allow us to perform statistical analysis. However, it must be borne in mind that isolated sIUGR is a rare pathology. In addition, we also found that TTTS was commonly associated with sIUGR. Such cases were excluded from analysis since they do not raise the same issues, in particular regarding the choice of treatment, since laser treatment is usually indicated.

Cases of type II or III sIUGR occurred two weeks earlier than cases of type I sIUGR and were complicated by higher perinatal morbidity, in particular a greater weight discrepancy. This was already hypothesized in Gratacòs' s classification [13]. Still, in our study, comparison of perinatal outcome may be biased because of the retrospective design and because only type II or III sIUGR underwent laser therapy. One of the factors proposed to account for the growth discrepancy is velamentous cord insertion [21]. The risk of selective restriction is thought to be three- or four-fold greater when the cord insertion of one of the twins is velamentous [22,23]. As it happens, in our study there were more velamentous insertions in type II or III than in type I cases.

Type I has a good perinatal prognosis whereas types II and III are associated with less favorable outcomes [6,11,24]. Where neonatal progress was favorable for both twins in type I cases, the IUFD rate was 44% for the fetus with type II or III sIUGR. This rate is higher than the 15% reported by Gratacós et al. [13], but this may be due to the fact that five patients were treated with laser in our series, thus increasing the risk of IUFD for the sIUGR twin. Furthermore, the occurrence of IUFD outside the context of laser intervention leads to increased morbidity in the surviving fetus: this is related to sudden fetal anemia due to hemodynamic decompensation, which can extend from tissue ischemia to death [25]. Hemodynamic decompensation of the surviving fetus due to sudden exsanguination would appear to occur just before or after IUFD of the other twin and results from the presence of intertwin vascular anastomoses, especially arteriovenous ones [26]. This may

Table 3
Ultrasound findings and perinatal outcomes in case of laser photocoagulation.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Ultrasound findings					
Gestational age at diagnosis (WG)	20.4	20.4	25.6	22.4	23.0
Percentile of the larger twin	39	13	12	50	86
Percentile of the sIUGR twin	0	0	0	1	0
Weight difference (%)	47	74	36	30	42
UD of the sIUGR twin	II	III	III	III	III
Gestational age at laser photocoagulation (WG)	22.6	20.6	26	22.6	25.2
Bichorionization by photocoagulation	Success	Success	Failure	Success	Success
UD of the sIUGR twin after laser	II	I	III	I	END
Perinatal outcomes					
Gestational age at delivery (SA)	32.4	30.1	28.2	33.2	32.5
Onset of labor	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Spontaneous
Mode of delivery	Vaginal birth	Cesarean	Vaginal birth	Vaginal birth	Vaginal birth
Percentile of the larger twin	31	36	68	38	79
Percentile of the sIUGR twin	0	0	8	0	0
Weight difference (%)	75	60	31	45	88
Perinatal outcome of the larger twin	Normal	Normal	END	Normal	Normal
Perinatal outcome of the sIUGR twin	IUFD	Normal	IUFD	Normal	IUFD

WG: weeks' gestation; UD: umbilical Doppler; END: early neonatal death; IUFD: intra-uterine fetal death.

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

therefore account for our rate of 22% early fetal death for larger fetuses in cases of type II or III sIUGR.

Selective IUGR carries a high risk of spontaneous prematurity, especially when associated with umbilical Doppler anomalies: 31 WG + 2 days versus 33 WG + 6 days when no Doppler anomalies are present. De Paepe et al. also underlined this risk of prematurity by showing that the gestational age on delivery was lower in cases of sIUGR than in eutrophic twins: 33 WG + 3 days versus 34 WG + 6 days ($P < 0.02$) [22]. A risk of induced prematurity has also been reported for the larger twin when emergency cesarean has been performed owing to non-reassuring fetal status in the sIUGR twin. This prematurity is also thought to raise the risk of neurological morbidity [24].

There are three possible options for managing cases of sIUGR: simple monitoring, cord occlusion of the smaller twin and placental bichorionization with laser photocoagulation. There are no studies showing that one particular strategy is better than the others. In cases of severe sIUGR with Doppler anomalies, it seems appropriate to propose therapeutic management since in the event of IUFD of the sIUGR twin the other twin is exposed to the risk of IUFD or neurological damage. We believe laser is an interesting option in these cases since it is the only technique enabling safeguard, in some cases, of the sIUGR twin [9]. In our study, photocoagulation of the anastomoses produced a favorable outcome for the eutrophic twin in all cases, but was complicated in two of the four cases by IUFD of the sIUGR fetus. According to Gratacós et al., the risk of IUFD is threefold higher for the sIUGR twin if laser is used [10]. This may be due to the fact that the anastomoses take on a protective role for the sIUGR fetus, the blood flow from the larger fetus acting as a "rescue" transfusion. It should be noted that if both twins survive the procedure, this may then raise the issue of an intertwin conflict of interest: prematurity may potentially be induced in the larger twin when severe sIUGR is present in the other twin. Lastly, laser photocoagulation in sIUGR cases is technically more difficult than in TTTS cases because of the absence of polyhydramnios, because the vascular equator is more often in the amniotic cavity of the sIUGR twin and because of the frequently wider anastomoses. However, if complete bichorionization has not been performed, the risks persist for the larger fetus, if IUFD of the sIUGR twin occur. In one of the cases in our series, laser photocoagulation was impossible for technical reasons and the outcome was unfavorable, with the loss of both twins. It is for these reasons that some authors propose immediate selective termination of pregnancy of the sIUGR twin, by bipolar forceps coagulation of the cord, in order to ensure that the larger fetus has the maximum likelihood of surviving [27]. In fact, cord occlusion is more easily achievable even though more radical. Comparing the cord occlusion series by Parra-Cordero et al. [28], Chalouhi et al. [29] and Bebbington [30] ($n = 136$) with the laser series of Peeva et al. [31], Quintero et al. [9] and Chalouhi et al. ($n = 192$), the rate of delivery before 32 weeks is 22% for cord occlusion versus 46% for laser, the larger fetal survival rate 91% for cord occlusion versus 69% for laser, and the sIUGR twin survival rate 0% for cord occlusion versus 37% for laser [32]. Unfortunately, there is no long-term data on the outcomes of these neonates, especially from a neurodevelopmental point of view. Still we can postulate that laser therapy ensures the best survival rate whereas cord occlusion provides a limited morbidity for the surviving twin. In sum, the decision about the treatment strategy to adopt depends on the severity of sIUGR and the technical constraints, but also — and primarily — on the choice of the parents.

Conclusion

Isolated sIUGR is associated with high perinatal morbidity and mortality. Early age of diagnosis, severity of sIUGR and the presence of umbilical Doppler anomalies are of poor prognosis. Bichorionization by laser photocoagulation is feasible but may be difficult technically. In some cases laser intervention enables safeguard of the sIUGR twin but reduces the chances of the larger twin compared to cord occlusion of the sIUGR twin. Another option is the cord occlusion, which is more easily achievable even though more radical. While awaiting further comparative studies on the different therapeutic approaches, treatment depends on the severity of sIUGR and technical constraints but also, and primarily, on the parents' choice.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Valsky DV, Eixarch E, Martinez JM, et al. Selective intrauterine growth restriction in monochorionic twins: pathophysiology, diagnostic approach and management dilemmas. Semin Fetal Neonatal Med 2010;15:342–8.
[2] Smith NA, Wilkins-Haug L, Santolaya-Forgas J, et al. Contemporary management of monochorionic diamniotic twins: outcomes and delivery recommendations revisited. Am J Obstet Gynecol 2010;203:e1–6.
[3] Hanley ML, Ananth CV, Shen-Schwarz S, et al. Placental cord insertion and birth weight discordancy in twin gestations. Obstet Gynecol 2002;99:477–82.
[4] Lewi L, Cannie M, Blickstein I, et al. Placental sharing, birthweight discordance, and vascular anastomoses in monochorionic diamniotic twin placentas. Am J Obstet Gynecol 2007;197:e1–8.
[5] Adegbite AL, Castille S, Ward S, et al. Neuromorbidity in preterm twins in relation to chorionicity and discordant birth weight. Am J Obstet Gynecol 2004;190:156–63.
[6] Chang Y-L, Chang S-D, Chao A-S, et al. Clinical outcome and placental territory ratio of monochorionic twin pregnancies and selective intrauterine growth restriction with different types of umbilical artery Doppler. Prenat Diagn 2009;29:253–6.
[7] Alam Machado RDC, Brizot MDL, Liao AW, et al. Early neonatal morbidity and mortality in growth-discordant twins. Acta Obstet Gynecol Scand 2009;88:167–71.
[8] Gratacós E, Carreras E, Becker J, et al. Prevalence of neurological damage in monochorionic twins with selective intrauterine growth restriction and intermittent absent or reversed end-diastolic umbilical artery flow. Ultrasound Obstet Gynecol 2004;24:159–63.
[9] Quintero RA, Bornick PW, Morales WJ, et al. Selective photocoagulation of communicating vessels in the treatment of monochorionic twins with selective growth retardation. Am J Obstet Gynecol 2001;185:689–96.
[10] Gratacós E, Antolin E, Lewi L, et al. Monochorionic twins with selective intrauterine growth restriction and intermittent absent or reversed end-diastolic flow (Type III): feasibility and perinatal outcome of fetoscopic placental laser coagulation. Ultrasound Obstet Gynecol 2008;31:669–75.
[11] Ishii K, Murakoshi T, Takahashi Y, et al. Perinatal outcome of monochorionic twins with selective intrauterine growth restriction and different types of umbilical artery Doppler under expectant management. Fetal Diagn Ther 2009;26:157–61.
[12] Van den Bos EM, van Klink JMM, Middeldorp JM, et al. Perinatal outcome after selective feticide in monochorionic twin pregnancies. Ultrasound Obstet Gynecol 2013;41:653–8.
[13] Gratacós E, Lewi L, Muñoz B, et al. A classification system for selective intrauterine growth restriction in monochorionic pregnancies according to umbilical artery Doppler flow in the smaller twin. Ultrasound Obstet Gynecol 2007;30:28–34.
[14] Valsky DV, Eixarch E, Martinez JM, et al. Selective intrauterine growth restriction in monochorionic diamniotic twin pregnancies. Prenat Diagn 2010;30:719–26.
[15] Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements – a prospective study. Am J Obstet Gynecol 1985;151:333–7.
[16] Quintero RA, Morales WJ, Allen MH, et al. Staging of twin-twin transfusion syndrome. J Perinato 1999;19:550–5.
[17] Lopriore E, Middeldorp JM, Oepkes D, et al. Twin anemia-polycythemia sequence in two monochorionic twin pairs without oligo-polyhydramnios sequence. Placenta 2007;28:47–51.
[18] Veujoz M, Sananès N, Severac F, et al. Evaluation of prenatal and postnatal diagnostic criteria for twin anemia-polycythemia sequence. Prenat Diagn 2015;35:281–8.

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

[19] Favre R, Koch A, Weingertner AS. Vascular pattern in monochorionic placentas with spontaneous TAPS and TTTS with residual anastomoses after laser: a case-control study. Prenat Diagn 2013;33:979–82.
[20] Mamelle N, Munoz F, Grandjean H. [Fetal growth from the AUDIPOG study. I. Establishment of reference curves]. J Gynecol Obstet Biol Reprod 1996;25:61–70.
[21] Lopriore E, Pasman SA, Klumper FJ, et al. Placental characteristics in growth-discordant monochorionic twins: a matched case-control study. Placenta 2012;33:171–4.
[22] De Paepe ME, Shapiro S, Young L, et al. Placental characteristics of selective birth weight discordance in diamniotic-monochorionic twin gestations. Placenta 2010;31:380–6.
[23] Kent EM, Breathnach FM, Gillan JE, et al. Placental cord insertion and birthweight discordance in twin pregnancies: results of the national prospective ESPRiT Study. Am J Obstet Gynecol 2011;205:e1–7.
[24] Weisz B, Hogen L, Yinon Y, et al. Perinatal outcome of monochorionic twins with selective IUGR compared with uncomplicated monochorionic twins. Twin Res Hum Genet 2011;14:457–62.
[25] Nicolini U, Pisoni MP, Cela E, et al. Fetal blood sampling immediately before and within 24 hours of death in monochorionic twin pregnancies complicated by single intrauterine death. Am J Obstet Gynecol 1998;179:800–3.
[26] Senat MV, Loizeau S, Couderc S, et al. The value of middle cerebral artery peak systolic velocity in the diagnosis of fetal anemia after intrauterine death of one monochorionic twin. Am J Obstet Gynecol 2003;189:1320–4.
[27] Has R, Kalelioglu I, Corbacioglu Esmer A, et al. Bipolar cord coagulation in the management of complicated monochorionic twin pregnancies. Fetal Diagn Ther 2014;36:190–5.
[28] Parra-Cordero M, Bennasar M, Martínez JM, et al. Cord occlusion in monochorionic twins with early selective intrauterine growth restriction and abnormal umbilical artery Doppler: A consecutive series of 90 cases. Fetal Diagn Ther 2016;39:186–91.
[29] Chalouhi GE, Marangoni MA, Quibel T, et al. Active management of selective intrauterine growth restriction with abnormal Doppler in monochorionic diamniotic twin pregnancies diagnosed in the second trimester of pregnancy. Prenat Diagn 2013;33:109–15.
[30] Bebbington M. Selective reduction in complex monochorionic gestations. Am J Perinatol 2014;31:S51–8.
[31] Peeva G, Bower S, Orosz L, et al. Endoscopic placental laser coagulation in monochorionic diamniotic twins with type ii selective fetal growth restriction. Fetal Diagn Ther 2015;38:86–93.
[32] Gratacos E.In: Selective IUGR II (and III). Expectant management and cord occlusion; 2016.

Please cite this article in press as: Koch A, et al. Expectant management and laser photocoagulation in isolated selective intra-uterine growth restriction: A single-center series. J Gynecol Obstet Hum Reprod (2017), http://dx.doi.org/10.1016/j.jogoh.2017.09.004

8.2. Uropathies obstructives basses

8.2.1. Standardisation de la prise en charge prénatale

Fetal Lower Urinary Tract Obstruction – A proposal of Standardized Multidisciplinary Prenatal Management based on Disease Severity

Rodrigo RUANO (MD, PhD) [1]; Nicolas SANANES (MD) [1]; Clark WILSON (MD) [2]; Jason AU (MD) [2]; Chester J. KOH (MD) [2]; Patricio GARGOLLO (MD) [2];Alireza A. SHAMSHIRSAZ (MD) [1]; Jimmy ESPINOZA (MD) [1]; Adnan SAFDAR (MD) [3]; Amirhossein MOADDAB (M.D.) [1]; Nicolas MEYER (MD, PhD) [7]; Darrell L. CASS (MD) [4]; Oluyinka O. OLUTOYE (MD, PhD) [4]; Olutoyin A. OLUTOYE (MD) [5]; Stephen WELTY (MD) [6]; David R. ROTH (MD) [2]; Michael C. BRAUN (MD) [3]; Michael A. Belfort (MD, PhD) [1]

[1] Department of Obstetrics and Gynecology, [2] Division of Pediatric Urology, [3] Division of Pediatric Nephrology, [4] Department of Pediatric Surgery, [5] Department of Anesthesiology and [6] Department of Pediatrics, Baylor College of Medicine and Texas Children's Fetal Center – Texas Children's Hospital Pavilion for Women, Houston, Texas, United States

[7] Department of public health, Strasbourg University Hospital, Strasbourg, France

Correspondance to: Rodrigo Ruano, Pavilion for Women – Texas Children's Fetal Center, 6651 Main Street, Suite F1020, Houston, Texas, United States, 77030, ruano@bcm.edu , rodrigoruano@hotmail.com , phone: 1.832.418.3310

Conflict of interest: none

All authors contributed to the present study

ABSTRACT

Objectives: To present a single center experience of a standardized prenatal multidisciplinary management protocol for fetal lower urinary tract obstruction (LUTO) and to propose a classification of fetal LUTO based on disease severity.

Methods: This is a retrospective cohort study of 25 consecutive fetal patients with prenatal diagnosis of primary LUTO. Fetal intervention was offered after evaluation by a multidisciplinary team. Analyses were conducted using Bayesian methodology.

Results: Fifteen of the 25 (60.0%) patients referred for evaluation survived to postnatal evaluation. Fetal vesicoamniotic shunt was placed in 14 (56.0%) patients with 12 survivors. Multivariate analysis suggested that fetal intervention (OR 6.97 [0.88-70.16], Pr(OR>1)=96.7%), anhydramnios (OR 0.12 [0.04-0.35], Pr (OR<1)=99.9%), and favorable fetal urine analysis (OR 3.98 [0.63-25.15], Pr(OR>1)=92.7%) were predictors of survival.

Conclusion: Fetal intervention and fetal renal function were independently associated with postnatal survival for fetuses with LUTO. A classification based on the severity of disease is proposed.

KEYWORDS: Fetal lower urinary tract obstruction, posterior urethral valves, cystoscopy, vesicoamniotic shunt, prenatal diagnosis, ultrasonography, laser, fetal surgery

INTRODUCTION

Fetal lower urinary tract obstruction (LUTO) or fetal bladder outlet obstruction is a spectrum of pathologies characterized by a dilated fetal bladder and bilateral hydronephrosis caused by an obstruction in the lower urinary tract.(1-4) The incidence is approximately 2.2 out of every 10,000 births and is commonly diagnosed during the late first or early second trimester of pregnancy.(1-4) The etiologies of LUTO include posterior urethral valves, urethral atresia and urethral stenosis.(5-7) Complete bladder outlet obstruction (severe LUTO) is associated with high perinatal mortality due to pulmonary hypoplasia and severe renal impairment/damage.(5-8)

There are three potential prenatal interventional therapies for LUTO – repeated vesicocentesis (uncommon), fetal vesicoamniotic shunt placement, and fetal cystoscopy, as well as termination of pregnancy.(2, 7-24) Prior reports have suggested that fetal intervention for LUTO improves postnatal survival, but improved long-term renal function from such interventions requires confirmation.(7, 25) Furthermore, the selection of candidates for fetal intervention in LUTO is clinically challenging and controversial, partly due to the lack of an accepted standardized staging classification. Previous publications have suggested that the use of fetal urinary biochemistry, ultrasonographic characteristics of the fetal kidneys, and various amniotic fluid indices can predict outcomes (26-32); however, systematic reviews have failed to confirm the individual predictive ability of these parameters probably because these prenatal parameters were considered in isolation (not in combination) and in a determined gestational age (without considering the *in utero* progression of the disease) .(33, 34) To date, a classification system for prognosis of fetal LUTO based on the disease severity as determined by clinical parameters has yet to be successfully validated.

One aim of the present study is to report our single center experience with fetal LUTO and fetal intervention after a multidisciplinary prenatal evaluation that considers a combination of prenatal parameters correlated to the gestational age as well as the in utero progression the disease. A second aim is to propose a standardized prenatal evaluation and a classification of fetal LUTO based on the findings of the most commonly applied tests in use today.

METHODS

Study design

A retrospective cohort study was performed on all fetuses with a prenatal diagnosis of primary LUTO referred to the Baylor College of Medicine/Texas Children's Fetal Center between April 2013 and December 2014. LUTO was diagnosed based on the ultrasound confirmation of a dilated bladder associated with bilateral hydroureter and bilateral hydronephrosis (8, 35). Cases of LUTO were considered primary if there were no associated fetal malformations (normal targeted ultrasound examination and fetal echocardiography) and a normal karyotype. Local institutional review board approval was received for this study.

Prenatal multidisciplinary management

All patients were evaluated according to a prenatal multidisciplinary LUTO management protocol. This protocol included an initial comprehensive ultrasound evaluation of fetal anatomy, detailed fetal echocardiogram, and prenatal genetic counseling followed by prenatal consultation with maternal-fetal (fetal intervention) specialists on the first day of evaluation. Ultrasound-guided amniocentesis or cordocentesis for fetal FISH and karyotype (or chromosomal microarray – CMA) were

offered in addition to ultrasound-guided vesicocentesis for fetal urinary analysis. A second evaluation was performed 48 hours after the initial evaluation and included a repeat fetal ultrasound evaluation with specific interest placed on the rate and degree of bladder refilling. In addition, a multidisciplinary team composed of pediatric nephrologists, pediatric urologists, and maternal-fetal medicine fetal interventionists evaluated the results of the fetal urinary analysis, imaging studies and genetic testing. The perinatal management options were discussed with the family on a case-by-case basis and included prenatal expectant management, termination of pregnancy (before 22weeks of gestation according to Texas state law) or fetal intervention (ultrasound-guided fetal vesicoamniotic shunt placement).

Fetal intervention was offered when there was a diagnosis of severe LUTO at gestational ages between 16 - 34weeks, in the presence of oligohydramnios/anhydramnios (consider only after 18 weeks) and normal renal functional (considered between 18 - 30 weeks of gestation) renal parameters. Normal fetal renal function was defined as the absence of bilateral renal dysplasia or renal cysts on ultrasonography (independent of gestational age) and/or 'favorable' urinary biochemistry (urinary sodium <100 mEq/L, chloride <90 mEq/L, osmolarity <200 mOsm/L and beta2-microglobulin <6 mg/L) between 18 and 30 weeks of gestation in the latest sample in a set of serial samples, out of a maximum of three samples collected over an interval of 48 hours (32, 36) Oligohydramnios (amniotic fluid index below the 5th percentile) was also considered as an inclusion criterion for fetal intervention if present after 18 weeks of gestation.(37)

Fetal intervention consisted of placing a fetal vesicoamniotic shunt percutaneously under ultrasound guidance. No fetal cystoscopic laser ablation of posterior urethral vales was performed in the present study. Either the Harrison Fetal

Bladder Stent Set (Cook Medical Inc., Bloomington, United States) (<18 weeks of gestation) or the Rocket KCH Fetal Bladder Drain Set (Rocket Medical plc, Washington, England) (≥ 18 weeks of gestation) was used. (16, 22, 38, 39). All shunt procedures were performed in the operating room with local anesthesia (0.25% bupivacaine to maternal skin and subcutaneous tissue). Fetal anesthesia/immobilization was provided by intramuscular injection of fentanyl (15 μg/Kg) and vecuronium (0.2 mg/Kg) to the fetus using a 22-gauge needle after 20 weeks, and was followed by an amnioinfusion using the same needle. Weekly ultrasound follow-up was performed after the procedure. In the event of shunt dislodgement or obstruction and recurrence of bladder enlargement, a repeat fetal vesicoamniotic shunt placement was offered.

Delivery timing and route were based on standard obstetric principles. After delivery, all surviving neonates were evaluated and followed by pediatric specialists for a period of 6 months, including pediatric urologists, nephrologists and neonatologists. A voiding cystourethrogram and postnatal cystoscopy were performed to evaluate the bladder and the urethra in all cases. Autopsy was offered if fetal or neonatal demise occurred.

Statistical analysis

The primary outcome measure was survival at 6 months of life. The secondary outcome measure was functional renal status at 6 months of life. Normal renal function was defined by serum creatinine < 0.5 mg/dL at 6 months of life.(40-42)

Collected data included gestational age at diagnosis, presence or absence of megacystis, severe hydronephrosis (grade ≥2 according to Grignon's classification (35)), hyperechogenic kidneys (renal tissue has similar echogenicity as the bones), presence of renal cortical cysts, renal dysplasia (enlarged hyperechogenic kidney, with no corticomedullary differentiation and small cysts in the cortex), presence of hydroureter

(dilated hydroureters), and anhydramnios, fetal urinary biochemistry (favorable vs. non-favorable) (32, 36), fetal intervention vs. no fetal intervention, gestational age at fetal intervention, total number of fetal interventions per patient and gestational age at delivery.

Continuous variables were expressed as mean and standard deviation or median and range as appropriate based on the distribution of the data. Categorical variables were expressed as absolute values and percentages. Statistical analyses were conducted using Bayesian methods (43-46) with R 3.0.0 software and WinBUGS software (estimation using Markov chain Monte Carlo (MCMC) with Gibbs sampling). After a burn-in of 5 000 updates, 100 000 iterations were performed and convergence was checked using trace plots of the sample values for each iteration. The primary and the secondary outcomes were modeled by logistic regression using Normal distribution ($\mathcal{N}$(mean, variance)). Results from the PLUTO trial (47) and a large recent study (48) were used to provide informative Bayesian priors for the logistic regression parameters [for survival vs. death: anhydramnios $\mathcal{N}(0.25, 6.58)$; 'favorable' fetal urinary biochemistry $\mathcal{N}(1.74, 1.43)$ and fetal intervention $\mathcal{N}(1.16, 0.358)$]. In the absence of prior information for the estimation of a proportion, a generic low-informative prior $\mathcal{N}(0.0, 6.58)$ was used for the logistic regression parameters.

Each result is presented as an odds ratio (OR) with a 95 % credibility interval, with probability of the OR being above or under 1. Bayesian analysis does not use the ('frequentist') p-value and the probability of exceeding 1 should not be confused with the p-value. The 95% credibility interval should not be confused with the 95% confidential interval. The 95% credibility interval is the interval where the true value of the parameter (OR for instance) lies within a probability of 0.95. It is important to

notice that Bayesian results should be interpreted or considered as a "unilateral view" when using 'frequentist language'. Thus, Pr(OR>1) >0.95 is considered significant, which is not imcompatible with a credibility interval that includes the value "1" since a 95% credibility interval leaves 2.5% probability below and 2.5% probability above the credibility interval. When the Pr(OR>1) is larger than 95% but is smaller than 97.5%, the result is significant (unilateral point of view) even though the OR credibility interval includes 1 (bilateral point of view). These are merely two different views of a same object, i.e. the posterior OR distribution, that can, in Bayesian analysis, be used simultaneously. (45, 49)

Multivariate analyses for prediction of survival were performed with no more than 3 variables in view of the small sample size. Selection of variables was performed according to clinical relevance and statistical criteria (credibility interval and deviance information criterion).

RESULTS

During the study period, a total of 25 consecutive fetuses with a diagnosis of primary LUTO were evaluated at our center. LUTO was diagnosed in the first trimester in 5 (20.0%) patients. At presentation to our center, the 'keyhole sign' was present in 22 (88.0%) fetuses, severe hydronephrosis in 21 (84.0%), bilateral hyperechogenic kidneys in 17 (68.0%) and renal cortical cysts in 7 (28.0%). Anhydramnios was seen in 19 (66.7%) fetuses. Fifteen of the 25 patients in the cohort (60.0%) survived. Ten (66.7%) infants had normal renal function at the age of 6 months.

Table 1 shows the demographics of the patients who underwent fetal intervention and those that did not. Fetal vesicoamniotic shunt was performed in 14

(56.0%) patients. Mean gestational age at diagnosis was 18.5+/-4.2 weeks. The following ultrasound characteristics were observed during the first evaluation: severe hydronephrosis (n=13; 92.9%), hyperechogenic kidney (n=8; 57.1%), renal cortical cysts (n=1; 7.1%) fetus and anhydramnios (n=10, 71.4%). None of the fetuses that underwent fetal intervention had ultrasound findings suggestive of 'renal dysplasia' at the initial examination. Among the 14 fetuses that underwent fetal intervention, 8 (57.1%) patients underwent repeated fetal vesico-amniotic shunt placement, with 2 out of 8 (14.3%) patients undergoing more than 3 procedures (3 and 5 shunts respectively). Mean gestational age at delivery was 33.0+/-4.4 weeks. At 6-month follow-up, the survival rate was 85.7% (12/14) and 8 (66.7%) infants had normal renal function.

Table 2 shows the characteristics of infants that survived vs. those that did not. A total of 15 (60.0%) fetuses survived to 6 months of age, whereas 10 (40.0%) did not. Among the patients that underwent fetal intervention (n=14), twelve (80.0%) infants survived while 2 (20%) infants died; one fetus died in utero at 18 weeks (1 week after fetal intervention); and another infant died due to complications related to extreme prematurity and sepsis (delivered at 27 weeks because of severe oligohydramnios and decreased fetal movement), but without signs of severe pulmonary hypoplasia or renal failure. In univariate analyses with non-informative priors, the following prenatal variables were negatively associated with survival at 6 months of life: presence of renal cortical cysts (OR: 0.07 [0.01-0.44], Pr(OR<1)=99.7%), presence of ultrasound findings suggestive of 'renal dysplasia' (OR: 0.06 [0.00-0.77], Pr(OR<1)=98.4%) and presence of anhydramnios (OR : 0.03 [0.00-0.67], Pr(OR<1)=99.2 %), Fetal intervention (OR : 12.9 was positively associated with survival at 6 months of life [2.28-89.40], Pr(OR>1)=99.8%). Multivariate analysis suggested that fetal intervention (OR 6.97 [0.88-70.16], Pr(OR>1)=96.7%), anhydramnios (OR 0.12 [0.04-0.35], Pr(OR<1)=99.9%),

favorable fetal urine analysis (OR 3.98 [0.63-25.15], Pr(OR>1)=92.7%) and absence of renal cortical cysts (OR 3.9 [0.66-24.2], Pr(OR>1)=93.3%) were predictors of survival.

Among the 15 infants that survived to 6 months of age, ten (66.7%) had normal renal function at this time (Table 3). Of those that had abnormal renal function at 6 months of life, there was a higher incidence of bilateral hyperechogenic kidneys and anhydramnios.

DISCUSSION

Principal findings

Our results suggest that postnatal outcome depends on the fetal intervention and the severity of LUTO, which seem to be independently associated with prognosis.

In our center, fetal vesicoamniotic shunt increased the chance of survival 13-fold, being associated with a survival rate of 85.7%, which seems to be higher than those previously reported in the literature (approximately 65%).(7, 25) There are possible explanations for the improved outcomes observed in our study. We treated fetuses with severe LUTO and "favorable" fetal renal biochemistry as urgent therapy – fetal intervention was offered as early as possible after the prenatal diagnosis was made usually within 2 days. In addition, close follow-up was carried out that included weekly fetal ultrasounds following placement of the shunt, and in the event of shunt migration or obstruction, the shunt was immediately replaced (***'aggressive proactive management'***). Another possible explanation could be the rigorous selection criteria used to screen candidates for fetal intervention based on a multidisciplinary evaluation of '***estimated fetal renal function***' according to gestational age.

Based on our results, we suggest that the selection of candidates for fetal intervention needs to be based on a multidisciplinary evaluation of multiple parameters according to gestational age. Both fetal urinary biochemistry and ultrasound characteristics of the fetal kidneys need to be considered to define ***'estimated fetal renal function'***. The presence of 'non-favorable' fetal urinary biochemistries, fetal renal cortical cysts, and/or fetal 'renal dysplasia' may suggest as hallmarks of '***abnormal fetal renal function'***. In this group of patients with abnormal fetal renal function, we did not offer fetal intervention. The utility of fetal urinary biochemistry has been validated only after 18 weeks of gestation.(26, 31-34, 50) Therefore, in our protocol, we only considered the fetal urinary biochemistry after this gestational age. Secondly, it is unclear in the literature if repeated fetal urinary biochemistry profiling improves the accuracy of this index in evaluating fetal renal function.(26, 32) In our center, we repeated fetal urinary biochemistry 48 hours after the first examination if there were no ultrasound findings suggesting fetal 'renal cortical cysts' or 'renal dysplasia'. If there was significant improvement in the fetal urinary biochemistry profile in association with other favorable ultrasound markers, we offered fetal vesicoamniotic shunt. In the event that repeat fetal urinary biochemistry markers were similar or worse compared to the initial results, the fetus was considered to have '***abnormal' fetal renal function***' and shunt was not offered

Based on our observations of 'fetal renal function, we suggest 3 groups of patients with LUTO (Table 4):

The first group of fetuses with LUTO (***Stage I LUTO***) usually had normal amniotic fluid levels [after 18 weeks of gestation] and 'normal fetal renal function' ('favorable' fetal urinary biochemistry and no evidence of fetal renal cysts/dysplasia). For this group, we recommend expectant prenatal management with weekly ultrasound follow-

up, where consideration for fetal intervention was only in the setting of oligohydramnios [after 18 weeks of gestation]. This group of patients had partial urethral obstruction. In our series, all fetuses in this group survival and had normal renal function at 6 months of life.

The second group of fetuses with LUTO (***Stage II LUTO***) had oligohydramnios (after 18 weeks of gestation) and severe bilateral hydronephrosis but with '***normal renal function'***. This group, in our opinion, would benefit from fetal intervention (vesicoamniotic shunt) with the objective of preventing severe pulmonary hypoplasia and further deterioration of renal function. In our series, we identified 16 patients with this profile. Fetal intervention was performed on 14 patients resulting in 12 survivors at 6 months of life. One fetus expired in utero one week after the procedure and another infant expired due to consequences of extreme prematurity. Overall the survival rate in this group was 75% with 33% of patients being dependent on dialysis (ESRD) by 6 months of life.

The third group of fetuses with severe LUTO (***Stage III LUTO***) had oligohydramnios (after 18 weeks) and severe bilateral hydronephrosis but with signs of already '***abnormal renal function'*** (ultrasound findings suggesting renal cortical cysts and/or 'renal dysplasia' and/or 'non-favorable' fetal urinary biochemistry). In this group, fetal intervention was thought not to be able to reduce the severity of renal impairment and therefore fetal vesicoamniotic shunting was not offered at our center. We identified 7 fetuses with this profile in our series; one pregnancy was terminated in this group. One infant did survive, though it had renal failure requiring dialysis. The remaining infants in this group died immediately after birth due to severe pulmonary hypoplasia. Further studies are necessary to investigate the possible benefit of

vesicoamniotic shunting or serial amnioinfusion in this population to prevent postnatal death due to severe pulmonary hypoplasia.

Strengths and weaknesses

The strengths of the present study were careful evaluation following a rigorous protocol with multidisciplinary evaluation, uniform prenatal management for all patients, and only 2 patients elected to terminate the pregnancy. The present study proposes a strategy for prenatal management of LUTO that classifies the patients based on the disease severity and the gestational age, which can guide clinicians and investigators to appropriately select candidates for fetal vesicoamniotic shunt or fetal cystoscopy and to accurately study the effectiveness of fetal interventions for specific groups of patients.

Our study also has limitations, including the retrospective nature of the analysis, the small number of patients. Additionally, we did not evaluate the long-term outcome regarding renal function and urological sequelae. However, all patients were prospectively followed and data was obtained from all patients. Bayesian statistical methods were used, because it considers prior information along with our results, which is of paramount importance in situations with small sample size. Additionally, results produced by this method are exact rather than approximated as is seen with 'frequentist' statistics and this analysis provides the probability that the actual OR is larger or smaller than a threshold value, being more intuitive for clinicians. (43, 45, 46)

Conclusion

The present study suggests that fetal intervention (vesicoamniotic shunt) improves postnatal outcome in a highly select group of fetuses with LUTO that have

favorable fetal renal function parameters. Fetal renal function can be evaluated by combining fetal urinary biochemistry profiles with ultrasound characteristics of fetal kidneys according to gestational age. The present study proposes a standardized multidisciplinary prenatal management plan based on disease severity (staging classification) and the gestational age. Further prospective studies are necessary to validate this proposal.

REFERENCES

1. Clayton DB, Brock JW, 3rd. Lower urinary tract obstruction in the fetus and neonate. Clin Perinatol. 2014;41(3):643-59. Epub 2014/08/27.
2. Tonni G, Vito I, Ventura A, Grisolia G, De Felice C. Fetal lower urinary tract obstruction and its management. Archives of gynecology and obstetrics. 2013;287(2):187-94. Epub 2012/11/10.
3. Wu S, Johnson MP. Fetal lower urinary tract obstruction. Clin Perinatol. 2009;36(2):377-90, x. Epub 2009/06/30.
4. Lissauer D, Morris RK, Kilby MD. Fetal lower urinary tract obstruction. Semin Fetal Neonatal Med. 2007;12(6):464-70. Epub 2007/09/01.
5. Robyr R, Benachi A, Daikha-Dahmane F, Martinovich J, Dumez Y, Ville Y. Correlation between ultrasound and anatomical findings in fetuses with lower urinary tract obstruction in the first half of pregnancy. Ultrasound Obstet Gynecol. 2005;25(5):478-82. Epub 2005/04/09.
6. Bernardes LS, Aksnes G, Saada J, Masse V, Elie C, Dumez Y, Lortat-Jacob SL, Benachi A. Keyhole sign: how specific is it for the diagnosis of posterior urethral valves? Ultrasound Obstet Gynecol. 2009;34(4):419-23. Epub 2009/07/31.
7. Ruano R, Sananes N, Sangi-Haghpeykar H, Hernandez-Ruano S, Moog R, Becmeur F, Zaloszyc A, Giron AM, Morin B, Favre R. Fetal intervention for severe lower urinary tract obstruction: a multicenter case-control study comparing fetal cystoscopy with vesicoamniotic shunting. Ultrasound Obstet Gynecol. 2015;45(4):452-8. Epub 2014/08/27.
8. Ruano R. Fetal surgery for severe lower urinary tract obstruction. Prenat Diagn. 2011;31(7):667-74. Epub 2011/03/18.
9. Sananes N, Favre R, Koh CJ, Zaloszyc A, Braun MC, Roth DR, Moog R, Becmeur F, Belfort MA, Ruano R. Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves - surgical technical aspects. Ultrasound Obstet Gynecol. 2014. Epub 2014/05/13.
10. Ruano R, Yoshizaki CT, Giron AM, Srougi M, Zugaib M. Fetal cystoscopic placement of transurethral stent in a fetus with urethral stenosis. Ultrasound Obstet Gynecol. 2013. Epub 2014/01/01.
11. Morris RK, Malin GL, Quinlan-Jones E, Middleton LJ, Hemming K, Burke D, Daniels JP, Khan KS, Deeks J, Kilby MD. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. Lancet. 2013;382(9903):1496-506. Epub 2013/08/21.
12. Ethun CG, Zamora IJ, Roth DR, Kale A, Cisek L, Belfort MA, Haeri S, Ruano R, Welty SE, Cassady CI, Olutoye OO, Cass DL. Outcomes of fetuses with lower urinary tract obstruction treated with vesicoamniotic shunt: a single-institution experience. J Pediatr Surg. 2013;48(5):956-62. Epub 2013/05/25.
13. Ruano R, Yoshisaki CT, Salustiano EM, Giron AM, Srougi M, Zugaib M. Early fetal cystoscopy for first-trimester severe megacystis. Ultrasound Obstet Gynecol. 2011;37(6):696-701. Epub 2011/02/22.
14. Morris RK, Ruano R, Kilby MD. Effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. Ultrasound Obstet Gynecol. 2011;37(6):629-37. Epub 2011/03/05.

15. Ruano R, Duarte S, Bunduki V, Giron AM, Srougi M, Zugaib M. Fetal cystoscopy for severe lower urinary tract obstruction--initial experience of a single center. Prenat Diagn. 2010;30(1):30-9. Epub 2009/12/08.
16. Brown C, Morris RK, Daniels J, Khan KS, Lilford RJ, Kilby MD. Effectiveness of percutaneous vesico-amniotic shunting in congenital lower urinary tract obstruction: divergence in prior beliefs among specialist groups. Eur J Obstet Gynecol Reprod Biol. 2010;152(1):25-9. Epub 2010/06/16.
17. Clifton MS, Harrison MR, Ball R, Lee H. Fetoscopic transuterine release of posterior urethral valves: a new technique. Fetal diagnosis and therapy. 2008;23(2):89-94. Epub 2007/11/24.
18. Hofmann R, Becker T, Meyer-Wittkopf M, Tekesin I, Sierra F, Schmidt S. Fetoscopic placement of a transurethral stent for intrauterine obstructive uropathy. J Urol. 2004;171(1):384-6. Epub 2003/12/11.
19. Welsh A, Agarwal S, Kumar S, Smith RP, Fisk NM. Fetal cystoscopy in the management of fetal obstructive uropathy: experience in a single European centre. Prenat Diagn. 2003;23(13):1033-41. Epub 2003/12/24.
20. Quintero RA, Homsy Y, Bornick PW, Allen M, Johnson PK. In-utero treatment of fetal bladder-outlet obstruction by a ureterocele. Lancet. 2001;357(9272):1947-8. Epub 2001/06/27.
21. Holmes N, Harrison MR, Baskin LS. Fetal surgery for posterior urethral valves: long-term postnatal outcomes. Pediatrics. 2001;108(1):E7. Epub 2001/07/04.
22. Agarwal SK, Fisk NM. In utero therapy for lower urinary tract obstruction. Prenat Diagn. 2001;21(11):970-6. Epub 2001/12/18.
23. Quintero RA, Shukla AR, Homsy YL, Bukkapatnam R. Successful in utero endoscopic ablation of posterior urethral valves: a new dimension in fetal urology. Urology. 2000;55(5):774. Epub 2000/05/03.
24. Quintero RA, Hume R, Smith C, Johnson MP, Cotton DB, Romero R, Evans MI. Percutaneous fetal cystoscopy and endoscopic fulguration of posterior urethral valves. Am J Obstet Gynecol. 1995;172(1 Pt 1):206-9. Epub 1995/01/01.
25. Morris RK, Malin GL, Quinlan-Jones E, Middleton LJ, Hemming K, Burke D, Daniels JP, Khan KS, Deeks J, Kilby MD. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. Lancet. 2013. Epub 2013/08/21.
26. Nicolini U, Spelzini F. Invasive assessment of fetal renal abnormalities: urinalysis, fetal blood sampling and biopsy. Prenat Diagn. 2001;21(11):964-9. Epub 2001/12/18.
27. Ciardelli V, Rizzo N, Farina A, Vitarelli M, Boni P, Bovicelli L. Prenatal evaluation of fetal renal function based on serum beta(2)-microglobulin assessment. Prenat Diagn. 2001;21(7):586-8. Epub 2001/08/09.
28. Tassis BM, Trespidi L, Tirelli AS, Pace E, Boschetto C, Nicolini U. Serum beta 2-microglobulin in fetuses with urinary tract anomalies. Am J Obstet Gynecol. 1997;176(1 Pt 1):54-7. Epub 1997/01/01.
29. Shaarawy M, el Mallah SY, el-Yamani AA. Clinical significance of urinary human tissue non-specific alkaline phosphatase (hTNAP) in pre-eclampsia and eclampsia. Ann Clin Biochem. 1997;34 (Pt 4):405-11. Epub 1997/07/01.
30. Qureshi F, Jacques SM, Seifman B, Quintero R, Evans MI, Smith C, Johnson MP. In utero fetal urine analysis and renal histology correlate with the outcome in fetal obstructive uropathies. Fetal Diagn Ther. 1996;11(5):306-12. Epub 1996/09/01.

31. Lipitz S, Ryan G, Samuell C, Haeusler MC, Robson SC, Dhillon HK, Nicolini U, Rodeck CH. Fetal urine analysis for the assessment of renal function in obstructive uropathy. Am J Obstet Gynecol. 1993;168(1 Pt 1):174-9. Epub 1993/01/01.
32. Nicolini U, Fisk NM, Rodeck CH, Beacham J. Fetal urine biochemistry: an index of renal maturation and dysfunction. Br J Obstet Gynaecol. 1992;99(1):46-50. Epub 1992/01/01.
33. Morris RK, Malin GL, Khan KS, Kilby MD. Antenatal ultrasound to predict postnatal renal function in congenital lower urinary tract obstruction: systematic review of test accuracy. BJOG. 2009;116(10):1290-9. Epub 2009/05/15.
34. Morris RK, Quinlan-Jones E, Kilby MD, Khan KS. Systematic review of accuracy of fetal urine analysis to predict poor postnatal renal function in cases of congenital urinary tract obstruction. Prenat Diagn. 2007;27(10):900-11. Epub 2007/07/05.
35. Grignon A, Filion R, Filiatrault D, Robitaille P, Homsy Y, Boutin H, Leblond R. Urinary tract dilatation in utero: classification and clinical applications. Radiology. 1986;160(3):645-7. Epub 1986/09/01.
36. Muller F, Dommergues M, Mandelbrot L, Aubry MC, Nihoul-Fekete C, Dumez Y. Fetal urinary biochemistry predicts postnatal renal function in children with bilateral obstructive uropathies. Obstet Gynecol. 1993;82(5):813-20. Epub 1993/11/01.
37. Hallak M, Kirshon B, Smith EO, Cotton DB. Amniotic fluid index. Gestational age-specific values for normal human pregnancy. J Reprod Med. 1993;38(11):853-6. Epub 1993/11/01.
38. Blaicher W, Hausler M, Gembruch U, Bollmann R, Terinde R, Rempen A, Deutinger J, Bernaschek G. Feto-amniotic shunting -- experience of six centres. Ultraschall Med. 2005;26(2):134-41. Epub 2005/04/27.
39. Johnson MP, Bukowski TP, Reitleman C, Isada NB, Pryde PG, Evans MI. In utero surgical treatment of fetal obstructive uropathy: a new comprehensive approach to identify appropriate candidates for vesicoamniotic shunt therapy. Am J Obstet Gynecol. 1994;170(6):1770-6; discussion 6-9. Epub 1994/06/01.
40. Daikha-Dahmane F, Dommergues M, Muller F, Narcy F, Lacoste M, Beziau A, Dumez Y, Gubler MC. Development of human fetal kidney in obstructive uropathy: correlations with ultrasonography and urine biochemistry. Kidney international. 1997;52(1):21-32. Epub 1997/07/01.
41. Eugene M, Muller F, Dommergues M, Le Moyec L, Dumez Y. Evaluation of postnatal renal function in fetuses with bilateral obstructive uropathies by proton nuclear magnetic resonance spectroscopy. American journal of obstetrics and gynecology. 1994;170(2):595-602. Epub 1994/02/01.
42. Dumez Y, Muller F, Dommergues M, Nihoul-Fekete C. [Antenatal diagnosis of obstructive uropathies]. Chirurgie; memoires de l'Academie de chirurgie. 1990;116(6-7):517-22. Epub 1990/01/01. Diagnostic antenatal des uropathies obstructives.
43. Freedman L. Bayesian statistical methods. BMJ. 1996;313(7057):569-70. Epub 1996/09/07.
44. Ntzoufras I. Bayesian Modeling Using WinBUGS: Wiley; 2009.
45. Spiegelhalter DJ, Myles JP, Jones DR, Abrams KR. Bayesian methods in health technology assessment: a review. Health Technol Assess. 2000;4(38):1-130. Epub 2001/01/03.
46. Freedman LS, Spiegelhalter DJ. Application of Bayesian statistics to decision making during a clinical trial. Statistics in medicine. 1992;11(1):23-35. Epub 1992/01/15.

47. Morris RK, Malin GL, Quinlan-Jones E, Middleton LJ, Hemming K, Burke D, Daniels JP, Khan KS, Deeks J, Kilby MD, Percutaneous vesicoamniotic shunting in Lower Urinary Tract Obstruction Collaborative G. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. Lancet. 2013;382(9903):1496-506.
48. Ruano R, Sananes N, Sangi-Haghpeykar H, Hernandez-Ruano S, Moog R, Becmeur F, Zaloszyc A, Giron AM, Morin B, Favre R. Fetal intervention for severe lower urinary tract obstruction: a multicenter case-control study comparing fetal cystoscopy with vesico-amniotic shunting. Ultrasound Obstet Gynecol. 2014. Epub 2014/08/27.
49. Schurink CA, Visscher S, Lucas PJ, van Leeuwen HJ, Buskens E, Hoff RG, Hoepelman AI, Bonten MJ. A Bayesian decision-support system for diagnosing ventilator-associated pneumonia. Intensive care medicine. 2007;33(8):1379-86. Epub 2007/06/19.
50. Abdennadher W, Chalouhi G, Dreux S, Rosenblatt J, Favre R, Guimiot F, Salomon LJ, Oury JF, Ville Y, Muller F. Fetal urine biochemistry at 13-23 weeks of gestation in lower urinary tract obstruction: criteria for in-utero treatment. Ultrasound Obstet Gynecol. 2015;46(3):306-11. Epub 2014/11/22.

Table 1: Demographics of patients who underwent fetal intervention and those that did not.

Variables	Fetal intervention (n=14)	Expectant prenatal management (n=11)
GA at diagnosis (weeks) - means and standard deviation	18.5 +/- 4.2	21.0 +/- 3.7
Megacystis at first trimester - N (%)	3/14 (21.4%)	2/11 (18.2%)
Severe hydronephrosis - N (%)	13 (92.9%)	8 (72.7%)
Hyperechogenic kidneys - N (%)	8 (57.1%)	9 (81.8%)
Renal cortical cysts - N (%)	1 (7.1%)	6 (54.5%)
Renal dysplasia - N (%)	0 (0%)	3 (27.3%)
Severe hydroureters - N (%)	3 (21.4%)	1 (9.1%)
Anhydramnios - N (%)	10 (71.4%)	9 (81.8%)
Favorable fetal urinary biochemistry - N (%)	10 (71.4%)	4 (36.4%)
GA at delivery (weeks) - means and standard deviation	33.0 +/- 4.4	32.7 +/- 8.0

GA: gestational age; OR: odds ratio; CI: credibility interval.

Continuous variables are expressed as means and standard deviations, categorical variables with numbers and percentages.

Each result is presented as an OR with a 95 % credibility interval, and a probability of the OR being above or under 1.

Table 2: Diagnostic data for comparisons between survivors and non-survivors.

Variables	Survivors (n=15)	Non-survivors (n=10)	Statistical analysis	
			OR for survival [CI], Pr (OR>1 or <1)	
			With non-informative prior	With informative prior
GA at diagnosis (weeks) - means and standard deviation	20 +/- 4.4	20 +/- 2.8	1.11 [0.93-1.34], Pr(OR>1)=87.7%	1.12 [0.93-1.36], Pr(OR>1)=87.5 %
Megacystis at first trimester - N (%)	3 (20.0%)	2 (20.0%)	1.04 [0.15-7.92], Pr(OR>1)=51.6%	/
Severe hydronephrosis - N (%)	13 (86.7%)	8 (80%)	2.74 [0.28-25.4], Pr(OR>1)=80.8%	/
Hyperechogenic kidneys - N (%)	8 (53.3%)	9 (90.0%)	0.18 [0.02-1.02], Pr(OR<1)=97.4%	/
Renal cortical cysts - N (%)	1 (6.7%)	6 (60.0%)	0.07 [0.01-0.44], Pr(OR<1)=99.7%	/
Renal dysplasia - N (%)	0 (0%)	3 (30.0%)	0.06 [0.00-0.77], Pr(OR<1)=98.4%	/
Severe hydroureters - N (%)	2 (13.3%)	2 (20.0%)	0.71 [0.08-5.84], Pr(OR<1)=63.2%	/
Anhydramnios - N (%)	9 (60.0%)	10 (100%)	0.03 [0.00-0.67], Pr(OR<1)=99.2 %	0.12 [0.04-0.33], Pr(OR<1)=100 %

Favorable fetal urinary biochemistry - N (%)	9 (60.0%)	5 (50.0%)	2.34 [0.35-16.98], Pr(OR>1)=80.3%	3.81 [0.81-19.34], Pr(OR>1)=95.3 %
Fetal intervention - N (%)	12 (85.7%)	2 (20.0%)	12.9 [2.28-89.40], Pr(OR>1)=99.8%	5.19 [1.94-13.5], Pr(OR>1)=99.9 %
GA at delivery (weeks) - means and standard deviation	34.6 +/- 3.9	30.3 +/- 4.1	1.13 [0.98-1.33], Pr(OR>1)=94.5%	/

GA: gestational age; OR: odds ratio; CI: credibility interval.

Continuous variables are expressed as means and standard deviations, categorical variables with numbers and percentages.

Each result is presented as an OR with a 95 % credibility interval, and a probability of the OR being above or under 1.

Table 3: Diagnostic data for comparisons between infants with normal renal function vs. those with abnormal renal function at 6 months of life.

Variables	Normal renal function at 6 months of life (n=10)	Abnormal renal function at 6 months of life (n=5)	Statistical analysis
			OR for survival [CI], Pr (OR>1 or <1)
			With non-informative prior
GA at diagnosis (weeks) - means and standard deviation	20 +/- 4.4	20 +/- 2.8	0.92 [0.73-1.13], Pr(OR<1)=22.5%
Megacystis at first trimester - N (%)	2 (20.0%)	1 (20.0%)	0.91 [0.06-10.5], Pr(OR>1)=47.3%
Severe hydronephrosis - N (%)	9 (90.0%)	5 (100.0%)	2.05 [0.09-72.69], Pr(OR>1)=67.3%
Hyperechogenic kidneys - N (%)	4 (40.0%)	4 (80.0%)	4.35 [0.53-44.12], Pr(OR>1)=91.5%
Renal cortical cysts - N (%)	0 (0%)	1 (20.0%)	7,50 [0.22-405.19], Pr(OR>1)=86,6%
Renal dysplasia - N (%)	0 (0%)	0 (0%)	0.98 [0.00-135.33], Pr(OR<1)=50.3%
Severe hydroureters - N (%)	2 (20.0%)	0 (0%)	0.17 [0.01-3.93], Pr(OR>1)=84,9%
Anhydramnios - N (%)	5 (50.0%)	4 (80.0%)	3.01 [0.0.38-33.28], Pr(OR>1)=85.1 %
Favorable fetal urinary biochemistry - N (%)	6 (60.0%)	3 (60.0%)	2.90 [0.15-76.51], Pr(OR>1)=76,3%
Fetal intervention - N (%)	8 (80.0%)	4 (80.0%)	0.93 [0.08-12.20], Pr(OR<1)=52,3%
GA at delivery (weeks) - means and standard deviation	34.6 +/- 3.9	30.3 +/- 4.1	0.89 [0.75-1.05], Pr(OR<1)=82,2%

GA: gestational age; OR: odds ratio; CI: credibility interval.

Continuous variables are expressed as means and standard deviations, categorical variables with numbers and percentages.

Each result is presented as an OR with a 95 % credibility interval, and a probability of the OR being above or under 1.

Table 4: Classification of LUTO according to the severity.

	Stage I **(Mild LUTO)**	**Stage II** **(Severe LUTO)** ***with prenatal findings suggestive of preserved fetal renal function***	**Stage III** **(Severe LUTO)** ***with prenatal findings suggestive of fetal abnormal renal function***
Amount of amniotic fluid	Normal	Oligohydramnios or anhydramnios	Oligohydramnios, but usually anhydramnios
Echogenicity of the fetal kidneys	Normal	Hyperechogenic	Hyperechogenic
Renal cortical cysts	Absent	Absent	Can be present
Renal dysplasia	Absent	Absent	Can be Present
Fetal urinary biochemistry	Favorable	Favorable within 3 consecutive evaluations	Not favorable after 3 consecutive evaluations

Fetal intervention	Not indicated	Indicated to prevent pulmonary hypoplasia and severe renal impairment	May be indicated to prevent pulmonary hypoplasia but not postnatal renal impairment. Further studies are necessary.

The disease can progress from stage I to stage II and then stage III during pregnancy.

Ultrasound Obstet Gynecol 2015; **45**: 452–458
Published online 2 March 2015 in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.14652

Fetal intervention for severe lower urinary tract obstruction: a multicenter case–control study comparing fetal cystoscopy with vesicoamniotic shunting

R. RUANO*†‡, N. SANANES§¶, H. SANGI-HAGHPEYKAR†‡, S. HERNANDEZ-RUANO**, R. MOOG††, F. BECMEUR††, A. ZALOSZYC‡‡, A. M. GIRON§§, B. MORIN§ and R. FAVRE*

**Department of Obstetrics and Gynecology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; †Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA; ‡Texas Children's Fetal Center, Texas Children's Hospital Pavilion for Women, Houston, TX, USA; §Service de Gynécologie Obstétrique, CMCO-HUS, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ¶INSERM, UMR-S 1121, Biomatériaux et Bioingénierie, Strasbourg, France; **Dr. Ademir C. Ruano Maternal-Fetal Clinic, São Paulo, São Paulo, Brazil; ††Service de Chirurgie Pédiatrique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ‡‡Service de Pédiatrie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; §§Department of Urology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil*

KEYWORDS: fetal cystoscopy; fetal surgery; fistula; lower urinary tract obstruction; posterior urethral valves

ABSTRACT

Objective *To evaluate the efficacy of fetal intervention using fetal cystoscopy or vesicoamniotic shunting in the treatment of severe lower urinary obstruction (LUTO).*

Methods *A cohort of 111 fetuses with severe LUTO attending two centers between January 1990 and August 2013 were included retrospectively. Fetuses were categorized into three groups based on the method of intervention: (1) fetal cystoscopy, (2) vesicoamniotic shunting or (3) no intervention. Multivariate analyses were performed to determine the probability of survival and normal renal function until 6 months of age by comparing fetal cystoscopy and vesicoamniotic shunting to no fetal intervention.*

Results *Of the 111 fetuses with severe LUTO that were included in the analysis, fetal cystoscopy was performed in 34, vesicoamniotic shunting was performed in 16 and there was no fetal intervention in 61. Gestational age at diagnosis, method of fetal intervention and cause of bladder obstruction were associated with prognosis. In multivariate analysis and after adjustment for potential confounders (considering all causes of LUTO) the overall probability of survival was significantly higher with fetal cystoscopy and vesicoamniotic shunting when compared to no intervention (adjusted relative risk (ARR), 1.86 (95% CI, 1.01–3.42;* $P = 0.048$*) and ARR, 1.73 (95% CI, 1.01–3.08;* $P = 0.04$*) respectively). A clear trend for normal renal function was present in the fetal cystoscopy group (ARR, 1.73 (95% CI, 0.97–3.08;* $P = 0.06$*)) but was not observed in the vesicoamniotic shunt group (ARR, 1.16 (95% CI, 0.86–1.55;* $P = 0.33$*)). In cases in which there was a postnatal diagnosis of posterior urethral valves, fetal cystoscopy was effective in improving both the 6-month survival rate and renal function (ARR, 4.10 (95% CI, 1.75–9.62;* $P < 0.01$*) and 2.66 (95% CI, 1.25–5.70;* $P = 0.01$*) respectively) while vesicoamniotic shunting was associated only with an improvement in the 6-month survival rate (ARR, 3.76 (95% CI, 1.42–9.97;* $P < 0.01$*)) with no effect on renal function (ARR, 1.03 (95% CI, 0.49–2.17,* $P = 0.93$*)).*

Conclusion *Fetal cystoscopy and vesicoamniotic shunting improve the 6-month survival rate in cases of severe LUTO. However, only fetal cystoscopy may prevent impairment of renal function in fetuses with posterior urethral valves. Our data support the idea of performing a subsequent randomized controlled trial to compare the effectiveness of fetal cystoscopy* vs *vesicoamniotic shunting for severe fetal LUTO.*

INTRODUCTION

Fetal lower urinary tract obstruction (LUTO) occurs in approximately two out of 10 000 pregnancies and is diagnosed on ultrasound examination by the presence of a distended bladder with bilateral hydronephrosis and severe oligohydramnios[1]. Severe LUTO is associated with high perinatal mortality and morbidity due to pulmonary hypoplasia and severe renal impairment or damage[2–8].

Correspondence to: Dr R. Ruano, Pavilion for Women, Texas Children's Fetal Center, 6651 Main Street, Suite F1020, Houston, TX, 77030, USA (e-mail: ruano@bcm.edu, rodrigoruano@hotmail.com)

Accepted: 7 August 2014

Classically, vesicoamniotic shunting has been considered the therapeutic modality of choice for LUTO and seems to reduce the incidence of postnatal mortality[9]. However, vesicoamniotic shunting is associated with complications such as shunt migration, obstruction and displacement of the shunt tubing which occur in more than 40% of cases[9–12].

Because of those complications, fetal cystoscopy has been proposed as a mode of intervention with theoretical advantages over the shunting procedure by permitting a more physiological drainage of the obstructed bladder and endoscopic examination of the dilated posterior urethra to help determine the etiology of the obstructive uropathy including posterior urethral valves (PUVs), prune belly syndrome or urethral atresia[13–20]. Another potential clinical advantage of fetal cystoscopy over vesicoamniotic shunting is avoidance of the usual amnioinfusion, often needed prior to shunting, which carries some additional risks[13,16,19].

Preliminary studies have yielded promising results with fetal cystoscopic laser fulguration of PUVs as opposed to no prenatal treatment, but they have been performed only in limited cohorts[13,19,21,22]. Also, there has been no clinical study to date comparing fetal cystoscopy to vesicoamniotic shunting as an intervention for severe LUTO. There is only one systematic review that compares both interventions for severe LUTO, based on different series with different perinatal approaches[23].

Based on the experience of a multicenter study of cases of severe LUTO, the objective of the present study was to compare the postnatal outcome of fetuses which underwent intervention with that of non-treated fetuses with similar underlying pathologies and prenatal characteristics.

SUBJECTS AND METHODS

We reviewed data from a cohort of male fetuses that were diagnosed prenatally with severe LUTO at two centers, one in Sao Paulo, Brazil between July 2006 and August 2013 and one in Strasbourg, France between January 1990 and August 2013. All data were collected prospectively, following the same research protocol and procedure for clinical management at both sites. Specifically, severe LUTO was diagnosed on detailed fetal ultrasound examination by confirming an extremely dilated bladder, with increased wall thickness (megabladder) associated with bilateral hydroureters and severe bilateral hydronephrosis[16,21,22,24]. Inclusion criteria for this study were: (1) severe LUTO confirmed on detailed fetal ultrasound examination, (2) absence of additional fetal malformations on targeted ultrasound examination including fetal echocardiography, (3) gestational age at diagnosis between 16 and 26 weeks, (4) 'favorable' fetal urinary analysis defined as sodium < 100 mEq/L, chloride < 90 mEq/L, calcium < 8 mg/dL, osmolarity < 210 mOsm/L and beta-2 microglobulin < 6 mg/dL in the latest sample, out of a maximum of three samples that were collected daily and (5) normal fetal karyotype.

Fetal intervention, either vesicoamniotic shunting or fetal cystoscopy, was offered to all consecutive patients who met the inclusion criteria, with normalization of the urinary analysis at the third sample. Patients were offered and consented to the following options: (1) expectant prenatal management, (2) fetal intervention (ultrasound-guided fetal vesicoamniotic shunting from 1990 onward or fetal cystoscopy with possible laser ablation of PUVs from 2006 onward) or (3) termination of pregnancy (TOP) in cases of poor prognosis. Local institutional review boards and fetal therapy boards approved the fetal interventions and the clinical study.

Fetal vesicoamniotic shunting was performed by percutaneous insertion of a pigtail catheter with either the King's College/Rocket introducer or the Harrison shunting set[25–28]. Fetal cystoscopy was performed using 1.0-mm or 1.3-mm telescopes (11510 A and 11540 AA, Karl Storz, Tuttlingen, Germany) as previously reported[16,22]. Both procedures were performed in operative rooms and maternal anesthesia was administered using maternal epidural or local skin anesthesia. Fetal anesthesia was administered by injecting fentanyl (15 μg/kg) and pancuronium (2 mg/kg) into the umbilical vein or fetal arms or legs using a 22-gauge needle under ultrasound guidance. During fetal cystoscopy, if PUVs were identified, the valves were fulgurated by pulsed shots of an Nd:YAG laser at maximal settings of 30 Watts and 100 Joules (SmartEpil, DEKA, Florence, Italy) or pulsed shots of a Diode laser at settings of 30–40 Watts (Multibeam, Dornier Medtech, Kennesaw, GA, USA). When urethral atresia was diagnosed during fetal cystoscopy, laser fulguration was not performed and the procedure was discontinued.

After delivery, all survivors were evaluated and followed up by urologists and pediatricians. Micturating cystourethrography and postnatal cystoscopy were performed to evaluate the bladder and the urethra and an autopsy was performed in the case of fetal or neonatal death. Clinical management remained consistent during the study period.

Statistical analysis

Primary outcome was defined as survival with normal renal function until 6 months of age. Normal renal function was defined by a serum creatinine level < 50 μmol/L (± 2 SD) as an average of the last five samples taken and absence of a need for dialysis[29].

Each treatment group (fetal cystoscopy or vesicoamniotic shunting) was compared with the group that did not undergo fetal intervention, and with each other, by a chi-square test for grouped variables, Student's *t*-test for continuous variables and Wilcoxon rank test for non-normally distributed data. Generalized estimating equation models (Proc Genmod in SAS) were used to determine the probability (relative risk, RR) of survival with normal renal function, comparing each treatment

group (cystoscopy or vesicoamniotic shunting) to the control group. RRs were adjusted for possible confounders, as determined by univariate analysis. Correlation of data from different fetal centers was controlled by including this variable in the repeated statement. In the analysis of survival data, since some pregnancies were terminated (TOP) subsequent to different perinatal management, a sensitivity analysis was performed, including results that combined TOP patients and those in whom neonatal or fetal death occurred. Results were presented overall and stratified by postnatal diagnosis of the cause of LUTO. Adjusted RRs (ARR) were calculated, followed by corresponding 95% CI values. $P < 0.05$ was considered to be statistically significant. All analyses were performed using SAS (version 9.3, Cary, NC, USA).

RESULTS

A total of 111 consecutive pregnancies were included in the present study (68 patients were seen in Sao Paulo, Brazil and 43 patients in Strasbourg, France). Details of 40 patients from Brazil were reported previously as case reports or part of a study cohort[13,16–18,20]. Mean gestational age at diagnosis was 15.4 ± 4.3 weeks. A first-trimester diagnosis of LUTO was made in 85 (76.6%) fetuses by the identification of megacystis and the diagnosis was made during the second trimester in 26 (23.4%) fetuses. All cases were diagnosed prenatally with severe LUTO (megacystis with increased wall thickness and bilateral severe hydronephrosis associated with reduced amniotic fluid). The keyhole sign was present in all but five (95.5%) fetuses. Mean ± SD fetal urinary levels of sodium, chloride, calcium, osmolarity and beta-2 microglobulin were 93.2 ± 19.3 mEq/L, 81.5 ± 7.6 mEq/L, 3.2 ± 1.7 mg/dL, 179.5 ± 25.2 mOsm/L and 3.4 ± 1.7 mg/dL, respectively, and the mean fetal serum level of beta-2 microglobulin was 4.8 ± 1.4 mg/dL. Fetal intervention was undertaken in 50 fetuses (34 cystoscopies and 16 vesicoamniotic shunt placements). Mean gestational age at the time of the procedure was 19.0 ± 3.3 weeks.

Table 1 provides obstetric characteristics and fetal outcome in each group. Similar demographics and prenatal characteristics were observed in all three groups; however, gestational age at diagnosis was significantly lower in the group that underwent fetal cystoscopy compared to the group that did not undergo intervention ($P < 0.01$), with no difference between the vesicoamniotic shunt group and the group without fetal intervention ($P = 0.26$). The survival rate was higher in the group with prenatal treatment (38.2% (13/34) in the fetal cystoscopy group and 43.8% (7/16) in the vesicoamniotic shunt group compared to 19.7% (12/61) the group that underwent no intervention). The 6-month survival rate was similar statistically between the fetal cystoscopy group and vesicoamniotic shunt group ($P = 0.76$). Normal renal function was observed in 75.0% (12/16), 60.0% (6/10) and 39.3% (11/28) of the fetal cystoscopy group, the vesicoamniotic shunt group and the no intervention group, respectively. Compared to the absence of intervention, fetal cystoscopy significantly improved renal function ($P = 0.03$) while vesicoamniotic shunting did not ($P = 0.29$).

The underlying cause of LUTO was associated statistically with the 6-month survival rate ($P = 0.03$) and normal renal function ($P = 0.01$). Outcome in patients with LUTO, classified according to fetal intervention and postnatal diagnosis of the cause of LUTO, is presented in Table 2. In the present series, PUVs were diagnosed postnatally in 57 (51.4%) patients; the 6-month survival rate

Table 1 Characteristics and outcome in 111 fetuses with severe lower urinary tract obstruction according to perinatal management

Characteristic	*Fetal cystoscopy (n = 34)*	*Fetal vesicoamniotic shunting (n = 16)*	*No fetal intervention (n = 61)*	P
GA at diagnosis (weeks)	13.7 ± 2.7‡	17.3 ± 5.3	15.9 ± 4.4	< 0.01
Ultrasound diagnosis of megacystis				
First trimester	30/34 (88.2)	10/16 (62.5)	45/61 (73.8)	0.10
Second trimester	34/34 (100)	16/16 (100)	61/61 (100)	1.00
Keyhole sign*	34/34 (100)	14/16 (87.5)	58/61 (95.1)	0.09
Severe oligohydramnios/anhydramnios*	19/34 (55.9)	5/16 (31.3)	37/61 (60.7)	0.11
Fetal urinary levels				
Sodium (mEq/L)	95.8 ± 15.0	84.8 ± 19.3	95.9 ± 21.5	0.15
Chloride (mEq/L)	81.9 ± 8.8	85.3 ± 3.1	80.23 ± 6.7	0.32
Calcium (mg/dL)	2.7 ± 1.5	2.3 ± 1.3	2.7 ± 1.6	0.58
Beta-2 microglobulin (mg/dL)	3.3 ± 1.7	3.9 ± 2.0	3.2 ± 1.7	0.40
Osmolarity (mOsm/mL)	184.6 ± 26.1	187.2 ± 11.9	172.0 ± 25.0	0.13
Fetal serum beta-2 microglobulin level (mg/dL)	1.5 ± 0.75	1.6 ± 0.73	1.4 ± 0.40	0.87
GA at fetal intervention (weeks)	18.5 ± 2.9	20.2 ± 3.9	—	0.10
GA at birth (weeks)	25.1 ± 7.9	29.2 ± 7.0	27.3 ± 8.2	0.48
Survival at 6 months of age (including TOP)	13/34 (38.2)§	7/16 (43.8)§	12/61 (19.7)	0.06
Survival at 6 months of age (excluding TOP)	13/22 (59.1)	7/13 (53.8)	12/35 (34.3)	0.15
Normal renal function at 6 months of age†	12/16 (75.0)¶	6/10 (60.0)	11/28 (39.3)	0.07

Data are given as mean ± SD or *n*/*N* (%). *Observed on second-trimester ultrasound examination. †Excluding termination of pregnancy, miscarriage and neonatal death within 48 hours of delivery. ‡$P < 0.01$, when compared to no fetal intervation. §$P = 0.04$, when compared to no fetal intervention. ¶$P = 0.03$, when compared to no fetal intervention. GA, gestational age; TOP, termination of pregnancy.

Table 2 Survival and normal renal function at 6 months of age according to fetal intervention and postnatal diagnosis of the cause of lower urinary tract obstruction (LUTO)

Cause of LUTO	*Outcome at 6 months of age*	*Fetal intervention*		
		Cystoscopy	*Vesicoamniotic shunting*	*None*
Posterior urethral valves ($n = 57$)				
	Survival ($n = 23$)	12	6	5
	Perinatal death ($n = 21$)	4	4	13
	TOP ($n = 13$)	2	0	11
	Normal renal function ($n = 21$)	11	5	5
Urethral atresia ($n = 24$)				
	Survival ($n = 0$)	0	0	0
	Perinatal death ($n = 11$)	3	1	7
	TOP ($n = 13$)	9	1	3
	Normal renal function ($n = 0$)	0	0	0
Urethral stenosis ($n = 4$)				
	Survival ($n = 2$)	1	0	1
	Perinatal death ($n = 0$)	0	0	0
	TOP ($n = 2$)	0	0	2
	Normal renal function ($n = 2$)	1	0	1
Prune belly syndrome ($n = 5$)				
	Survival ($n = 0$)	0	0	0
	Perinatal death ($n = 1$)	0	0	1
	TOP ($n = 4$)	0	1	3
	Normal renal function ($n = 0$)	0	0	0
Vesicoureteral reflux ($n = 7$)				
	Survival ($n = 5$)	0	1	4
	Perinatal death ($n = 0$)	0	0	0
	TOP ($n = 2$)	0	1	1
	Normal renal function ($n = 4$)	0	1	3
Cloacal dystrophy ($n = 7$)				
	Survival ($n = 0$)	0	0	0
	Perinatal death ($n = 2$)	0	0	2
	TOP ($n = 5$)	1	0	4
	Normal renal function ($n = 0$)	0	0	0
Megalourethra ($n = 4$)				
	Survival ($n = 2$)	0	0	2
	Perinatal death ($n = 1$)	0	1	0
	TOP ($n = 1$)	0	0	1
	Normal renal function ($n = 2$)	0	0	2
Megacystis-microcolon-intestinal hypoperistalsis syndrome ($n = 3$)				
	Survival ($n = 0$)	0	0	0
	Perinatal death ($n = 2$)	2	0	0
	TOP ($n = 1$)	0	0	1
	Normal renal function ($n = 0$)	0	0	0
All cases ($n = 111$)				
	Survival ($n = 32$)	13	7	12
	Perinatal death ($n = 38$)	9	6	23
	TOP ($n = 41$)	12	3	26
	Normal renal function ($n = 29$)	12	6	11

Data are given as *n*. TOP, termination of pregnancy.

was 66.7% (12/18), 60.0% (6/10) and 17.2% (5/29) for the fetal cystoscopy group, the vesicoamniotic shunt group and the no intervention group, respectively ($P < 0.001$), while normal renal function was 61.1% (11/18), 50.0% (5/10) and 17.2% (5/29), respectively for the groups that underwent fetal cystoscopy or vesicoamniotic shunting or had no intervention ($P = 0.01$). Urethral atresia was diagnosed in 24 (21.6%) cases, with deaths in all cases, independent of fetal therapy. Urethral stenosis was identified in four cases; two fetuses survived with normal renal function (fetal cystoscopy was performed in one case and since the other had normal amniotic fluid with mild bilateral hydronephrosis there was no fetal intervention). Prune belly syndrome was diagnosed in five (4.5%) cases, with no survival. Postnatal vesicoureteral reflux was observed in seven patients; two had undergone fetal vesicoamniotic shunting, of whom one infant survived with normal renal function; and five had expectant prenatal management, of whom four survived and three infants had normal renal function. Cloacal dystrophy was diagnosed in seven cases; five pregnancies were terminated and two infants died. Megalourethra

was diagnosed in four infants; two survived with normal renal function, in whom no fetal intervention was performed. Megacystis-microcolon-intestinal hypoperistalsis syndrome was identified in three fetuses; all of them died.

After performing a multivariate analysis and adjusting for all variables including gestational age at diagnosis of LUTO, type of fetal intervention given, postnatal diagnosis of the cause of LUTO and fetal center location, as well as considering TOP, fetal cystoscopy was associated with a significant improvement in the 6-month survival rate (ARR, 1.86; 95% CI, 1.01–3.42; $P = 0.048$) and a clear trend for normal renal function (ARR, 1.73; 95% CI, 0.97–3.08; $P = 0.06$). However, fetal vesicoamniotic shunting was associated with significant improvement in the 6-month survival rate (ARR, 1.73; 95% CI, 1.01–3.08; $P = 0.04$) but with no improvement in normal renal function (ARR, 1.16; 95% CI, 0.86–1.55; $P = 0.33$).

When considering patients with PUV confirmed by postnatal diagnosis, both fetal cystoscopy (ARR, 4.10; 95% CI, 1.75–9.62; $P < 0.01$) and fetal vesicoamniotic shunting (ARR, 3.76; 95% CI, 1.42–9.97; $P < 0.01$) were associated with an improved 6-month survival rate. However, only fetal cystoscopy was associated with normal renal function (ARR, 2.66; 95% CI, 1.25–5.70; $P = 0.01$) and fetal vesicoamniotic shunting was not (ARR, 1.03; 95% CI, 0.49–2.17; $P = 0.93$).

PUVs were suspected in 20 fetuses and confirmed postnatally in 18 infants (one infant had a cloacal dystrophy and one had megacystis-microcolon-intestinal hypoperistalsis syndrome). All cases of urethral atresia that were suspected on fetal cystoscopy were confirmed postnatally. Additional postnatal PUV ablation was required in 5/12 (41.7%) infants with PUV who survived to 6 months.

Regarding complications of fetal interventions, the shunt became obstructed or migrated in five (31.3%) fetuses, which required a repeat procedure; three of them survived, one died and one pregnancy was terminated. Among the 34 fetuses that underwent fetal cystoscopy, there were three (8.8%) with urological fistulas (two fetuses survived and one pregnancy was terminated) and two (5.9%) had a recurrence of severe LUTO requiring a second intervention (both infants were delivered preterm at 24 and 25 weeks' gestation, respectively, and died after birth).

DISCUSSION

The present study evaluated perinatal outcome in fetuses with LUTO, comparing two modalities of fetal therapy: fetal cystoscopy and vesicoamniotic shunting. Based on our results, both fetal vesicoamniotic shunting and fetal cystoscopy improve the 6-month survival rate, especially when PUVs are present. However, fetal cystoscopic PUV laser ablation was associated statistically with normal renal function while fetal vesicoamniotic shunting did not prevent severe renal impairment.

Our data also confirmed that fetal LUTO comprises a spectrum of different diseases, manifesting with similar prenatal characteristics including megacystis, keyhole sign, bilateral hydrenophrosis and bilateral hydroureters. In our experience, PUVs were present in 51% of the cases, followed by urethral atresia in 22%. There were other causes in the remaining 27% of cases of LUTO, including prune belly syndrome, urethral stenosis, megalourethra, vesicoureteral reflux, cloacal dystrophy and megacystis-microcolon intestinal hypoperistalsis syndrome. The prognosis of fetal LUTO was associated statistically with the cause of the disease, independent of the type of fetal intervention. Therefore, fetal cystoscopy may have an advantage over fetal vesicoamniotic shunting by allowing prenatal diagnosis of the etiology of LUTO[13,16,19,20]. In our experience, the sensitivity and specificity of fetal cystoscopy in diagnosing LUTO correctly was 100.0% and 87.5%, respectively.

When considering the two most common pathological conditions, PUV and urethral atresia, fetal intervention improved outcomes successfully in the former but not in the latter cause of LUTO. The 6-month survival rate and the rate of normal renal function were higher in fetuses with PUV that underwent cystoscopy (66.7% and 61.1%) than in those that underwent vesicoamniotic shunting (60.0% and 50.0%) and those that did not undergo fetal intervention (17.2% and 17.2%), respectively. Considering urethral atresia, in our experience the mortality rate was 100%, which was completely independent of prenatal management. In the literature, however, there are some rare cases of urethral atresia with survival after vesicoamniotic shunting or with spontaneous reopening of the urachus allowing fetal urine flow[30,31].

As our objective was to investigate outcomes after fetal intervention in cases of severe LUTO, a multivariate analysis was performed, adjusting for those variables associated with outcome. When considering all diseases, both vesicoamniotic shunting and fetal cystoscopy were effective in improving the 6-month survival rate. However, a clear trend existed for fetal cystoscopy to preserve normal renal function effectively in infants with LUTO, while vesicoamniotic shunting was not effective. If we consider the diagnosis of PUV, both methods allowed improvement of the 6-month survival rate, but only fetal cystoscopy effectively prevented renal damage in our patients.

Therefore, our results suggest that the fetal cystoscopic procedure seems to be more effective than vesicoamniotic shunting since it improves both the 6-month survival rate and normal renal function, specifically when PUVs are present. In our opinion, the main explanation for these results may be the fact that fetal cystoscopy is also a diagnostic procedure allowing for a more specific treatment.

In the present series, we did not offer vesicoamniotic shunting after identification of other findings, especially when urethral atresia was present, because of the severity of the disease, which was confirmed by our results that have shown 100% mortality even after vesicoamniotic shunting. In one case, urethral stenosis was diagnosed during fetal cystoscopy and a transurethral catheter was

placed during the fetal cystoscopic procedure[20]. Our study, therefore, highlights the importance of determining the etiology of LUTO for counseling patients regarding the prognosis of the disease with or without fetal intervention. For this purpose, fetal cystoscopy may be a better option.

To the best of our knowledge, no clinical study in the literature compared different fetal interventions for severe LUTO with a control (i.e. no fetal therapy). Morris *et al.*[13] performed a systematic review including a series of studies comparing vesicoamniotic shunting *vs* no fetal therapy and fetal cystoscopy *vs* no fetal therapy. According to this study, fetal cystoscopy seemed to have a similar effect on the improvement of perinatal survival rates (odds ratio, 1.49; 95% CI, 0.13–16.97). Our clinical experience confirms that observation.

Recently, a randomized controlled trial has compared vesicoamniotic shunting with no fetal intervention for severe LUTO[9]. According to this study, vesicoamniotic shunting improves the 2-year survival rate with an RR of 4.27 (95% CI, 1.07–16.96) but does not prevent impairment of renal function (83.3% of survivors had impaired renal function at 2 years of age). Our results were, again, in agreement with that study.

Based on all this information, vesicoamniotic shunting improves survival rates, and it appears that fetal cystoscopy has a similar effect. However, fetal vesicoamniotic shunting does not appear to improve renal function postnatally. Our study contributes to the literature since we observed similar results regarding survival rate, but we have also provided further information concerning renal function, suggesting that fetal cystoscopy is more effective in preventing renal impairment at least until 6 months of age.

Our study has some limitations, most notably that it is a retrospective analysis and not a randomized controlled trial. Despite this limitation, similar characteristics were observed in the three groups of patients. A randomized controlled trial comparing fetal cystoscopy and vesicoamniotic shunting has been proposed to investigate the two interventions (Clinicaltrials.gov, identifier NCT01552824). In addition, this study did not provide long-term follow-up regarding renal and bladder function.

We have investigated the complications related to both interventions. Mean gestational age at delivery was similar in both groups in the present study and fetal cystoscopy was associated with urological fistulas and recurrence of severe LUTO in 8.8% and 5.9% of cases. We have recently reported complications related to technical aspects of fetal cystoscopic PUV laser ablation, including urological fistulas that may occur in approximately 10% of cases following this procedure and are associated with surgical technique such as type, energy and power settings of the laser, instrumentation and absence of fetal anesthesia, as well as limited operator experience with this particular surgery[32]. The main complication in our series related to vesicoamniotic shunting was migration/obstruction of the shunt (31.3%) that required repeat of the procedure. Our results are very similar to those reported in the literature (approximately 40%)[9,33]. The clinical relevance of such complications is being evaluated in a randomized controlled trial (Clinicaltrials.gov, identifier NCT01552824).

In the present study, fetal renal biochemical data were similar in the three groups and, therefore, we did not investigate the effect of fetal urinalysis to predict outcome. Further studies are necessary for longitudinal assessment (samples from different days) of fetal urinary biochemistry.

In conclusion, our study suggests that both fetal cystoscopy and vesicoamniotic shunting improve the 6-month survival rate in cases of severe LUTO, but only fetal cystoscopy may prevent impairment of renal function until the age of 6 months, especially for the group of patients with PUV. Our data support the idea of performing a subsequent randomized controlled trial to compare the effectiveness of fetal cystoscopy *vs* vesicoamniotic shunting for the treatment of severe fetal LUTO.

ACKNOWLEDGMENT

This study was funded by the CNPq (Conselho Nacional de Desenvolvimento de Pesquisa e Tecnologia do Brasil), process number 311121/2010-1.

REFERENCES

1. Anumba DO, Scott JE, Plant ND, Robson SC. Diagnosis and outcome of fetal lower urinary tract obstruction in the northern region of England. *Prenat Diagn* 2005; **25**: 7–13.
2. Favre R, Kohler M, Gasser B, Muller F, Nisand I. Early fetal megacystis between 11 and 15 weeks of gestation. *Ultrasound Obstet Gynecol* 1999; **14**: 402–406.
3. Liao AW, Sebire NJ, Geerts L, Cicero S, Nicolaides KH. Megacystis at 10–14 weeks of gestation: chromosomal defects and outcome according to bladder length. *Ultrasound Obstet Gynecol* 2003; **21**: 338–341.
4. Sepulveda W. Megacystis in the first trimester. *Prenat Diagn* 2004; **24**: 144–149.
5. Kari JA, El-Desoky S, Farag YM, Mosli H, Altyieb AM, Al Sayad A, Radawi O, Ghabra H, Basnawi F, Bahrawi O, Singh A, Farsi H. Renal impairment in children with posterior urethral valves. *Pediatr Nephrol* 2013; **28**: 927–931.
6. Wu S, Johnson MP. Fetal lower urinary tract obstruction. *Clin Perinatol* 2009; **36**: 377–390, x.
7. Morris RK, Malin GL, Khan KS, Kilby MD. Antenatal ultrasound to predict postnatal renal function in congenital lower urinary tract obstruction: systematic review of test accuracy. *BJOG* 2009; **116**: 1290–1299.
8. Lissauer D, Morris RK, Kilby MD. Fetal lower urinary tract obstruction. *Semin Fetal Neonatal Med* 2007; **12**: 464–470.
9. Morris RK, Malin GL, Quinlan-Jones E, Middleton LJ, Hemming K, Burke D, Daniels JP, Khan KS, Deeks J, Kilby MD; Percutaneous vesicoamniotic shunting in Lower Urinary Tract Obstruction (PLUTO) Collaborative Group. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. *Lancet* 2013; **382**: 1496–1506.
10. Morris RK, Kilby MD. An overview of the literature on congenital lower urinary tract obstruction and introduction to the PLUTO trial: percutaneous shunting in lower urinary tract obstruction. *Aust N Z J Obstet Gynaecol* 2009; **49**: 6–10.
11. Morris RK, Khan KS, Kilby MD. Vesicoamniotic shunting for fetal lower urinary tract obstruction: an overview. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**: F166–168.
12. Biard JM, Johnson MP, Carr MC, Wilson RD, Hedrick HL, Pavlock C, Adzick NS. Long-term outcomes in children treated by prenatal vesicoamniotic shunting for lower urinary tract obstruction. *Obstet Gynecol* 2005; **106**: 503–508.
13. Morris RK, Ruano R, Kilby MD. Effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. *Ultrasound Obstet Gynecol* 2011; **37**: 629–637.
14. Quintero RA, Hume R, Smith C, Johnson MP, Cotton DB, Romero R, Evans MI. Percutaneous fetal cystoscopy and endoscopic fulguration of posterior urethral valves. *Am J Obstet Gynecol* 1995; **172**: 206–209.
15. Quintero RA, Johnson MP, Romero R, Smith C, Arias F, Guevara-Zuloaga F, Cotton DB, Evans MI. In-utero percutaneous cystoscopy in the management of fetal lower obstructive uropathy. *Lancet* 1995; **346**: 537–540.
16. Ruano R, Duarte S, Bunduki V, Giron AM, Srougi M, Zugaib M. Fetal cystoscopy for severe lower urinary tract obstruction--initial experience of a single center. *Prenat Diagn* 2010; **30**: 30–39.
17. Ruano R, Pimenta EJ, Duarte S, Zugaib M. Four-dimensional ultrasonographic imaging of fetal lower urinary tract obstruction and guidance of percutaneous cystoscopy. *Ultrasound Obstet Gynecol* 2009; **33**: 250–252.

18. Ruano R, Yoshisaki CT, Salustiano EM, Giron AM, Srougi M, Zugaib M. Early fetal cystoscopy for first-trimester severe megacystis. *Ultrasound Obstet Gynecol* 2011; **37**: 696–701.
19. Welsh A, Agarwal S, Kumar S, Smith RP, Fisk NM. Fetal cystoscopy in the management of fetal obstructive uropathy: experience in a single European centre. *Prenat Diagn* 2003; **23**: 1033–1041.
20. Ruano R, Yoshizaki CT, Giron AM, Srougi M, Zugaib M. Fetal cystoscopic placement of transurethral stent in a fetus with urethral stenosis. *Ultrasound Obstet Gynecol* 2014; **44**: 238–240.
21. Ruano R. Fetal surgery for severe lower urinary tract obstruction. *Prenat Diagn* 2011; **31**: 667–674.
22. Ruano R, Yoshisaki CT, Salustiano EM, Giron AM, Srougi M, Zugaib M. Early fetal cystoscopy for first-trimester severe megacystis. *Ultrasound Obstet Gynecol* 2011; **37**: 696–701.
23. Morris RK, Ruano R, Kilby MD. The effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. *Ultrasound Obstet Gynecol* 2011; **37**: 629–637.
24. Grignon A, Filion R, Filiatrault D, Robitaille P, Homsy Y, Boutin H, Leblond R. Urinary tract dilatation in utero: classification and clinical applications. *Radiology* 1986; **160**: 645–647.
25. Brown C, Morris RK, Daniels J, Khan KS, Lilford RJ, Kilby MD. Effectiveness of percutaneous vesicoamniotic shunting in congenital lower urinary tract obstruction: divergence in prior beliefs among specialist groups. *Eur J Obstet Gynecol Reprod Biol* 2010; **152**: 25–29.
26. Blaicher W, Häusler M, Gembruch U, Bollmann R, Terinde R, Rempen A, Deutinger J, Bernaschek G. Feto-amniotic shunting - experience of six centres. *Ultraschall Med* 2005; **26**: 134–141.
27. Agarwal SK, Fisk NM. In utero therapy for lower urinary tract obstruction. *Prenat Diagn* 2001; **21**: 970–976.
28. Johnson MP, Bukowski TP, Reitleman C, Isada NB, Pryde PG, Evans MI. In utero surgical treatment of fetal obstructive uropathy: a new comprehensive approach to identify appropriate candidates for vesicoamniotic shunt therapy. *Am J Obstet Gynecol* 1994; **170**: 1770–1776; discussion 1776–1779.
29. Muller F, Dommergues M, Mandelbrot L, Aubry MC, Nihoul-Fekete C, Dumez Y. Fetal urinary biochemistry predicts postnatal renal function in children with bilateral obstructive uropathies. *Obstet Gynecol* 1993; **82**: 813–820.
30. Chilakala SK, Boulden TF, Pourcyrous M. Ruptured remnant of urachal diverticulum: an unusual cause of congenital urinary ascites. *J Perinatol* 2012; **32**: 978–980.
31. Stalberg K, Gonzalez R. Urethral atresia and anhydramnios at 18 weeks of gestation can result in normal development. *J Pediatr Urol* 2012; **8**: e33–35.
32. Sananes N, Favre R, Koh CJ, Zaloszyc A, Braun MC, Roth DR, Moog R, Becmeur F, Belfort MA, Ruano R. Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves: surgical technical aspects. *Ultrasound Obstet Gynecol* 2015; **45**: 183–189.
33. Won HS, Kim SK, Shim JY, Lee PR, Kim A. Vesicoamniotic shunting using a double-basket catheter appears effective in treating fetal bladder outlet obstruction. *Acta Obstet Gynecol Scand* 2006; **85**: 879–884.

DOI: 10.1002/pd.4771 PRENATAL **DIAGNOSIS**

ORIGINAL ARTICLE

Two-year outcomes after diagnostic and therapeutic fetal cystoscopy for lower urinary tract obstruction†

Nicolas Sananes[1,2], Rogelio Cruz-Martinez[3,4], Romain Favre[5], Ricardo Ordorica-Flores[6], Raphaël Moog[7], Ariane Zaloszy[8], Amilcar Martins Giron[9] and Rodrigo Ruano[1]*

[1]Department of Obstetrics and Gynecology, Baylor College of Medicine and Texas Children's Fetal Center, Texas Children's Hospital, Houston, TX, USA
[2]INSERM, UMR-S 1121, 'Biomatériaux et Bioingénierie', Strasbourg, France
[3]Department of Fetal SurgeryChildren's and Women's Specialty Hospital of Queretaro, Queretaro, Mexico
[4]Unidad de Investigación en Neurodesarrollo, Instituto de Neurobiología, UNAM-Juriquilla, Queretaro, Mexico
[5]Maternal Fetal Medicine Department, Strasbourg University Hospital, Strasbourg, France
[6]Department of Pediatric Surgery, Children's Hospital Mexico 'Federico Gomez' (HIMFG), Mexico City, Mexico
[7]Pediatric Surgery Department, Strasbourg University Hospital, Strasbourg, France
[8]Pediatric Nephrology Department, Strasbourg University Hospital, Strasbourg, France
[9]Department of Urology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
*Correspondence to: Rodrigo Ruano. E-mail: ruano@bcm.edu; rodrigoruano@hotmail.com
†This study was presented orally at the 34th Annual Meeting of the International Fetal Medicine and Surgery Society and 14th World Congress in Fetal Medicine, in Hersonissos, Crete, Greece.

ABSTRACT

Objectives Our objective is to report long-term outcome after fetal cystoscopy for lower urinary tract obstruction (LUTO), as well as to investigate the accuracy of fetal cystoscopy in diagnosing the cause of bladder outlet obstruction.

Methods This is a retrospective cohort study of all fetuses who underwent cystoscopy for prenatal diagnosis of LUTO in three tertiary referral centers. Fetal diagnostic cystoscopy was performed to determine prenatally the cause of LUTO and to ablate the posterior urethral valves (PUV).

Results A total of 50 fetal cystoscopies were performed, revealing PUV in 31 (62%) fetuses, urethral atresia (UA) in 14 (28%) fetuses, and urethral stenosis (US) in 5 (10%) fetuses. Two fetuses had trisomy 18 diagnosed after fetal cystoscopy and were excluded from the present analysis. Fetal cystoscopy was accurate in the diagnosis of the etiology of LUTO in 32/35 (91.4%). There were no survivors in the UA group. One fetus with US underwent urethral stenting and survived with normal renal function at 2 years of life. Among the infants with PUV, 17/30 (56.7%) infants survived, and 13/17 (76.5%) had normal renal function at 1 year of life; 15/28 (53.6%) infants survived, and 11/15 (73.3%) had normal renal function at 2 years.

Conclusions Fetal cystoscopy is accurate in the diagnosis of the etiology of LUTO and serves as a guide to the specific prenatal treatment. This procedure is associated with modest long-term survival (54%) but with adequate preserved normal renal function in two thirds of the infants among fetuses with PUV. © 2016 John Wiley & Sons, Ltd.

Funding sources: None
Conflicts of interest: None declared

INTRODUCTION

Fetal lower urinary tract obstruction (LUTO) is an obstructive uropathy at the level of the neck of the bladder, caused by different entities such as posterior urethral valves (PUV), urethral atresia (UA), and urethral stenosis (US).[1,2] It occurs in approximately two out of 10 000 births and is associated with a high perinatal mortality and morbidity due to pulmonary hypoplasia and renal dysfunction.[3–10] Relief of the urinary obstruction by prenatal vesicoamniotic shunting (VAS) is commonly offered because it may reduce the postnatal mortality.[11,12] However, VAS requires an amnioinfusion prior to shunt placement and is associated with complications such as migration and obstruction.[11,13–15]

Fetal cystoscopy has been described as an alternative option to VAS by avoiding amnioinfusion, by providing an etiological diagnosis for the obstruction prior to prenatal management, and consequently by directing the specific prenatal treatment for the cause of LUTO.[16–25] However, there are limited data on outcomes after fetal cystoscopy, especially those outcomes related to long-term follow-up. In addition, significant perinatal complications have been reported related to the surgical techniques.[25–27]

The primary objective of this present study was to report perinatal and long-term outcomes after fetal cystoscopy for LUTO, especially after laser ablation of PUV. The secondary objective was to evaluate the accuracy of fetal cystoscopy to diagnose the etiology of the obstructive uropathy.

MATERIALS AND METHODS

Study design and setting

This is a retrospective cohort study of all fetuses who underwent cystoscopy for LUTO in three tertiary referral centers (Sao Paulo, Brazil; Queretaro, Mexico; and Strasbourg, France) between January 2004 and December 2013. All data were collected prospectively until April 2015. The local institutional review boards approved the studies related to the experimental procedure (fetal cystoscopy) for severe LUTO. The present study was standardized in the different centers using the same patient selection criteria, surgical technique, and research protocol, according to a publication by Ruano *et al.* in 2010.[19]

Participants

Fetal LUTO was defined at detailed ultrasound examination by the combination of extremely dilated bladder with increased wall thickness ('megabladder') and dilatation of the posterior urethra ('keyhole sign').[19] LUTO was considered severe in case of bilateral hydronephrosis and oligohydramnios.[19,25] Cystoscopy was offered after extensive counseling and informed consent in cases of LUTO in male fetuses without suggestive signs of prune-belly syndrome, renal dysplasia, additional malformations, as an experimental procedure and an alternative to VAS, expectant management, or termination of pregnancy. Fetal karyotype was systematically analyzed before surgery, but in two cases fetal karyotype was performed from a sample of fetal urine that was collected at the time of fetal cystoscopy. Cases with chromosomal anomalies were excluded from outcome analysis. In addition, fetal cystoscopy was offered in cases of oligohydramnios and 'favorable' fetal urinary analysis (defined as sodium < 100 mEq/L, chloride < 90 mEq/L, calcium < 8 mg/dL, osmolarity < 210 mOsm/L, and beta-2 microglobulin < 6 mg/dL in the latest sample, out of a maximum of three samples that were collected daily) if gestational age is >18 weeks.[28] Before 18 weeks, the amount of amniotic fluid and fetal biochemistry were not used to select the candidates for fetal intervention.[20] In those cases before 18 weeks, fetal urine for biochemistry was collected at fetal cystoscopy for research purposes.

Fetal interventions

Fetal cystoscopy was performed under maternal epidural or local anesthesia by three operators, one in each center (R. R., R. F., and R. C. M.) using the same technique described previously.[19,25] Fetal anesthesia was conducted via the injection of fentanyl (15 μg/kg) and pancuronium (2 mg/kg) under ultrasound guidance of a 22-gauge needle into the umbilical vein or fetal arms or legs. A curved sheath and a 1.0- or 1.3-mm fetoscope (11510A and 11540AA, Karl Storz, Germany) were introduced into the fetal bladder percutaneously (Figure 1).

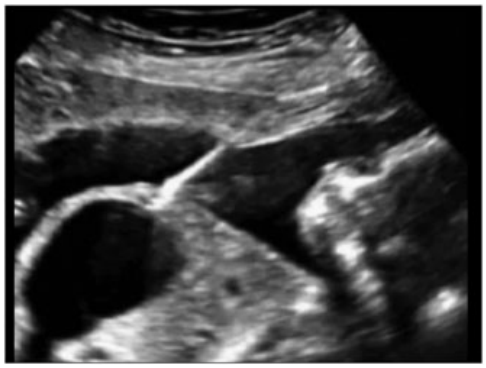


Figure 1 Ultrasound image shows the insertion of the fetoscopic trocar inside the bladder of a fetus at 16 weeks of gestation

The endoscope was then advanced towards the bladder neck and the dilated proximal urethra. When PUV were identified (Figure 2a), the valves were fulgurated using 400- and 600-μm laser fibers by pulsed shots of a Nd:YAG laser (SmartEpil, DEKA, Florence, Italy) at maximal settings of 30 W and 100 J or continuous shots of diode (Multibeam, Dornier Medtech, Kennesaw, GA, USA) laser at settings of 30 to 40 W and 100 J (Figure 2b).[25] Fulguration was performed by almost touching the valves with the 400- or 600-μm laser fiber. Fulguration was considered accomplished when the bladder was found to be empty, and power Doppler ultrasound confirmed passage of fluid through the patent penile urethra into the amniotic cavity after flushing fluid into the fetal bladder through the fetoscope. [16,19] When UA or US was diagnosed during fetal cystoscopy (Figure 2c and d), laser fulguration was not performed, and the procedure was ended.

Follow-up and outcomes

Patients were discharged within 1 or 2 days after fetal intervention, and an ultrasound follow-up was performed on a weekly or bi-monthly basis. After delivery, all the survivors were evaluated and followed by pediatric urologists and nephrologists. Micturating cystourethrography and postnatal cystoscopy were performed to evaluate the bladder and the urethra. Autopsy was always offered in cases of termination of pregnancy, intrauterine death, or neonatal death.

Outcomes were survival (neonatal, 1 year, and 2 years), renal function, and need for dialysis. Renal function was assessed on the glomerular filtration rate (GFR) using the Schwartz formula, taking into account cystatin C or serum creatinine when cystatin C was not known.[29] Infants with GFR < 90 mL/min/1.73 m^2 were considered to have abnormal renal function. We also considered end-stage kidney disease when dialysis and transplant were indicated.

RESULTS

Flowchart and overall outcome

A total of 50 fetal cystoscopies were performed for severe LUTO: 29 (58%) in Sao Paulo, Brazil; 15 (30%) in Queretaro, Mexico; and 6 (12%) in Strasbourg, France (Figure 3). The

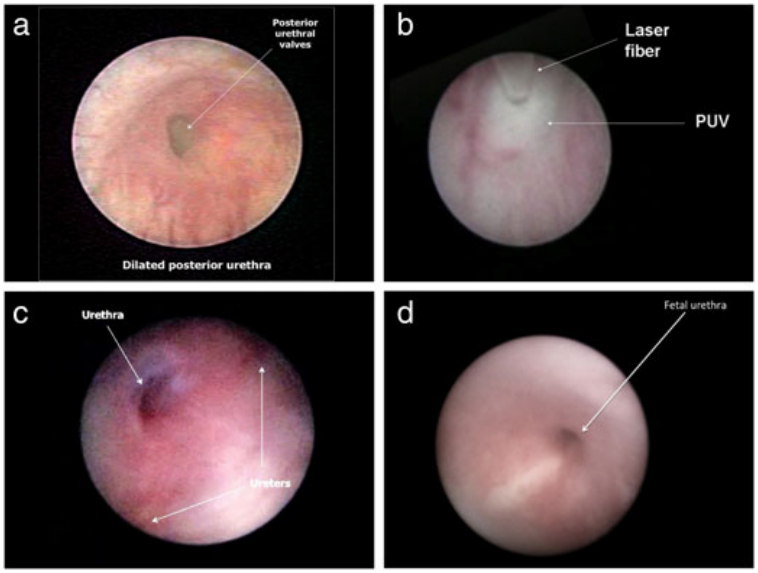


Figure 2 (A) Fetal cystoscopic view of the dilated posterior urethra caused by posterior urethral valves. (B) Fetal cystoscopic fulguration of the posterior urethral valves (PUV). (C) Fetal cystoscopic view of urethral atresia (there was no dilated posterior urethra). (D) Fetal cystoscopic view of urethral stenosis

perinatal outcome is reported on 34 patients.[25] The mean gestational age at procedure was 19.4 ± 3.9 weeks (range 14–29 weeks). Severe bilateral hydronephrosis was identified in 40 fetuses (80.0%), while 10 cases had mild forms. Preterm premature rupture of the membranes (PPROM) occurred in 11 (22.0%) cases at mean gestational age of 29.4 ± 9.8 weeks (range from 19.0 to 35.0 weeks). Mean gestational age at delivery was 32.4 ± 4.6 weeks (excluding medical termination of pregnancy).

All fetuses who underwent fetal cystoscopy after 18 weeks had severe LUTO ($n = 30$), severe hydronephrosis, oligo hydramnios/anhydramnios, and 'favorable' fetal urinary analysis. PPROM occurred in seven (23.3%) patients.

Fetal cystoscopy was performed in 20 fetuses before 18 weeks [14 fetuses had a normal amount of amniotic fluid while 6 had oligohydramnios; fetal urinary biochemistry was considered 'favorable' in 13 fetuses, while 'non-favorable' in 4 patients (not collected in 3 cases)]. Bilateral severe hydronephrosis was seen 14 fetuses (70.0%), while 6 cases had mild forms.

Among the 48 cases of LUTO with normal karyotype who underwent fetal cystoscopy, termination of pregnancy was

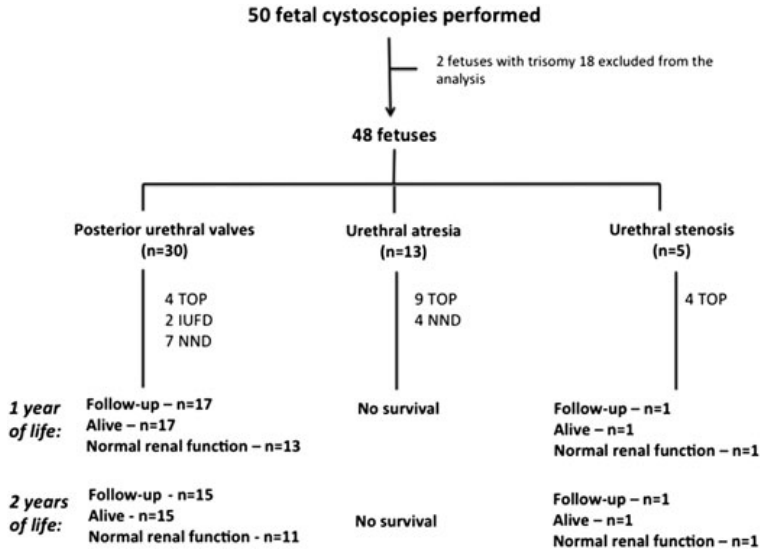


Figure 3 Flowchart of the study population. LUTO: lower urinary tract obstruction; TOP: termination of pregnancy; IUFD: intrauterine fetal death; NND: neonatal death

performed in 17 (35.4%). There were 2 (4.2%) cases of intrauterine fetal demise after fetal intervention. Eleven (22.9%) infants died during the neonatal period. There was a 1-year follow-up for all the 18 survivors and a 2-year follow-up for 16 patients (2 infants were younger than 2 years at the moment of the present analysis). All infants who survived the neonatal period survived to 1 and 2 years of life. At 1 year of life, 18/48 (37.5%) infants survived, and 14 (77.8%) infants had normal renal function. At 2 years of life, 16/46 (34.8%) infants survived, and 12 (75.0%) infants had normal renal function.

The etiology for the obstructive uropathy was assessed in all 18 survivors and in 17 autopsies. Diagnosis for the cause of obstruction was correct in 32 of 35 cases (91.4%). In one case, PUV was suspected at fetal cystoscopy examination; however, it was a hypoperistalsis-microcolon-megabladder syndrome. In two cases, PUV were associated with distal US that was not diagnosed at the time of the cystoscopy.

Fetal cystoscopy in case of urethral atresia

None of the 13 fetuses with a fetal cystoscopic diagnosis of UA underwent laser fulguration. There were no survivors in case of UA, because of either termination of pregnancy ($n=9$) or neonatal death ($n=4$). Vesicoamniotic shunt placement was attempted in two patients: one chose to terminate the pregnancy thereafter because of persistent oligohydramnios; the other one delivered at 25 weeks, and the baby died within the neonatal period.

Fetal cystoscopy in case of urethral stenosis

None of the five fetuses with a fetal cystoscopic diagnosis of US underwent laser fulguration. A total of four patients decided for termination of pregnancy. The only survivor in the US group was a fetus in whom a transurethral stent was successfully placed. This case has been published previously, and the renal function was still normal at 6 years of age.[22]

Fetal cystoscopic laser ablation of posterior urethral valves

A total of 30 cases of PUV were identified prenatally and treated by laser ablation. In one case, a small fetal bleeding at the level of the abdominal wall was observed at the end of the cystoscopy, during fetoscope withdrawal. A fetal demise occurred within 24 h probably because of an umbilical vein laceration since the postnatal examination showed a peri-umbilical ecchymosis (parents refused autopsy). There was no other intra-operative significant complication.

Recurrence of LUTO occurred in 6/30 (20.0%) cases. An additional procedure was performed in 3/30 (10.0%) cases (two others cystoscopies and one VAS): two neonates survived; the other one was born at 28 weeks after a PPROM and died within the neonatal period.

Parents decided for termination of pregnancy in 4/30 (13.3%) cases because of recurrence of LUTO ($n=2$), PPROM at 26 weeks ($n=1$), and urologic fistula with significant perineal defect seen on ultrasound ($n=1$).[26]

There were 4/30 (13.3%) cases of urological fistulas, either urethral-rectal or urethral-cutaneous, among which three have been previously reported.[26] The diagnosis has been made prenatally in one patient who decided to terminate the pregnancy. In the three other cases, fistulas were treated within the first year of life with a cystostomy and a colostomy in one case. A right-hip dysplasia and a right clubfoot were associated with the fistula in this last case and necessitated an orthopedic management. This child is currently 2 years old; he has a unilateral flaccid paralysis and needs a splint. Lastly, there was a case of a small herniation of the epiploon through the fetal abdominal wall that required a neonatal surgical correction.

A total of 24/30 (80.0%) patients delivered a live-born baby at a mean gestational age of 34.6 ± 2.5 weeks (range 28–37 weeks), but seven infants died within the neonatal period, because of prematurity, lung hypoplasia, or renal failure. Among the 17/30 (56.7%) infants who survived the neonatal period, 10/17 (58.8%) underwent an additional postnatal cystoscopic ablation of PUV, while additional postnatal ablation of PUV was not necessary in 7/17 (41.2%) cases.

All 17 infants who survived the neonatal period were alive to 1 year of age. At 1 year of life, a total of 17/30 (56.7%) infants survived, and 13/17 (76.5%) children had normal renal function. Four infants underwent peritoneal dialysis during the first year of life.

At 2 years of life, 15/28 (53.6%) patients survived, and 11/15 (73.3%) had normal renal function (two patients were not 2 years old at the moment of the present analysis). One infant was under dialysis within the first year of life and another one within 2 years.

Discussion

Principal findings

Our study shows that fetal cystoscopy can diagnose accurately the etiology of the obstructive uropathy, which may be useful to select adequate candidates for fetal intervention and to determine appropriated specific prenatal treatment based on the cause of the LUTO. Our results suggest that half of the infants with prenatal diagnosis of PUV who undergo fetal cystoscopic laser ablation survive at 2 years of life, and 70% of the survivors at this age have normal renal function. When considering all the patients who underwent cystoscopy for severe fetal LUTO, only one quarter of the cases are survivors with normal renal function at 2 years.

Strengths and weaknesses

To our knowledge, this is the largest report of fetal cystoscopies for severe LUTO. We provide data on the accuracy of fetal cystoscopy for the diagnosis of the obstructive etiology and complications, as well as the outcomes at 2 years of life (survival and renal function). In the present study, we evaluated the renal function based on the Schwartz equation.[29] Evaluation of adequate renal function in children is challenging, specifically when considering the determination of the true GFR. Schwartz equation is the most common formula used in children, which considers both serum creatinine and cystatin C, providing accurate assessment of GFR and renal function in children.[30–32]

The limitations of the study include a retrospective design with no control group. However, we reported previously a

case–control study of fetal cystoscopy versus vesicoamniotic shunt.[25] Our present objective was to report further outcomes and to focus on specific issues of fetal cystoscopy. We acknowledge that some secondary outcomes were not evaluated: valve bladder syndrome, atonic or hypertonic bladder, dribbling, vesicourethral reflux, and recurrent urinary tract infections.

Interpretation

Our study provides information that may be useful for the prenatal clinical management of fetuses with severe LUTO and for further investigations. The first conclusion is that fetal cystoscopy can accurately diagnose the cause of the bladder outlet obstruction. This may be the most important role of this procedure. In our experience, fetal cystoscopy correctly diagnosed the cause of LUTO in 90% of our cases. In the present series, one case of PUV diagnosed at fetal cystoscopy was revised to be a hypoperistalsis-microcolon-megabladder syndrome. A similar case has been already reported in the series of Martinez *et al.*[33] Laser fulguration cannot improve the urological outcome in this case, and the prognosis of this condition is totally different from isolated PUV.

The definitive diagnosis of the underlying etiology of LUTO is impossible using prenatal ultrasonography alone.[1,34] Fetal cystoscopy allows an endoscopic examination of the dilated posterior urethra and identification of PUV, UA, or US. Previous studies have demonstrated that the etiology of LUTO is one of the major factors associated with prognosis.[23,34]

Consequently to the adequate prenatal diagnosis of the cause of LUTO, an appropriated specific perinatal therapeutic management can be planned and performed as part of the prenatal counseling. For instance, fetal cystoscopic laser therapy can be attempted only in cases of PUV. In cases of UA, cystoscopy cannot be used as a therapeutic procedure, and the laser therapy should not be performed because it can lead to fistulas and damages to surrounding tissues. The only way to relieve the obstruction in those cases with UA is to place a VAS. However, the prognosis of UA is considered poor even after VAS in the literature.[25] Further studies are necessary to investigate the effectiveness of VAS in fetuses with UA. In a case of US, a transurethral stent may be a therapeutic option.

There is a lack of evidence for the effectiveness of fetal cystoscopy over VAS for LUTO.[25,35] Some potential benefits have been suggested such as lack of need for amnioinfusion and more physiological drainage of the bladder. However, our study suggests the main advantage of the fetal cystoscopy over VAS is to provide the correct diagnosis of the etiology of LUTO and consequently to allow a better selection of candidates for fetal therapy that contribute to better long-term outcome related to renal function. This can clearly be seen when we compare our results with the PLUTO trial [the randomized controlled trial (RCT) that evaluated the effectiveness of VAS versus no fetal intervention]; at 2 years of life, the survival rate was 16/50 (32%) in our series versus 7/14 (50%) in the PLUTO trial, but we observed normal renal function in 12/16 (75%) in our patients versus 2/7 (29%) in the PLUTO trial.

This information is important for the prenatal counseling of the families with fetal LUTO. Prenatal intervention management can include the following: (1) a primary fetal VAS placement independently of knowing the etiology of LUTO, which may be associated with better survival rate or alternatively (2) an initial fetal cystoscopy that may allow a better selection of those cases with more chance of having better long-term renal function. If fetal cystoscopy is considered initially, the final and specific treatment can be performed at the same time of the fetal cystoscopy (laser therapy for PUV or shunt for UA or urethral stent for US) or at a second procedure (a two-step approach) where further prenatal therapeutic options can be discussed with the family (Figure 4). In order to compare these two approaches, our

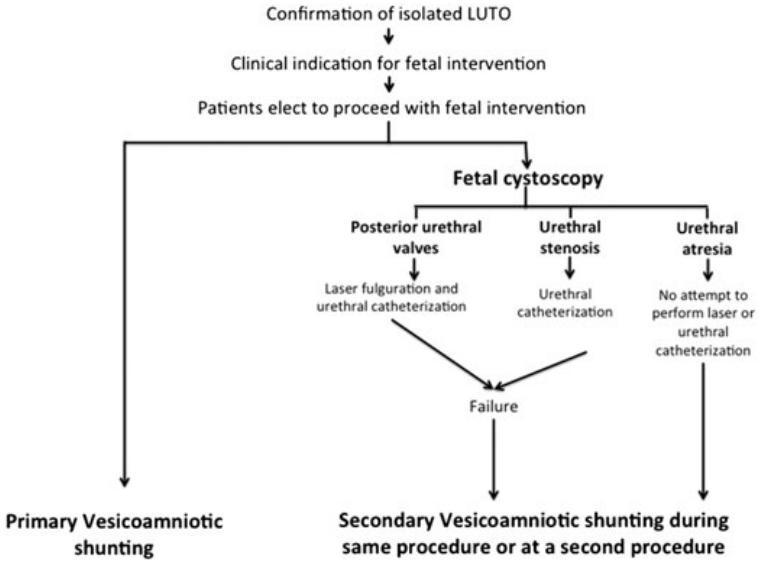


Figure 4 Proposed algorithm for fetuses with severe lower urinary tract obstruction (LUTO) using fetal cystoscopy

group will start an RCT comparing fetal cystoscopy versus VAS (NCT01552824).

Both procedures are associated with complications. Issues of VAS include shunt migration or obstruction, which require another fetal intervention.[11,12,14,15] In the present series of fetal cystoscopy, we report 19.4% of LUTO recurrence after laser ablation of PUV. This may be explained either by an insufficient fulguration or by a subsequent postlaser scar stenosis. On the other hand, fetal cystoscopy has the theoretical advantage over VAS to permit a more physiological drainage of the obstructed bladder, and postnatal ablation of PUV was not necessary in 41.2% of the cases in our series.

Fetal cystoscopy is still a challenging procedure; the risk for a urinary fistula is around 10%.[26,33] Technical aspects definitely need to be optimized. Other techniques should be investigated (microscissors, guidewire, and balloon disruption). We also report here a case of fetal demise that may be related to an umbilical vein laceration. That may be explained by the fact that in the cystoscopic procedure, the trocar must be inserted in the upper part of the fetal bladder, unlike the VAS. Fetal cystoscopy needs to involve a multidisciplinary team (maternal fetal medicine, radiology, and pediatric urology) in a specialized center with extensive fetal intervention experience and neonatal expertise. In our opinion, this procedure should be offered in very highly experienced fetal therapy centers, where adequate training and surgical instrumentations are available. After adequate training and pursuing the correct instrumentation, those centers will be able to participate in the proposed RCT (NCT01552824). In addition, severe LUTO is considered a rare condition; we also recommend that fetal cystoscopy should not be offered in centers that are not participating in the registered trial.

CONCLUSION

In conclusion, fetal cystoscopy can accurately diagnose the cause of the bladder outlet obstruction, allowing adequate selection of candidates for fetal intervention and specific perinatal therapeutic management of those patients. Selecting the adequate candidates for fetal intervention and performing the specific fetal treatment may be responsible for improving long-term renal function in those patients. A prospective RCT is necessary to evaluate this hypothesis. Additionally, fetal cystoscopy remains a challenging procedure, where technical improvements may allow for decreased fetal morbidity and improved accuracy in therapy for LUTO.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Fetal cystoscopy has been proposed as an alternative option to the vesicoamniotic shunt for severe LUTO with the main advantage of providing an adequate prenatal diagnosis and specific treatment of the cause of bladder outlet obstruction. However, there is no information about either the accuracy of fetal cystoscopy in diagnosing the etiology of LUTO or the long-term outcome of infants who underwent this procedure.

WHAT DOES THIS STUDY ADD?

- The present study provides new information to the literature regarding the accuracy of the fetal cystoscopy in diagnosing the etiology of LUTO, as well as the long-term outcome (survival and renal function to 2 years of life) of infants that underwent this intervention. In addition, the present study proposes an algorithm where both fetal cystoscopy and vesicoamniotic shunt can be considered in a single procedure or a two-step procedure.

REFERENCES

1. Robyr R, Benachi A, Daikha-Dahmane F, *et al.* Correlation between ultrasound and anatomical findings in fetuses with lower urinary tract obstruction in the first half of pregnancy. Ultrasound Obstet Gynecol 2005;25(5):478–82.
2. Kilby MD, Morris RK. Fetal therapy for the treatment of congenital bladder neck obstruction. Nat Rev Urol 2014;11(7):412–9.
3. Favre R, Kohler M, Gasser B, *et al.* Early fetal megacystis between 11 and 15 weeks of gestation. Ultrasound Obstet Gynecol 1999;14(6):402–6.
4. Liao AW, Sebire NJ, Geerts L, *et al.* Megacystis at 10–14 weeks of gestation: chromosomal defects and outcome according to bladder length. Ultrasound Obstet Gynecol. 2003;21(4):338–41.
5. Sepulveda W. Megacystis in the first trimester. Prenat Diagn 2004;24(2):144–9.
6. Kari JA, El-Desoky S, Farag Y, *et al.* Renal impairment in children with posterior urethral valves. Pediatr Nephrol 2013;28(6):927–31.
7. Wu S, Johnson MP. Fetal lower urinary tract obstruction. Clin Perinatol 2009;36(2):377–90. x.
8. Morris RK, Malin GL, Khan KS, *et al.* Antenatal ultrasound to predict postnatal renal function in congenital lower urinary tract obstruction: systematic review of test accuracy. BJOG 2009;116(10):1290–9.
9. Lissauer D, Morris RK, Kilby MD. Fetal lower urinary tract obstruction. Semin Fetal Neonatal Med 2007;12(6):464–70.
10. Anumba DO, Scott JE, Plant ND, *et al.* Diagnosis and outcome of fetal lower urinary tract obstruction in the northern region of England. Prenat Diagn 2005;25(1):7–13.
11. Morris RK, Malin GL, Quinlan-Jones E, *et al.* Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. Lancet 2013;382(9903):1496–506.
12. Morris RK, Malin GL, Khan KS, *et al.* Systematic review of the effectiveness of antenatal intervention for the treatment of congenital lower urinary tract obstruction. BJOG 2010;117(4):382–90.
13. Morris RK, Kilby MD. An overview of the literature on congenital lower urinary tract obstruction and introduction to the PLUTO trial: percutaneous shunting in lower urinary tract obstruction. Aust N Z J Obstet Gynaecol 2009;49(1):6–10.
14. Morris RK, Khan KS, Kilby MD. Vesicoamniotic shunting for fetal lower urinary tract obstruction: an overview. Arch Dis Child Fetal Neonatal Ed 2007;92(3):F166–8.
15. Biard JM, Johnson MP, Carr MC, *et al.* Long-term outcomes in children treated by prenatal vesicoamniotic shunting for lower urinary tract obstruction. Obstet Gynecol 2005;106(3):503–8.
16. Welsh A, Agarwal S, Kumar S, *et al.* Fetal cystoscopy in the management of fetal obstructive uropathy: experience in a single European centre. Prenat Diagn 2003;23(13):1033–41.
17. Quintero RA, Hume R, Smith C, *et al.* Percutaneous fetal cystoscopy and endoscopic fulguration of posterior urethral valves. Am J Obstet Gynecol 1995;172(1 Pt 1):206–9.
18. Quintero RA, Shukla AR, Homsy YL, *et al.* Successful in utero endoscopic ablation of posterior urethral valves: a new dimension in fetal urology. Urology 2000;55(5):774.

19. Ruano R, Duarte S, Bunduki V, *et al.* Fetal cystoscopy for severe lower urinary tract obstruction – initial experience of a single center. Prenat Diagn 2010;30(1):30–9.
20. Ruano R, Yoshisaki CT, Salustiano EM, *et al.* Early fetal cystoscopy for first-trimester severe megacystis. Ultrasound Obstet Gynecol 2011;37(6):696–701.
21. Sago H, Hayashi S, Chiba T, *et al.* Endoscopic fetal urethrotomy for anterior urethral valves: a preliminary report. Fetal Diagn Ther 2008;24(2):92–5.
22. Ruano R, Yoshizaki CT, Giron AM, *et al.* Fetal cystoscopic placement of transurethral stent in a fetus with urethral stenosis. Ultrasound Obstet Gynecol 2014;44(2):238–40.
23. Ruano R, Pimenta EJ, Duarte S, *et al.* Four-dimensional ultrasonographic imaging of fetal lower urinary tract obstruction and guidance of percutaneous cystoscopy. Ultrasound Obstet Gynecol 2009;33(2):250–2.
24. Ruano R. Fetal surgery for severe lower urinary tract obstruction. Prenat Diagn 2011;31(7):667–74.
25. Ruano R, Sananes N, Sangi-Haghpeykar H, *et al.* Fetal intervention for severe lower urinary tract obstruction: a multicenter case-control study comparing fetal cystoscopy with vesicoamniotic shunting. Ultrasound Obstet Gynecol 2015;45(4):452–8.
26. Sananes N, Favre R, Koh CJ, *et al.* Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves – surgical technical aspects. Ultrasound Obstet Gynecol 2015;45(2):183–9.
27. Luks FI. Re: Fetal intervention for severe lower urinary tract obstruction: a multicenter case–control study comparing fetal cystoscopy with vesicoamniotic shunting. R. Ruano, N. Sananes, H. Sangi-Haghpeykar, S. Hernandez-Ruano, R. Moog, F. Becmeur, A. Zaloszyc, A. M. Giron, B. Morin and R. Favre. Ultrasound Obstet Gynecol 2015;45:452–458. Ultrasound Obstet Gynecol. 2015;45(4):375–6.
28. Muller F, Dommergues M, Mandelbrot L, *et al.* Fetal urinary biochemistry predicts postnatal renal function in children with bilateral obstructive uropathies. Obstet Gynecol 1993;82(5):813–20.
29. Schwartz GJ, Munoz A, Schneider MF, *et al.* New equations to estimate GFR in children with CKD. J Am Soc Neph: JASN 2009;20(3):629–37.
30. Lemmens AS, Mekahli D, Devlieger R, *et al.* Population-specific serum creatinine centiles in neonates with posterior urethral valves already predict long-term renal outcome. J Mater-fetal & Neon Med: Off J Europ Assoc of Per Med, FedAsia and Oceania Per Soc, Int Soc of Per Obs 2015;28(9):1026–31.
31. Pohl M, Mentzel HJ, Vogt S, *et al.* Risk factors for renal insufficiency in children with urethral valves. Pediatr Nephrol 2012;27(3):443–50.
32. Lopez Pereira P, Espinosa L, Martinez Urrutina MJ, *et al.* Posterior urethral valves: prognostic factors. BJU Int 2003;91(7):687–90.
33. Martinez JM, Masoller N, Devlieger R, *et al.* Laser ablation of posterior urethral valves by fetal cystoscopy. Fetal Diagn Ther 2015;37(4):267–73.
34. Bernardes LS, Aksnes G, Saada J, *et al.* Keyhole sign: how specific is it for the diagnosis of posterior urethral valves? Ultrasound Obstet Gynecol 2009;34(4):419–23.
35. Morris RK, Ruano R, Kilby MD. Effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. Ultrasound Obstet Gynecol 2011;37(6):629–37.

Ultrasound Obstet Gynecol 2015; **45**: 183–189
Published online in Wiley Online Library (wileyonlinelibrary.com). **DOI:** 10.1002/uog.13405

Urological fistulas after fetal cystoscopic laser ablation of posterior urethral valves: surgical technical aspects

N. SANANES*†, R. FAVRE*†, C. J. KOH‡§, A. ZALOSZYC¶, M. C. BRAUN§**, D. R. ROTH‡§, R. MOOG††, F. BECMEUR††, M. A. BELFORT§‡‡ and R. RUANO§‡‡

**Department of Obstetrics and Gynecology, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; †INSERM, UMR-S 1121, Biomatériaux et Bioingénierie, Strasbourg, France; ‡Division of Pediatric Urology, Baylor College of Medicine, Houston, TX, USA; §Texas Children's Fetal Center, Texas Children's Hospital Pavilion for Women, Houston, TX, USA; ¶Department of Pediatrics, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; **Division of Pediatric Nephrology, Baylor College of Medicine, Houston, TX, USA; ††Department of Pediatric Surgery, Hôpitaux Universitaires de Strasbourg, Strasbourg, France; ‡‡Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, USA*

KEYWORDS: fetal cystoscopy; fetal surgery; fistula; lower urinary tract obstruction; urethral valves

ABSTRACT

Objective *To describe the surgical technical aspects associated with the development of urological fistulas after fetal antegrade cystoscopic laser fulguration of the posterior urethral valves (PUV).*

Methods *The perioperative data for all fetal cystoscopies performed between January 2004 and August 2013 at three institutions in the USA, France and Brazil were reviewed, with particular emphasis on surgical technical aspects of the procedure and the complications encountered.*

Results *A total of 40 fetal cystoscopies were performed at the three institutions. Laser fulguration of the PUV was performed in 23 of these cases, with a survival rate of 60.9% (14/23) and normal renal function in 85.7% (12/14) of these infants. Urological fistulas were diagnosed postnatally in four (10%) newborns. The presence of fistulas was associated with a higher gestational age at diagnosis of PUV (*$P < 0.01$*) and with the use of semi-curved rather than curved sheaths (*$P < 0.01$*), the use of a diode laser (*$P < 0.01$*) and the use of higher laser power and energy (*$P < 0.01$ *and* $P < 0.01$*, respectively), as well as with less operator experience (*$P < 0.01$*) and with absence of fetal anesthesia/immobilization (*$P = 0.02$*).*

Conclusion *Urological fistulas are a severe complication of fetal cystoscopic laser fulguration of PUV and are associated with type, energy and power settings of the laser and instrumentation. The use of appropriate technique and proper training of the operator are necessary to perform this fetal intervention safely.*

INTRODUCTION

Fetal lower urinary tract obstruction (LUTO) occurs in approximately 2.2 of every 10 000 births[1]. Severe LUTO with bladder outlet obstruction is often diagnosed during the first trimester of pregnancy and is associated with high perinatal mortality due to pulmonary hypoplasia and severe renal impairment or damage[2–8]. Vesicoamniotic shunting is a treatment option for relief of the urinary obstruction associated with severe LUTO but this procedure is associated with complications such as migration, obstruction and displacement of the shunt tubing[9–12]. Antegrade fetal cystoscopy has theoretical advantages over vesicoamniotic shunting because it permits a more physiological drainage of the obstructed bladder and enables an endoscopic examination of the dilated posterior urethra to help determine the etiology of the obstructive uropathy (posterior urethral valves (PUV), prune belly syndrome or urethral atresia)[13–24]. Another potential clinical advantage of fetal cystoscopy as compared to *in-utero* vesicoamniotic shunting is avoidance of amnioinfusion, which is often needed for shunting, and its well-described risks[16,22–24]. Promising results of fetal cystoscopic laser fulguration of PUV have been described in the past, but in a limited number of patients[16,22–24]. The objective of the present study was to review our perioperative experience and the complications involved with fetal cystoscopic laser fulguration of PUV at three centers in the USA, France and Brazil, with particular emphasis on the surgical technical aspects of the procedure.

SUBJECTS AND METHODS

We reviewed perioperative data for all male fetuses that underwent fetal cystoscopic laser fulguration of

Correspondence to: Dr R. Ruano, Pavilion for Women, Texas Children's Fetal Center, 6651 Main Street, Suite F1020, Houston, TX 77030, USA (e-mail: ruano@bcm.edu; rodrigoruano@hotmail.com)

Accepted: 25 April 2014

PUV at three tertiary referral centers (Sao Paulo, SP, Brazil; Strasbourg, Alsace, France; Houston, Texas, USA) between January 2004 and August 2013. All data were prospectively collected. The local Institutional Fetal Therapy Boards and Institutional Review Board approved the fetal interventions and the clinical study, respectively.

In Brazil, the fetal cystoscopy technique used has been described previously[21,23,24]. Maternal anesthesia was achieved using an epidural or local skin anesthesia. Fetal anesthesia was performed in every case by injecting fentanyl (15 μg/kg) and pancuronium (2 mg/kg) into the umbilical vein or fetal arms or legs, with ultrasound guidance, using a 22-gauge needle. Inclusion criteria for fetal cystoscopy have been reported previously and are very restrictive: (1) singleton pregnancy, (2) severe LUTO confirmed on detailed fetal ultrasound examination, including an extremely dilated bladder with increased wall thickness ('megabladder') associated with a dilated urethra ('keyhole sign'), bilateral hydroureters and severe bilateral hydronephrosis, (3) no additional fetal malformations observed by normal targeted ultrasound examination and fetal echocardiography, (4) anhydramnios or severe oligohydramnios after 18 weeks of gestation, (5) gestational age less than 26 weeks, (6) favorable fetal urinary analysis (sodium < 100 mEq/L, chloride < 90 mEq/L, osmolarity < 210 mOsm/L and β-2-microglobulin < 6 mg/dL) and (7) normal fetal karyotype[21,23–25]. Fetal cystoscopy was performed by R.R. (Operator 1) in 29 fetuses that met all inclusion criteria (findings in 19 fetuses were previously reported)[19,21,24,25], using a 1.0-mm fetoscope (11 510 A, Karl Storz, Tuttlingen, Germany) with a 2.2-mm custom curved sheath. When PUV were identified ($n = 17$), the valves were fulgurated by pulsed shots of an Nd:YAG laser (SmartEpil, DEKA, Florence, Italy) using a maximal setting of 30 Watts and 100 Joules and, before 20 weeks' gestation, a 400-μm or, after 20 weeks' gestation, a 600-μm contact laser fiber. When urethral atresia ($n = 11$) or urethral stenosis ($n = 1$) was identified, no laser fulguration was performed. Among the 11 cases with urethral atresia, the pregnancy was medically terminated in eight patients; two infants died after birth because of severe pulmonary hypoplasia and one fetus died *in utero*. The fetus diagnosed with urethral stenosis was treated during fetal cystoscopy with a transurethral catheter placement; the infant was doing well with normal renal function when the case was reported[19,21,24,25].

In France, maternal anesthesia was conducted using local skin anesthesia and fetal anesthesia was not performed, as is usual for this center. Fetal cystoscopy was performed in six fetuses (not reported previously) by R.F. (Operator 2) using a 1.3-mm fetoscope (11 540 AA, Karl Storz) and a 2.7-mm semi-curved sheath (11 540 KB, Karl Storz). Laser fulguration was performed when PUV were identified ($n = 5$; one fetus was diagnosed by fetal cystoscopy with urethral atresia and the pregnancy was terminated medically at 16.1 weeks; the diagnosis of urethral atresia was confirmed postnatally) using a multibeam diode laser (Dornier Medtech, Kennesaw, GA, USA) at settings of 30–40 Watts and 800–1000 Joules, continuous fire of 2 s, and a 600-μm contact laser fiber.

In the USA, the procedure was performed in five fetuses under maternal local anesthesia and fetal intramuscular anesthesia, using a 2.0-mm fetoscope (26120BA, Karl Storz, Germany) and 3.3-mm semi-curved sheath (26120 KB, Karl Storz, Germany) by R.R. and M.A.B. (Operator 3). Fetal cystoscopic laser ablation of the PUV was performed in only one fetus, by R.R. (Operator 1) using a multibeam diode laser (Dornier Medtech) at settings of 30–40 Watts and 800–1100 Joules, continuous fire of 2 s, and a 600-μm contact laser fiber. The semi-curved sheath was used instead of the curved sheath which has not received regulatory approval in the USA. In four fetuses it was not possible to identify the bladder obstruction precisely and, therefore, laser fulguration was not performed (Operator 3). These fetuses required multiple vesicoamniotic shunt placements as two had PUV and two had prune belly syndrome; they have survived with normal renal function up to the time of writing.

Surgical complications such as fistulas, fetal bleeding or fetal demise that were related to fetal intervention were evaluated. The following technical aspects were analyzed: gestational age at procedure, laser technique (type, fiber type, power, energy, pulsed *vs* continuous, contact *vs* non-contact laser fiber) and type of fetoscope and sheath.

Statistical analysis was performed using IBM SPSS for Windows version 20 (IBM Inc., Armonk, NY, USA). Fisher's exact test, a chi-square test and the Mann–Whitney *U*-test were used for comparisons between the groups (surgical complications *vs* non-surgical complications). $P < 0.05$ was considered statistically significant.

RESULTS

A total of 40 fetal cystoscopies were performed in the three centers (29 procedures in Brazil, six in France and five in the USA). The mean gestational age at diagnosis of PUV and at fetal cystoscopy was 13.9 ± 2.8 weeks and 18.6 ± 3.4 weeks, respectively. Laser fulguration of PUV was performed in 23 (57.5%) fetuses, with a survival rate of 60.9% (14/23 infants). Among the 14 that had fetal cystoscopic laser ablation of the PUV and survived, normal renal function was observed in 12 (85.7%). Urological fistulas were observed in four (10.0%) cases that underwent fetal cystoscopy (Table 1), but no cases of fetal bleeding or demise or other complications were noted. The first three fetuses had urethrocutaneous fistulas (in Case 2 the pregnancy was terminated at 20 weeks' gestation and the other two infants required postnatal surgical repair) and the last case had a low urethrorectal fistula that was managed with the use of urethral catheter drainage and closed spontaneously. Among the patients who underwent fetal cystoscopic laser ablation of PUV ($n = 23$), termination of pregnancy was performed in four (17.4%) patients because of an associated anomaly revealed after fetal cystoscopy ($n = 2$), persistent severe

Table 1 Characteristics and follow-up of fetuses that developed a urological fistula after fetal cystoscopic laser fulguration of posterior urethral valves (PUV)

	Case			
Characteristic	*1*	*2*	*3*	*4*
City	Strasbourg	Strasbourg	Strasbourg	Houston
Maternal age (years)	25	34	19	23
GA at diagnosis of PUV (weeks)	16.5	15.6	24.4	20.0
Megacystis in first trimester	Y	Y	N	N
Megabladder in second trimester	Y	Y	Y	Y
Keyhole sign	Y	Y	Y	N
Bilateral hydronephrosis	Y	Y	Y	Y
Amniotic fluid volume before cystoscopy	Normal	Normal	Normal	Anhydramnios
Urinary sodium (mEq/L)	119	123	57	NA
Urinary chloride (mEq/L)	113	NA	108	NA
GA at cystoscopy (weeks)	16.5	15.6	24.4	29.0
Diagnosis at cystoscopy	PUV	PUV	PUV	Partial PUV
Laser fulguration	Y	Y	Y	Y
Surgical aspects	1.3-mm fetoscope; 2.7-mm semi-curved operating sheath; 600-μL laser fiber; multibeam diode*	1.3-mm fetoscope; 2.7-mm semi-curved operating sheath; 600-μL laser fiber; multibeam diode*	1.3-mm fetoscope; 2.7-mm semi-curved operating sheath; 600-μL laser fiber; multibeam diode*	2.0-mm fetoscope; 3.5-mm semi-curved operating sheath; 600-μL laser fiber; multibeam diode*
Touching laser	Y	Y	Y	Y
Power (Watts)	40	40	40	35
Energy (Joules)	1000	1000	1000	1100
Laser fire	Continuous	Continuous	Continuous	Continuous
GA at delivery (weeks)	36.5	20	34.2	30.0
Postnatal diagnosis	PUV	—	PUV	PUV
Complications of laser fulguration	Repeat procedure at 24 weeks; urethrorectal-cutaneal fistula and atrophy of gluteal muscle	Urethrocutaneous fistula; perineal defect and limb atrophy (TOP)	Urethrocutaneous fistula	Preterm delivery at 30 weeks; low urethrorectal fistula
Follow-up	Alive at 2 months; fistula closed surgically; temporary vesicotomy and colostomy performed	TOP	Alive at 1 month; vesicocutaneous fistula diagnosed postnatally and treated by bladder catheter drainage (urethral)	Alive at 4 months; fistula closed spontaneously with use of urethral catheter drainage; infant discharged home at 4 months
Renal function	Normal (creatinine, 0.7 mg/dL)	—	Normal (creatinine, 0.6 mg/dL)	Abnormal but dialysis not needed† (creatinine, 2.9 mg/dL)

*Dornier Medtech, Kennesaw, GA, USA. †At time of writing. GA, gestational age; N, no; NA, not available; TOP, termination of pregnancy; Y, yes.

oligohydramnios ($n = 1$; Case 6 from Ruano *et al.*[21]) or a urological fistula ($n = 1$; Case 2 of the present study) (Table 1 and Figures 1–5).

Fetuses with a urological fistula after fetal cystoscopic laser fulguration of PUV were noted to have a higher gestational age at the diagnosis of PUV when compared to those without a fistula ($P < 0.01$). In addition, a higher incidence of urological fistulas was associated with the use of semi-curved instruments ($P < 0.01$), the use of a multibeam diode laser ($P < 0.01$), higher laser power settings ($P < 0.01$), higher laser energy settings ($P = 0.01$) as well as with less operator experience in fetal cystoscopy ($P < 0.01$) and with absence of fetal anesthesia/immobilization ($P = 0.02$) (Table 2).

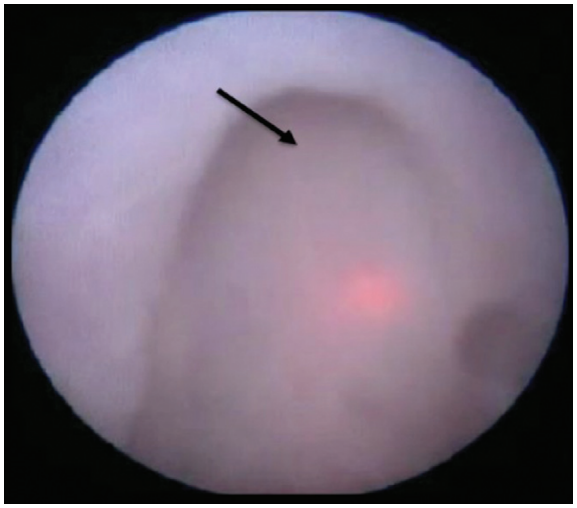


Figure 1 Case 1: cystoscopic view of posterior urethral valves (arrow).

DISCUSSION

For treatment of fetal LUTO, fetal cystoscopy is considered a potential option, since vesicoamniotic shunting has been associated with limited short-term effectiveness[10,12,26]. Antegrade fetal cystoscopy has potential advantages over vesicoamniotic shunting, including improvement in distinguishing between PUV, prune belly syndrome and urethral atresia during the fetal period, physiological relief of the urinary obstruction via the urethra with restoration of normal bladder cycling and avoidance of amnioinfusion prior to the procedure[16,21,24]. However, fetal cystoscopy is more complex technically and usually requires special instrumentation and multidisciplinary training[23]. As with all surgical procedures, this procedure is associated with potential complications.

To our knowledge, this study is the first to report complications (urethrorectal thermal fistula) after fetal cystoscopic laser fulguration of PUV. The fistulas varied in severity and may eventually impact the postnatal course of these patients. Since the urethrorectal fistulas were associated with technical issues related to available equipment, the goal of the present report was to identify the risks related to this novel procedure that might be ameliorated in the future, with the use of more appropriate instruments and by assuring that operators have an adequate level of training and that the selection of candidates for this advanced fetal intervention is appropriate.

The use of a true-curved sheath, as opposed to a straight sheath that was bent into a semi-curved shape, led to a statistically significant difference in fistula formation in this small series of patients. A manufactured curved sheath was available in Brazil but not in the USA due to regulatory controls. For access to the dilated posterior urethra and the urethral obstruction, this sheath was able to adapt to the curved anatomy of the vesicourethral

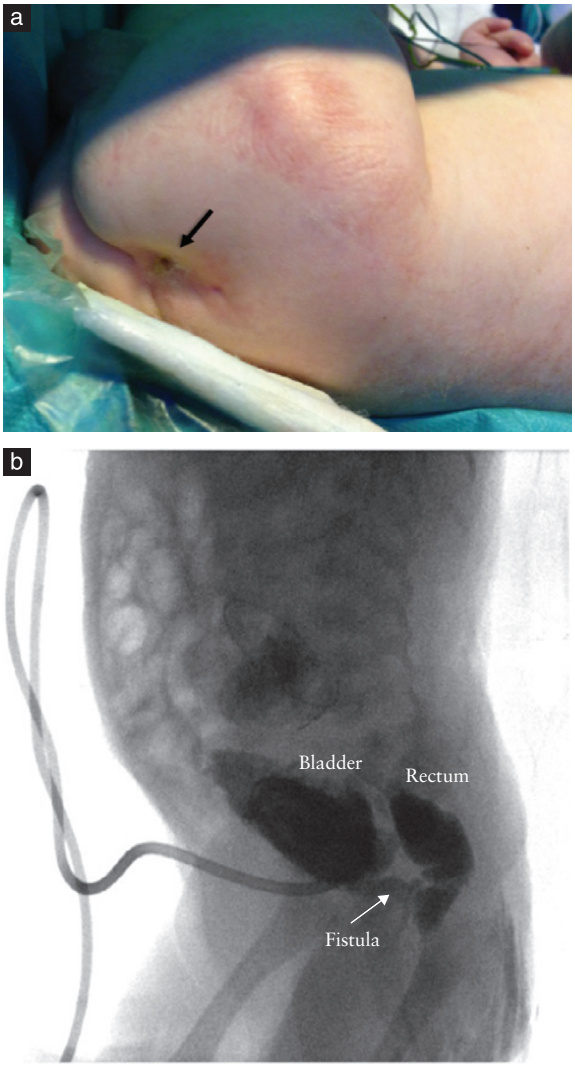


Figure 2 (a) Image showing perineal fistula (arrow) on the back of a newborn (Case 1) that underwent fetal laser fulguration of posterior urethral valves. (b) Cystography of same newborn showing the urethrorectal fistula.

angle, allowing access to the bladder neck and valves. The modified straight sheath did not gain the necessary exposure to the posterior urethra and jeopardized the procedure by forcing the laser beam more posterior to the dorsal aspect of the verumontanum. We believe that the use of the curved fetal cystoscopy sheath, available in Brazil, would have allowed for a greater chance of success. Analysis of sheath type would be an ideal project for the FDA P50 Pediatric Medical Device Consortia program, of which over 200 pediatric medical-device projects have been assisted over the past 4 years[27].

Other technical issues associated with an increased rate of fistulas included the use of a diode laser which fulgurates a surface area larger than that of the Nd:YAG laser. The most important technical aspects determined were the power and energy settings, which should be

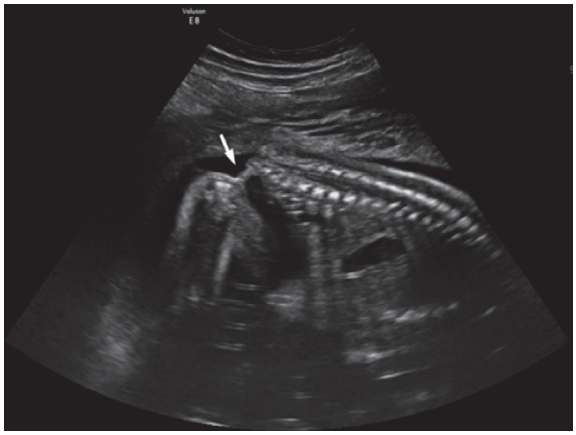


Figure 3 Case 2: ultrasound image revealing a perineal defect in fetal rectal area at 20 + 5 weeks' gestation.

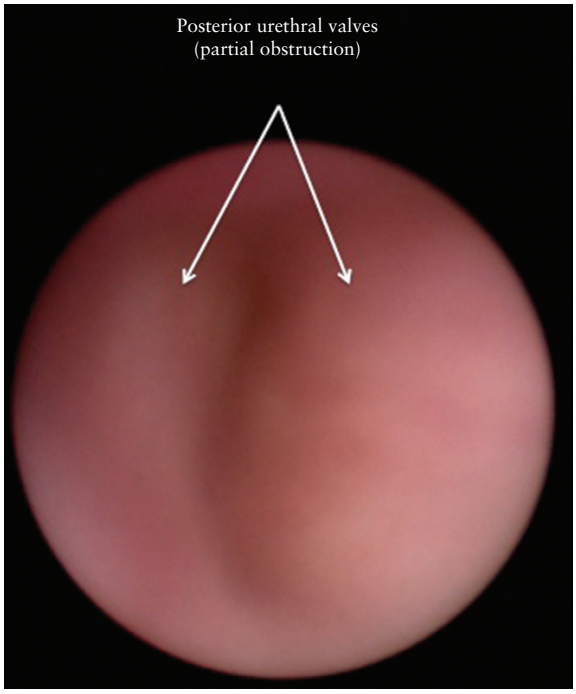


Figure 4 Case 4: fetal antegrade cystoscopic view of posterior urethral valves.

adjusted to the lowest possible levels to ablate the valves, thereby decreasing the chance of collateral iatrogenic peripheral tissue damage. This is also crucial to avoid burning the distal tissues, such as skin and muscles. In our opinion, this was the major factor related to the severity of the iatrogenic fistula (urethrorectal-cutaneous fistula with muscle atrophy in Cases 1 and 2). Future studies are necessary to investigate the possibility of other non-laser techniques, such as the use of microscissors, guidewires and balloon disruption.

The absence of fetal anesthesia/immobilization was associated statistically with the presence of urological

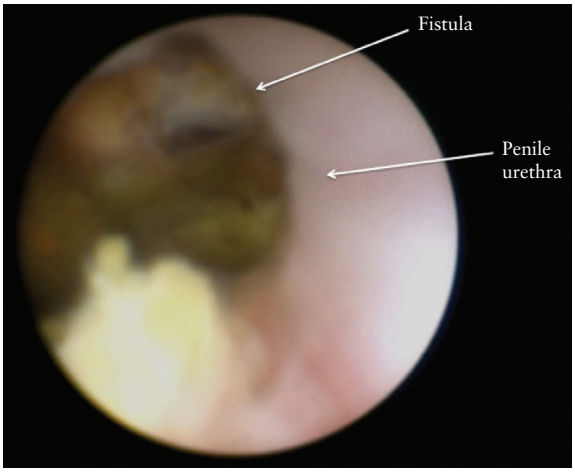


Figure 5 Case 4: postnatal cystoscopic view of urethrorectal fistula.

fistulas. The present study highlights the importance of sedating and immobilizing the fetus, even at gestational ages before 18 weeks. Our hypothesis is that any fetal movement can lead to displacement of the bladder outlet obstruction, which may cause iatrogenic fulguration of the surrounding tissue of the valves.

The selection of appropriate candidates for fetal cystoscopy can help to reduce the risk of complications. In Case 4, there was no 'keyhole sign' and no convincing evidence of posterior urethra dilation on prenatal ultrasonography, despite an enlarged bladder and bilateral hydronephrosis (signs of LUTO). The 'keyhole sign' is an important marker for LUTO, but with lower specificity for PUVs[28]. An attempt to fulgurate presumed valves in the urethra by laser led to iatrogenic fulguration of the verumontanum and a resulting urethrorectal fistula because the urethral obstruction was distally located, leading to an extremely difficult visualization of this point.

The optimal gestational age to perform fetal cystoscopic laser ablation of PUV has yet to be determined. Previous studies have suggested that early fetal cystoscopy at 16 weeks' gestation may help to prevent future renal impairment. When fetal cystoscopy is performed earlier (at 16–18 weeks), as opposed to later in gestation, we have noted a less acute angle of the vesicourethral junction but precise anatomic studies are not available. However, size of the fetus, bladder and the urethra at that early stage of gestation render fetal cystoscopy a more challenging procedure.

In the present series, occurrence of urological fistulas was associated with the operator. Operator 1 had performed 30 fetal cystoscopies with 18 fetal cystoscopic laser ablations of PUV and one case of urological fistula, while Operator 2 had performed five cystoscopies, with three cases of urological fistula. It seems that urological complications can be related to a less experienced operator, specifically in fetal cystoscopy. However, Operator 2 has more than 30 years of experience with other types of fetal surgery and, in addition,

Table 2 Comparison between fetuses that had a urological fistula *vs* those that did not after fetal cystoscopic laser fulguration of the posterior urethral valves (PUV)

Characteristic	*Urological fistula (n = 4)*	*No complication (n = 19)*	P
Gestational age at diagnosis of PUV (weeks)	18 (16–24)	13 (11–20)	< 0.01
Megacystis in first trimester	2 (50.0)	17 (89.5)	0.13
Megabladder in second trimester	4 (100)	19 (100)	1
Absence of keyhole sign	1 (25)	0 (0)	0.17
Bilateral hydronephrosis	4 (100)	19 (100)	1
Oligohydramnios/anhydramnios	1 (25.0)	12 (63.2)	0.28
Gestational age at fetal cystoscopy	20.0 (15.6–29.0)	19.0 (16.0–22.0)	0.36
Operator performing surgery			
1	1	17	< 0.01
2	3	2	
Fetal anesthesia/immobilization	1 (25)	17 (89.5)	0.02
Fetoscopic sheath			
Curved	0 (0)	17 (89.5)	< 0.01
Semi-curved	4 (100)	2 (11.1)	
Laser fiber			
400 µm	0 (0)	9 (47.4)	0.13
600 µm	4 (100)	10 (52.6)	
Laser type			
Diode	4 (100)	2 (10.5)	< 0.01
ND:YAG*	0 (0)	17 (89.5)	
Touching fulguration	4 (100)	19 (100)	1
Power (Watts)	40.0 (35.0–40.0)	30.0 (25.0–40.0)	< 0.01
Energy (Joules)	1000 (1000–1100)	100 (90–1000)	< 0.01

Data are given as median (range) or *n* (%). *SmartEpil, DEKA, Florence, Italy.

other variables were present in cases performed by Operator 2, such as semi-curved sheath, diode laser, higher laser energy and absence of fetal anesthesia. The major limitation of the present study was the very small sample size that did not allow for investigation of the independence of the factors causing urological fistulas.

In conclusion, urological fistulas can occur after fetal cystoscopic laser ablation of PUV if (1) an inappropriate fetoscopic sheath is used, precluding the correct angle to assess the fetal bladder outlet obstruction, (2) higher laser power and energy are applied, leading to iatrogenic ablation of the surrounding tissue, (3) absence of fetal anesthesia/immobilization, (4) experience of the operator with fetal cystoscopy is insufficient. Our group recommends strict adherence to the protocol previously described in order to maintain consistency and avoid complications[19,21,23–25].

ACKNOWLEDGMENT

This research was supported in part by FDA 1P50 FD004896-01 (awarded to C.J.K.).

REFERENCES

1. Anumba DO, Scott JE, Plant ND, Robson SC. Diagnosis and outcome of fetal lower urinary tract obstruction in the northern region of England. *Prenat Diagn* 2005; **25**: 7–13.
2. Favre R, Kohler M, Gasser B, Muller F, Nisand I. Early fetal megacystis between 11 and 15 weeks of gestation. *Ultrasound Obstet Gynecol* 1999; **14**: 402–406.
3. Liao AW, Sebire NJ, Geerts L, Cicero S, Nicolaides KH. Megacystis at 10–14 weeks of gestation: chromosomal defects and outcome according to bladder length. *Ultrasound Obstet Gynecol* 2003; **21**: 338–341.
4. Sepulveda W. Megacystis in the first trimester. *Prenat Diagn* 2004; **24**: 144–149.
5. Kari JA, El-Desoky S, Farag Y, Mosli H, Altyieb AM, Al Sayad A, Radawi O, Ghabra H, Basnawi F, Bahrawi O, Singh A, Farsi H. Renal impairment in children with posterior urethral valves. *Pediatr Nephrol* 2013; **28**: 927–931.
6. Wu S, Johnson MP. Fetal lower urinary tract obstruction. *Clin Perinatol* 2009; **36**: 377–390.
7. Morris RK, Malin GL, Khan KS, Kilby MD. Antenatal ultrasound to predict postnatal renal function in congenital lower urinary tract obstruction: systematic review of test accuracy. *BJOG* 2009; **116**: 1290–1299.
8. Lissauer D, Morris RK, Kilby MD. Fetal lower urinary tract obstruction. *Semin Fetal Neonatal Med* 2007; **12**: 464–470.
9. Morris RK, Kilby MD. An overview of the literature on congenital lower urinary tract obstruction and introduction to the PLUTO trial: percutaneous shunting in lower urinary tract obstruction. *Aust N Z J Obstet Gynaecol* 2009; **49**: 6–10.
10. Morris RK, Khan KS, Kilby MD. Vesicoamniotic shunting for fetal lower urinary tract obstruction: an overview. *Arch Dis Child Fetal Neonatal Ed* 2007; **92**: F166–168.
11. Biard JM, Johnson MP, Carr MC, Wilson RD, Hedrick HL, Pavlock C, Adzick NS. Long-term outcomes in children treated by prenatal vesicoamniotic shunting for lower urinary tract obstruction. *Obstet Gynecol* 2005; **106**: 503–508.
12. Morris RK, Malin GL, Quinlan-Jones E, Middleton LJ, Hemming K, Burke D, Daniels JP, Khan KS, Deeks J, Kilby MD. Percutaneous vesicoamniotic shunting in Lower Urinary Tract Obstruction (PLUTO) Collaborative Group. Percutaneous vesicoamniotic shunting versus conservative management for fetal lower urinary tract obstruction (PLUTO): a randomised trial. *Lancet* 2013; **382**: 1496–1506.
13. Quintero RA, Shukla AR, Homsy YL, Bukkapatnam R. Successful in utero endoscopic ablation of posterior urethral

valves: a new dimension in fetal urology. *Urology* 2000; **55**: 774.
14. Agarwal SK, Fisk NM. In utero therapy for lower urinary tract obstruction. *Prenat Diagn* 2001; **21**: 970–976.
15. Quintero RA, Homsy Y, Bornick PW, Allen M, Johnson PK. In-utero treatment of fetal bladder-outlet obstruction by a ureterocele. *Lancet* 2001; **357**: 1947–1948.
16. Welsh A, Agarwal S, Kumar S, Smith RP, Fisk NM. Fetal cystoscopy in the management of fetal obstructive uropathy: experience in a single European centre. *Prenat Diagn* 2003; **23**: 1033–1041.
17. Hofmann R, Becker T, Meyer-Wittkopf M, Tekesin I, Sierra F, Schmidt S. Fetoscopic placement of a transurethral stent for intrauterine obstructive uropathy. *J Urol* 2004; **171**: 384–386.
18. Clifton MS, Harrison MR, Ball R, Lee H. Fetoscopic transuterine release of posterior urethral valves: a new technique. *Fetal Diagn Ther* 2008; **23**: 89–94.
19. Ruano R, Pimenta EJ, Duarte S, Zugaib M. Four-dimensional ultrasonographic imaging of fetal lower urinary tract obstruction and guidance of percutaneous cystoscopy. *Ultrasound Obstet Gynecol* 2009; **33**: 250–252.
20. Quintero RA, Gomez Castro LA, Bermudez C, Chmait RH, Kontopoulos EV. In utero management of fetal lower urinary tract obstruction with a novel shunt: a landmark development in fetal therapy. *J Matern Fetal Neonatal Med* 2010; **23**: 806–812.
21. Ruano R, Duarte S, Bunduki V, Giron AM, Srougi M, Zugaib M. Fetal cystoscopy for severe lower urinary tract obstruction--initial experience of a single center. *Prenat Diagn* 2010; **30**: 30–39.
22. Morris RK, Ruano R, Kilby MD. The effectiveness of fetal cystoscopy as a diagnostic and therapeutic intervention for lower urinary tract obstruction: a systematic review. *Ultrasound Obstet Gynecol* 2011; **37**: 629–637.
23. Ruano R. Fetal surgery for severe lower urinary tract obstruction. *Prenat Diagn* 2011; **31**: 667–674.
24. Ruano R, Yoshisaki CT, Salustiano EM, Giron AM, Srougi M, Zugaib M. Early fetal cystoscopy for first-trimester severe megacystis. *Ultrasound Obstet Gynecol* 2011; **37**: 696–701.
25. Ruano R, Yoshizaki CT, Giron AM, Srougi M, Zugaib M. Fetal cystoscopic placement of transurethral stent in a fetus with urethral stenosis. *Ultrasound Obstet Gynecol* 2014; **44**: 238–240.
26. Morris RK, Malin GL, Khan KS, Kilby MD. Systematic review of the effectiveness of antenatal intervention for the treatment of congenital lower urinary tract obstruction. *BJOG* 2010; **117**: 382–390.
27. Ulrich LC, Joseph FD, Lewis DY, Koenig RL. FDA's pediatric device consortia: national program fosters pediatric medical device development. *Pediatrics* 2013; **131**: 981–985.
28. Bernardes LS, Aksnes G, Saada J, Masse V, Elie C, Dumez Y, Lortat-Jacob SL, Benachi A. Keyhole sign: how specific is it for the diagnosis of posterior urethral valves? *Ultrasound Obstet Gynecol* 2009; **34**: 419–423.

8.3. Myéloméningocèle

8.3.1. Protocole de foetoscopie au gaz chez le singe

Demande d'Autorisation de Projet utilisant des Animaux à des Fins Scientifiques

Ce formulaire a pour objectif de rassembler les informations permettant au comité d'éthique dont relève l'établissement utilisateur où sera réalisé le projet utilisant des animaux à des fins scientifiques, d'évaluer éthiquement le projet et au Ministère de l'Enseignement Supérieur et de la Recherche d'autoriser le projet suite à l'évaluation éthique.

1. INFORMATIONS GÉNÉRALES

1.1. TITRE DU PROJET :

Évaluation des effets de la foetoscopie au gaz (CO_2 et Hélium) sur la physiologie cardio-respiratoire et l'état acido-basique materno-foetal, dans le modèle du macaque

1.2. Durée du projet :

5 ans

1.3. Date prévue de début du projet :

Dès que possible

2. RÉSUMÉ NON TECHNIQUE

La chirurgie mini-invasive du fœtus par voie endoscopique est en plein essor grâce à la miniaturisation des fibres optiques et le développement de l'instrumentation chirurgicale. Ainsi certaines pathologies (syndrome transfuseur-transfusé, hernie de coupole diaphragmatique et myéloméningocèle) peuvent bénéficier d'une prise en charge anténatale, ce qui permet de diminuer la morbidité voire la mortalité néonatale.

Concernant la chirurgie prénatale de la myéloméningocèle, la foetoscopie reste cependant une approche difficile car elle implique la réalisation de dissections et de sutures en milieu liquidien (le liquide amniotique). Certaines équipes ont proposé de remplacer, le temps de la chirurgie, une partie du liquide amniotique par du gaz. Un total de vingt-six cas de réparation foetoscopique de myéloméningocèle avec insufflation de CO_2 a été publié à ce jour. Cette nouvelle

technique permet d'optimiser et de faciliter la chirurgie car l'intervention se déroule en milieu aérien (comme une coelioscopie classique) plutôt qu'en milieu liquidien.

Cette nouvelle approche constitue donc potentiellement un nouveau paradigme dans le domaine de la chirurgie fœtale, mais la sécurité de l'insufflation d'un gaz dans l'utérus pendant la grossesse doit encore être démontrée. Le CO_2 est le gaz le mieux connu et le plus utilisé en coelioscopie. Cependant, il pourrait entraîner entre autres une hypercapnie voire une acidose chez le fœtus. L'Hélium pourrait être une alternative car sans effet sur l'équilibre acido-basique mais il est moins soluble que le CO_2, ce qui peut poser problème en cas d'absorption vasculaire.

Des expérimentations animales sont nécessaires pour évaluer les effets de la foetoscopie au gaz sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal. Certaines évaluations ont déjà été conduites chez la brebis gestante mais elles ne sont malheureusement pas extrapolables à l'humain car la placentation chez la brebis est syndesmo-choriale et non hémo-choriale, c'est-à-dire que, contrairement à l'humain, il n'y a pas de contact entre le sang maternel et l'épithélium chorial. D'où l'intérêt du modèle du macaque, chez qui la placentation est de type hémo-choriale et très proche de celle de l'humain. L'équipe partenaire a développé la foetoscopie chez le macaque depuis 2009 et a aujourd'hui l'expérience la plus importante de ce modèle au niveau mondial.

L'objectif est d'évaluer les effets de la fœtoscopie au gaz (CO_2 et Hélium) sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal, dans le modèle du macaque.

L'intervention consistera en une simple fœtoscopie dans le troisième tiers de la gestation. Il n'y aura pas de geste chirurgical réalisé sur le fœtus mais seulement la voie d'abord elle-même, c'est-à-dire l'introduction de l'endoscope dans la cavité utérine. Une insufflation de gaz dans la cavité utérine sera réalisée pendant 2 heures et les données pertinentes seront collectées chez la mère et le fœtus, en particulier pour ce qui concerne l'état acido-basique.

La femelle gestante est remise dans son groupe social dès le lendemain. L'utilisation de l'endoscopie, les protocoles d'anesthésie et d'analgésies (aussi bien généraux que locaux) permettront de réduire au minimum le mal être de l'animal en raffinant (Raffinement) au maximum chaque étape du protocole.

Une série exploratoire avec la collection de données sur 16 interventions (8 avec insufflation au CO_2 et 8 avec insufflation à l'Hélium) sera réalisée dans un premier temps, et donnera lieu à une analyse avant d'envisager d'autres éventuelles interventions, dans le but de réduire (Reduction) autant que possible le nombre d'animaux nécessaires. Les animaux utilisés lors de ces interventions seront des macaques cynomolgus (Macaca fascicularis, 16 animaux : 8 adultes et 8 foetus) et des macaques rhésus (Macaca mulatta, 16 animaux : 8 adultes et 8 foetus).

Le projet nécessitant l'utilisation d'animaux vivants afin de considérer l'impact de cette méthode sur leur organisme, il n'est pas possible d'utiliser ni des programmes informatiques, ni des cultures cellulaires (Remplacement).

3. INFORMATIONS ADMINISTRATIVES ET RÉGLEMENTAIRES

3.1. L'établissement utilisateur (EU)

3.1.1. Agrément de l'EU où seront utilisés les animaux :

- Nom: SILABE- ADEUIS

3.1.2. Comité d'éthique agréé par le MESR dont relève l'EU :

CREMEAS Strasbourg

3.2. Le personnel

- **Compétences des personnes participant au projet :**

  - **la conception des procédures expérimentales et des projets** ☒ **oui** ○ **non**
  - **l'application de procédures expérimentales aux animaux** ☒ **oui** ○ **non**
  - **les soins aux animaux** ☒ **oui** ○ **non**
  - **la mise à mort des animaux** ☒ **oui** ○ **non**

3.3. Le projet

3.3.1. L'objectif du projet est-il :

- **justifié du point de vue éducatif ?** ○
- **requis par la loi ?** ○
- **justifié du point de vue scientifique ?** **X**

Informations concernant cette (ces) justification(s) :

Ce projet est financé par une dotation MIGAC (Missions d'Intérêt Général et d'Aide à la Contractualisation) sous la forme d'une dotation annuelle.

3.3.2. Description du projet :

L'objectif est d'évaluer les effets de la fœtoscopie au gaz (CO_2 et Hélium) sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal, dans le modèle du macaque.

Les macaques (rhésus et cynomolgus) femelles gestantes seront opérés au troisième tiers de la gestation. Le moment de la chirurgie sera déterminé par une mesure échographique du diamètre biparietal en début de gestation (Voluson E GE ®).

L'intervention consistera en une simple fœtoscopie. Il n'y aura pas de geste chirurgical réalisé sur le fœtus mais seulement la voie d'abord elle-même, c'est-à-dire l'introduction de l'endoscope dans la cavité utérine. Une insufflation de gaz dans la cavité utérine sera réalisée pendant 2 heures et les données pertinentes seront collectées chez la mère et le fœtus, en particulier pour ce qui concerne l'état acido-basique.
La description précise de la procédure expérimentale est rapportée dans le paragraphe 4.2.1.

Les paramètres de surveillance recueillis toutes les 20 minutes pendant les 2 heures d'insufflation de gaz (0-20-40-60-80-100-120) seront les suivants :

- Au niveau fœtal : fréquence cardiaque, échographie cardiaque (pour évaluer la fonction cardiaque), gazométrie veineuse (ou capillaire) et lactates veineux (ou capillaires).

- Au niveau maternel : fréquence cardiaque, tension artérielle, fréquence respiratoire et gazométrie dans l'air expiré, température, échographie cardiaque (pour surveiller l'absence de bulles de gaz), gazométrie artérielle, lactates et électrolytes.

- Au niveau utérin : pression utérine, débit et volume de gaz instillé.

A l'issue de l'intervention, les femelles gestantes resteront en isolement et seront surveillées jusqu'au matin suivant où elles seront remises en groupe social.

3.3.3. Précisez, le cas échéant, la (ou les) méthode(s) de mise à mort prévue(s) :

Pas de mise à mort prévue

3.3.4. Précisez, le cas échéant, les éléments scientifiques justifiant la demande de dérogation concernant la méthode de mise à mort envisagée :

NA

3.3.5. Stratégie d'expérimentation ou d'observation et approche statistique utilisée afin de réduire au minimum le nombre d'animaux, la douleur, la souffrance et l'angoisse, infligées et l'impact environnemental, le cas échéant – si une étude statistique est prévue, indiquez et justifiez les tests choisis :

Le geste opératoire est réalisé sous anesthésie générale de l'animal. Un anesthésique locale est ajouté au niveau de la zone chirurgicale. La technique en elle-même (endoscopie) est à la fois peu invasive et peu douloureuse. Il n'y a pas de nécessité d'isoler l'animal de son groupe avant ce protocole chirurgical ce qui limite fortement le stress de l'animal.
Une anesthésie foetale est également réalisée de manière à limiter la douleur liée à la ponction veineuse.

Afin d'obtenir des résultats significatifs, 6 femelles gestantes par groupe (Hélium/CO2) sont prévues. Par mesure de précaution, 8 animaux par groupe sont prévus au cas où il faille stopper une ou plusieurs interventions en cours de recueil de données.
Il s'agit d'une série exploratoire avec la collection de données sur 16 interventions (8 avec insufflation au CO_2 et 8 avec insufflation à l'Hélium) dans un premier temps.
Nous prévoyons donc un nombre de cas limité, à l'issue desquels nous réaliserons une analyse afin de déterminer la légitimité ou non de réaliser d'autres interventions.
Cette démarche a pour but de réduire autant que possible le nombre d'animaux nécessaires.

3.4. Les animaux

3.4.1. Justifiez d'avoir recours à des animaux pour atteindre les objectifs du projet :

L'utilisation de l'animal permet d'évaluer la sécurité de la pratique de la fœtoscopie avec insufflation de gaz, avant d'étendre cette technique chez l'humain.

3.4.2. Espèces animales ou types d'animaux utilisés :

Singe cynomolgus (*Macaca fascicularis*)	X
Singe rhésus (*Macaca mulatta*)	X

3.4.3. Justifiez la pertinence de l'(des) espèce(s) choisie(s) :

Des expérimentations animales sont nécessaires pour évaluer les effets de la fœtoscopie au gaz sur la physiologie cardio-respiratoire et l'état acido-basique materno-fœtal. Certaines évaluations ont déjà été conduites chez la brebis gestante mais elles ne sont malheureusement pas extrapolables à l'humain car la placentation chez la brebis est syndesmo-choriale et non hémo-choriale, c'est-à-dire que, contrairement à l'humain, il n'y a pas de contact entre le sang maternel et l'épithélium chorial.
Le modèle macaque est le seul modèle de taille suffisante possédant une placentation hémo-choriale comme chez l'humain.
L'équipe partenaire a développé la fœtoscopie chez le macaque depuis 2009 et a aujourd'hui l'expérience la plus importante de ce modèle au niveau mondial.

3.4.4. S'agit-il de spécimens d'espèces menacées énumérées à l'annexe A du règlement (CE) n° 338/97 du Conseil du 9 décembre 1996 relatif à la protection des espèces de faune et de flore sauvages par le contrôle et leur commerce ?

○oui X non

3.4.5. S'agit-il de spécimens de primates non humains ? **X oui ○non**

Si oui, éléments scientifiques démontrant que la finalité de la procédure expérimentale ne peut être atteinte en utilisant d'autres espèces de primates non humains

Le modèle primate non-humain est le seul modèle de taille suffisante possédant une placentation hémo-choriale comme chez l'humain.
Parmi les primates non-humains, les macaques ont une taille compatible avec ce projet. En effet, la taille du fœtus au troisième tiers de la grossesse est compatible avec la taille du fœtus humain lors de la réalisation de la chirurgie *in utero* de cure de myéloméningocèle au deuxième trimestre de la grossesse.

3.4.6. S'agit-il d'animaux capturés dans la nature ? **○oui X non**

3.4.7. S'agit-il d'animaux d'espèces domestiques, errants ou vivant à l'état sauvage ? **○ oui X non**

3.4.8. Catégorie des animaux utilisés dans le projet :

Animaux tenus en captivité (domestiques ou non domestiques)	**X**
Animaux non domestiques non tenus en captivité	☐
Animaux génétiquement altérés	☐

3.4.9. Origine des animaux tenus en captivité :

- **Les animaux destinés à être utilisés dans les procédures expérimentales appartenant aux espèces dont la liste est fixée réglementairement sont-ils élevés à cette fin et proviennent-ils d'éleveurs ou de fournisseurs agréés ?**

X oui
○non

- **Si oui, nombre d'établissements éleveur ou fournisseur agréés fournissant tout ou partie des animaux du projet :**

1 établissement fournisseur durant le projet

Etablissement :

- **Nom :**

Plateforme SILABE

- **Adresse postale :**

Fort Foch, 67207 Niederhausbergen

- **Animaux fournis :**

Tous

3.4.10. Nombre estimé d'animaux utilisés dans le projet :

32

- **Justification de ce nombre pour chacune des espèces animales utilisées :**

16 femelles gestantes (et donc 16 fœtus après le 3ème tiers de gestation) macaques cynomolgus ou rhésus seront utilisées lors de ce projet afin d'obtenir des données complètes sur au moins 6 individus par groupe. Soit 6 femelles gestantes et 6 foetus dans le groupe CO2 et 6 femelles gestantes et 6 foetus dans le groupe helium.
A priori, 8 femelles macaques rhésus et 8 femelles macaques fascicularis seront utilisées.

3.4.11. Indiquez à quel(s) stade(s) de développement les animaux seront utilisés et le justifier :

Les animaux utilisés sont des femelles matures sexuellement afin de pouvoir être gestantes et permettre l'opération et des fœtus au 3ème tiers de gestation.

3.4.12. Indiquez le sexe des animaux utilisés et le justifier :

Dans ce projet seules des femelles gestantes sont utilisées ainsi que leur fœtus de sexe indifférent

3.4.13. Indiquez pour chaque espèce les points limites adaptés, suffisamment prédictifs et précoces pour permettre de limiter la douleur à son minimum, sans remettre en cause les résultats du projet :

Les points limites sont définis comme suit
- Une échographie est réalisée avant le début de la chirurgie pour vérifier l'état de santé du bébé, sa position et la position du placenta et valider que tous ces paramètres sont compatibles avec la chirurgie.
- La femelle gestante sera monitorée durant la chirurgie. Une chute trop importante des paramètres de surveillance entrainera l'arrêt le plus rapide possible de la chirurgie. On peut noter parmi ces paramètres : une température corporelle descendant sous les 34°C, un arrêt respiratoire (possibilité de prendre le relais avec un respirateur), troubles importants du rythme cardiaque.

4. LES PROCÉDURES EXPÉRIMENTALES

4.1 Objet(s) visés par les procédures expérimentales

A - La recherche fondamentale. ☐

B - Les recherches translationnelles : X

4.2 Nombre de procédures expérimentales :

1

4.2.1 NOM DE LA PROCÉDURE EXPÉRIMENTALE N° 1 :

Foetoscopie avec insufflation de gaz chez le macaque

- PROPOSITION DE CLASSIFICATION DE LA PROCÉDURE SELON LE DEGRÉ DE SÉVÉRITE (conformément à l'annexe de l'arrêté relatif à l'autorisation de projet) **:**
Oclasse légère
Xclasse modérée
Oclasse sévère
Oclasse sans réveil

- Description détaillée de la procédure expérimentale :

- Pertinence et justification de la procédure expérimentale :

Les macaques (rhésus et cynomolgus) femelles gestantes seront opérées au troisième tiers de la gestation. Le moment de la chirurgie sera déterminé par une mesure échographique du diamètre biparietal en début de grossesse (Voluson E GE ®). Cette échographie a lieu après la détection par palpation de la grossesse (pendant des interventions de routine type contrôle sanitaire) et se fait sous anesthésie générale (Kétamine 1000, 10 mg/kg, IM).

Les femelles gestantes seront capturées le jour de la chirurgie, entre 130 et 150 jours de gestation. Elles seront sédatées par de la Kétamine (10 mg/kg, IM) avant anesthésie générale par Propofol (5 à 10 mg/kg, IV) et 1 à 3% d'Isoflurane après intubation trachéale. Une couverture chauffante sera utilisée pour maintenir la température corporelle. Une antibioprophylaxie par Amoxicillin (15 mg/kg, IM) sera administrée avant le début de la chirurgie. Une voie artérielle sera mise en place, à partir de laquelle seront réalisés les prélèvements sanguins maternels itératifs.

Après repérage échographique du point d'entrée optimal, une injection de 5 mL de Xylocaïne 2% sera injectée de manière percutanée dans le myomètre de la femelle gestante afin d'apporter une anesthésie locale complémentaire.

En fonction de la disposition du placenta, du cordon ombilical et de certaines conditions techniques, 2 types d'abord endoscopique pourront être utilisés :

Soit par endoscopie percutanée, c'est-à-dire en introduisant le trocart d'endoscopie dans l'utérus, au travers de la peau de la femelle gestante, sous contrôle échographique permanent (technique privilégiée).

Soit en introduisant le trocart d'endoscopie directement au travers de l'utérus, après avoir réalisé une laparotomie à la femelle gestante et exposé l'utérus. Concernant la laparotomie, elle consistera en une incision médiane de la symphyse pubienne jusqu'au-dessus de l'ombilic. La taille de la laparotomie sera la taille minimale suffisante pour permettre une bonne exposition de l'utérus. Ensuite l'ouverture se fera plan par plan, avec une hémostase soigneuse : peau, tissu sous-cutané, aponévrose des muscles grands droits au niveau de la ligne blanche et péritoine pariétal. L'accès à l'utérus sera toujours réalisé par endoscopie, après une mise en place de 2 points transfixiants de fil monofilament 2.0 pour fixer les membranes à l'utérus au niveau de ce que sera le point d'entrée du trocart.

La suite du protocole est identique quelle que soit l'abord utilisé.

L'accès à la cavité utérine, qu'il soit percutané ou directement au travers de l'utérus, sera réalisé selon la technique de Seldinger modifiée sous contrôle échographique permanent. Une aiguille de 17 G est d'abord introduite dans la cavité utérine et une amnioinfusion de 200 à 300 mL de sérum physiologique est réalisée. Un guide est introduit au travers de l'aiguille puis le set d'introduction d'un trocard de 10 Fr est mis en place en suivant ce guide. Un endoscope de 1,3 mm de diamètre sera mis en place dans une chemise de 3,3 mm avec 3 canaux opérateurs (11540AA and 11540KE, Karl Storz ®). Enfin, 200 à 300 mL de liquide amniotique seront retirés (autant que ce qui a été infusé).

L'accès à la veine ombilicale fœtale, qu'il soit percutané ou directement au travers de l'utérus, sera réalisé selon les mêmes principes. La veine ombilicale du fœtus sera ponctionnée par une aiguille de 18 ou 20 G au niveau de l'insertion placentaire (ou au niveau intra-abdominal ou par ponction directe du cordon sous contrôle visuel fœtoscopique) et un cathéter sera éventuellement laissé en place pour permettre les prélèvements sanguins fœtaux itératifs. Une anesthésie et curarisation fœtale seront réalisées par une injection d'un mélange de Fentanyl (5-10 μg/kg), Vecuronium (0,3 mg/kg) et Atropine (20 μg/kg). Au cas où il n'est pas possible de ponctionner la veine ombilicale, les prélèvements fœtaux consisteront en une simple ponction cutanée sous contrôle de la vue et recueil de sang d'origine capillaire.

Du gaz (CO_2 ou Hélium) humidifié (95%) et chauffé (38° C) sera insufflé dans la cavité utérine au moyen d'un insufflateur Insufflo (Lexion Medical ®) à un débit de 0,5 L/min et maintenu à une pression de 6-8 cm d'eau. Le gaz sera retiré au travers du trocart en fin d'intervention.

Les paramètres de surveillance recueillis toutes les 20 minutes pendant les 2 heures d'insufflation de gaz (0-20-40-60-80-100-120) seront les suivants :

- Au niveau fœtal : fréquence cardiaque, échographie cardiaque (pour évaluer la fonction cardiaque), gazométrie veineuse (ou capillaire) et lactates veineux (ou capillaires).

- Au niveau maternel : fréquence cardiaque, tension artérielle, fréquence respiratoire et gazométrie dans l'air expiré, température, échographie cardiaque (pour surveiller l'absence de bulles de gaz), gazométrie artérielle, lactates et électrolytes.

- Au niveau utérin : pression utérine, débit et volume de gaz instillé.

A l'issue de l'intervention, les femelles gestantes resteront en isolement et seront surveillées jusqu'au matin suivant où elles seront remises en groupes sociaux.

Cette procédure permet de reproduire le plus fidèlement possible les conditions de la fœtoscopie chez l'humain et de recueillir les prélèvements permettant de juger de la sécurité de l'insufflation de gaz dans la cavité utérine.

- Indiquez le nombre de lots et le nombre d'animaux par lots, et les justifier :

16 femelles gestantes (et donc 16 fœtus après le 3ème tiers de gestation) macaques cynomolgus ou rhésus seront utilisées lors de ce projet afin d'obtenir des données complètes sur au moins 6 individus par groupe. Soit 6 femelles gestantes et 6 foetus dans le groupe CO2 et 6 femelles gestantes et 6 foetus dans le groupe helium.

- Indiquez le cas échéant le prélèvement, ainsi que la fréquence et le(s) volume(s) prélevés :

Prélèvement sanguin artériel chez la femelle gestante toutes les 20 minutes pendant 2 heures, soit 7 prélèvements de 2 mL.

Prélèvement sanguin veineux chez le fœtus toutes les 20 minutes pendant 2 heures, soit 7 prélèvements de 0,5 mL.
Le volume de prélèvement maximal chez un macaque étant de 8,5 ml/kg (tous les mois) et le femelles macaques (rhésus ou cynomolgus) adultes pesant au minimum 4 kg, le volume prélevé (17,5 ml) sera bien inférieur au volume prélevable (34 ml).

Les anesthésiques utilisés lors de cette chirurgie sont
- Kétamine : IM 10 mg/kg (Prémédication)
- Propofol : IV 5 à 10 mg/kg (pour l'intubation)
- Isoflurane : Anesthésie gazeuse 1 à 3 % (Durant la chirurgie, soit 2h30)
- Xylocaïne 2% : SC 5 mL (anesthésie locale de la zone d'incision);

Les animaux sont remis en contact avec leur groupe d'origine dès le lendemain.
Ces animaux sont hébergés dans de grandes volières intérieures et extérieures en groupes sociaux.
De plus les techniques d'endoscopie sont les moins invasives des techniques chirurgicales.

- Indiquez le cas échéant les dispositions prises en vue de réduire, d'éviter et d'atténuer toute forme de souffrance des animaux de la naissance à la mort :

Ces animaux sont hébergés en groupe sociaux.
De plus, un programme d'enrichissement est mis en place sur tout le site de la Plateforme Silabe.

- Dispositions prises pour éviter tout double emploi injustifié des procédures expérimentales, le cas échéant :

Certaines équipes ont proposé de remplacer, le temps de la chirurgie, une partie du liquide amniotique par du gaz. Un total de vingt-six cas de réparation foetoscopique de myéloméningocèle avec insufflation de CO_2 a été publié à ce jour. Mais la sécurité de cette technique n'a jamais encore été investiguée. Ce protocole est réalisé en collaboration avec les équipes américaines ayant publié ces cas.
Aucune étude évaluant la foetoscopie avec insufflation de gaz n'a encore été réalisé chez le singe et à notre connaissance, aucune n'est prévue.

- Devenir des animaux à la fin de cette procédure expérimentale :

- mise à mort ? ☐
- animal gardé en vie ? X

précisez les animaux concernés et si la décision a été prise par le vétérinaire ou toute autre personne compétente désignée par le responsable du projet :

Aucune détérioration de l'état clinique n'est attendue lors de ce projet. La décision est prise par le vétérinaire qui est également le responsable du projet.

- placement ou mise en liberté des animaux ?

4.3. Si le projet utilise des animaux réutilisés d'un projet antérieur :

- GRAVITÉ RÉELLE DES PROCÉDURES EXPÉRIMENTALES ANTÉRIEURES :

X légère
X modérée
☐sévère