A Novel Translational Model of Percutaneous Fetoscopic Endoluminal Tracheal Occlusion – Baboons (*Papio* spp.)

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Key Words

Fetoscopic endoluminal tracheal occlusion ·
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Abstract

Introduction: Percutaneous fetoscopic endoluminal reversible tracheal occlusion (FETO) was developed to prevent the pulmonary complications of fetal congenital diaphragmatic herniation. There is an urgent need to establish the closest to human translational model of FETO in order to improve fetal outcomes and to determine new clinical approaches and applications. **Material and Methods:** Seven non-human primates underwent two subsequent surgeries: the first, the FETO in the experimental group (n = 3) or sham operation in the control animals (S-FETO, n = 4) at 132–142 days of gestation (dGA); the second, the reversal of occlusion or sham operation at 162 ± 5 dGA. Maternal stress axis, complete blood count, and biochemical parameters were evaluated and newborn tracheal radiography was performed. **Results:** The

average pregnancy duration and neonatal weights in the FETO group did not differ from the animals in the S-FETO group. There was no bleeding or premature fetal membrane rupture during the procedures in any of the baboons. The maximal tracheal width was 7.02 ± 0.6 mm in the FETO versus 5.46 ± 0.6 mm in S-FETO group. **Discussion:** This is the very first report of a successful FETO model in non-human primates. Similarities to human tracheomegaly were for the first time documented in any model studied.

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Introduction

Congenital diaphragmatic hernia (CDH) has an incidence of 1/2,000–1/5,000 pregnancies worldwide [1]. The associated perinatal mortality ranges from 10 to 40% [2]. The cause of mortality is mostly pulmonary hypoplasia and severe pulmonary hypertension [3]. Percutaneous fetoscopic endoluminal tracheal occlusion (FETO) by a

Table 1. List of the animals (*Papio* spp.) and performed procedures

Animal ID	First surgery	Second surgery	Fetal outcome	Total number of surgeries
<i>S-FETO</i>				
T	sham procedure	sham procedure	spontaneous vaginal delivery at term	two surgeries
SQ	sham procedure	sham procedure	C-section after term/stillbirth	two surgeries
F	sham procedure	no	natural preterm delivery ¹	one surgery
S	sham procedure	sham procedure, amniorrhexis during second surgery	spontaneous vaginal delivery at term/ balloon residuals removed at 1 day of age	two surgeries
<i>FETO</i>				
Tlp	balloon placement	fetoscopy performed, followed by ultrasound guided puncture	spontaneous vaginal delivery at term	two surgeries
C	balloon placement	removed via fetoscopy	spontaneous vaginal delivery at term	two surgeries
L	balloon placement	removed via fetoscopy	spontaneous vaginal delivery at term	two surgeries

¹ Preterm infant was nursery reared, is in good condition.

balloon is a promising technique to improve fetal lung growth in the fetuses with CDH [4]. However, despite the application in humans, there is an urgent need to understand the mechanisms behind this procedure to develop the adjuvant therapies and new approaches in order to decrease long-term and short-term complications of CDH.

Several experimental models have been used to study the effect of tracheal occlusion on fetal lung development including sheep [5, 6], rats [7], mice [8] and rabbits [9, 10]. Each model has its own advantages. However, the lung development, placental structure and function and thus, the endocrinology of regulation of fetal organs' growth in these animal models differ from primates, including humans [11]. For example, leptin – an important regulator of fetal lung development, is synthesized by the placenta in primates, but not in rodents and ruminants [12].

Non-human primates (NHP) have been used for pregnancy-related research for decades. The similarity to human type of placentation [13], fetal endocrine responses to the manipulation of maternal environment [12], the well-described mechanism of lung development [14], and the similarity to the human fetus make this model ideal for both the development of fetal instrumentation and the study of fetal organogenesis. Baboons (*Papio* spp.) have been successfully used in models involving repeated cordocenteses [15, 16]; however, attempts to perform repeated in utero surgical procedures in these species are non-existent. Here, we describe the development of a baboon model to study the mechanism of FETO influence on fetal lung development.

Material and Methods

Animals Housing and Handling

Seven time-dated pregnant baboons (*Papio hamadryas anubis*) were used in this study (age range 6–15 years, weight 17.2 ± 0.4 kg). Animals were transported from the University of Oklahoma Health Sciences Center Baboon Research Resource (Oklahoma, Okla., USA) and housed in a single cage environment at the AALAC approved facilities of the Department of Comparative Medicine, University of Tennessee Health Science Center (UTHSC). Animals received enrichment in the form of video/audio recordings, plastic toys, and fruits/vegetables. They were fed standard Monkey diet (LEO5, Purina, St. Louis, Miss., USA) and given water ad libitum. Animals were constantly monitored with video cameras (Axis M3204 Camera – HD, Axis Communications AB, Lund, Sweden) connected through the secure network to the computer desks of the investigators and veterinarians (exacq-Vision, Fishers, Ind., USA). All procedures were approved by the Animal Care and Use Committee at UTHSC (protocol No. 2330).

Experimental Design

Following the initial acclimation period of 3 weeks, each animal underwent two subsequent surgeries: the first, FETO in the experimental group ($n = 3$) or a sham tracheoscopy in the control animals ($n = 4$, S-FETO); the second, reversal of occlusion or sham tracheoscopy (table 1). The gestational age at first surgery was 137 ± 5 days of gestation (dGA; 0.73–0.79 gestation; the length of the gestation is 180 dGA) and 162 ± 5 dGA (0.92 gestation) at the second intervention.

Animal Characteristics

The mean maternal age (MA) and parity (P) did not differ between the FETO (MA = 9.7 ± 0.3 years, P = 4) and the S-FETO (MA = 13 ± 4 years, P = 7) groups. One animal in the S-FETO group had previously delivered one stillborn fetus. The average gestational length was calculated for each animal based on the data retrieved from the animal records (table 1). The duration of the current pregnancy was expressed as the percentage of usual gesta-

tional length for each animal. As an indicator of the gestational length in both groups, this parameter is superior to the gestational age calculated as the number of days spent after the day of conception, since the delivery at term is individual for each animal and ranges from 163 to 190 dGA.

Procedural Animal Care

Preoperative, Operative, and Postoperative Care

On the day prior to surgery food was withheld, but access to water was ad libitum. Ketamine hydrochloride (10 mg/kg; Putney Ketamine HCl® for Injection C-III, Putney Inc., Portland, Me., USA) was given intramuscularly. Anesthesia was induced with isoflurane inhalation (Isothesia®, Butler Schein Animal Health, Memphis, Tenn., USA) via mask, and subsequently the animal was intubated. A 25-mg indomethacin suppository (Letco Medical, Decatur, Ala., USA) was placed rectally. The urinary bladder was emptied via a Foley catheter, which was left in place for the duration of the surgery. An intravenous catheter was placed into the left cephalic vein and blood was drawn for a complete blood cell count and a general chemistry profile (described below). Intravenous 5% dextrose solution (Abbott Laboratories, Abbott Park, Ill., USA) with MgSO₄ added at a concentration of 3,000 mg/250 ml was begun. The fluids were administered throughout the surgical procedure for a total volume of 75 ml which provided a total dose of 900 mg of MgSO₄. The abdomen was then shaved and a surgical prep was performed. The animal was transported to the surgery suite, where it was placed in a supine position and a final skin prep was performed. Ampicillin and sulbactam (Unasyn®, 30 mg/kg i.m., Sagent Pharmaceuticals, Schaumburg, Ill., USA) were administered prior to surgery, and buprenorphine hydrochloride (Buprenex®, 0.015 mg/kg, Reckitt Benckiser Pharmaceuticals Inc., Richmond, Va., USA) was administered at the end of the surgery. After completion of surgery, the anesthesia was stopped, and once a swallowing reflex returned, the animal was extubated and returned to the home cage and monitored until it became sternal. The status of housed animals was constantly observed by staff personnel. Animals exhibiting signs of stress, depression, or abnormal behavior were evaluated and discussed with the facility supervisor and attending veterinarian. Postsurgically, the animals were evaluated several times a day and during the night. Oral indomethacin chews (25 mg, cherry or banana flavored; Peoples Custom Rx, Memphis, Tenn., USA) were given every 6 h for 48 h, and Unasyn was given every 12 h for 3 days.

Operative Technique

Following orientation of the fetus and placenta in utero, a 22-gauge needle (Echotip disposable amniocentesis needle; Cook, Spencer, Ind., USA) was inserted into the amniotic cavity and sterile saline solution at 37°C was injected. Next, gentle external version was undertaken in order to bring the fetal snout to a surgically accessible area away from the placenta. A 14-gauge suprapubic trocar needle (Cook; Spencer) was inserted through a 5-mm skin incision into the amniotic cavity. After that, a guide wire was advanced through this needle into the amniotic cavity as described elsewhere [17]. The needle was withdrawn over the guide wire and then a thin-walled flexible Teflon cannula (10-Fr Check Flo® Cook; Bloomington, Ind., USA) loaded with a dilator was advanced over the guide wire (Seldinger technique). The cannula was directed towards the snout and the fetoscopic instruments were

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Fig. 1. Fetal endoscopic anatomy of the baboon. **a** Fingers. **b** Mouth with whiskers. **c** Lips and tongue. **d** Lips, tongue and mandible. **e** Back of the tongue. **f** Hard palate. **g** Frenulum. **h** Pharynx. **i** Vocal cords.

inserted. These instruments included a slightly bent 7-Fr sheath with a connection for amniotransfusion at body temperature. Next, a 1.3-mm 10,000-pixel fiber endoscope (Karl Storz, Tubingen, Germany) was introduced. The balloon occlusion system, consisting of a catheter (Baltacci®, Balt Extrusion, Montmorency, France) loaded with a detachable valve balloon (GoldBal2 balloon, Balt Extrusion) was advanced through the sleeve (described in detail in Deprest et al. [18]). The fetoscope was connected to the light source and video recorder (×8,000 Light Source, SDC3, Stryker, San Jose, Calif., USA).

The landmarks were not as obvious as in humans. The nose was more difficult to find; therefore, the cannula was inserted at the level of the tip of the snout. The fetal extremities could at times be identified in front of the mouth (fig. 1a). Eventually, the hairs (whiskers or moustache) would identify the fetal parts and the upper lip (fig. 1b, c).

The endoscope was introduced into the fetal mouth and directed over the tongue (fig. 1d, e) along the midline by visualizing the raphe of the palate (fig. 1f) and uvula (fig. 1g), to the pharynx (fig. 1h) then to the larynx, with the epiglottis as a landmark, and finally through the vocal cords (fig. 1i) to the trachea (fig. 2a, b) and carina (fig. 2c). In the 3 animals that underwent balloon placement, the catheter was positioned to deliver the balloon just above the carina (fig. 2d, e). Of the 4 animals that underwent the sham tracheoscopy, the balloon was inserted, but not inflated in one animal. In the 3 remaining animals, the balloon was not inserted. At the end of the procedure, the excessive amount of infused fluid was drained until the depth of the maximal pocket of the amniotic fluid did not exceed 3–4.5 cm.

Fig. 2. Fetal endoscopic anatomy of the baboon. **a** Epiglottis. **b** Vallecular. **c** Trachea. **d** Carina. **e** Insertion of the balloon. **f** Tip of the inflated balloon. **g** Removal of the balloon.

The second surgery was performed in the same manner. The balloon was endoscopically punctured with a sharp stylet (11506P Karl Storz) and the residuals of the balloon were removed using a 3-Fr grasping forceps (11510C Karl Storz; fig. 2f). In one animal, the balloon was punctured under ultrasound guidance.

Complete Blood Count and Biochemical Indices

Prior to each surgical procedure, 20 ml of maternal blood was obtained, processed as previously described [19] and included a complete blood count (CBC) and serum chemistries, described by Schlabritz-Loutsevitch et al. [19] (Antech Diagnostic, Southaven, Miss., USA).

Adrenocorticotrophic Hormone and β -Endorphin Evaluation

To evaluate the maternal stress prior to the procedure, adrenocorticotrophic hormone (ACTH) was measured in serum using an immunoassay kit (ALPCO Diagnostic, No. 21-ACTHU-E01, Salem, N.H., USA). All measurements were performed in triplicate. Intraassay validation for immunoassay was 2.27–6.71%. The ACTH ELISA has a calculated sensitivity of 0.22 pg/ml. The β -endorphin was measured in serum using an immunoassay kit [Peninsula Laboratories, LLC, San Carlos, Calif., USA, No. S-1245, β -endorphin (bovine, camel, mouse)]. All measurements were performed in duplicate. The sensitivity (Av. IC50) of the assay is 0.29 ng/ml. Intraassay coefficient of variance was 0.4%.

Newborn Procedures

Within 24 h after delivery, the dam was sedated with i.m. ketamine (10 mg/kg, Putney Ketamine HCl for Injection C-III; Putney Inc.). The neonate was transported to the imaging suite, where it was examined and weighed without anesthesia. At all times, every effort was made to keep the neonate warm by wrapping it in blankets and providing external heat. Next, the neonate was anesthetized via a facemask with 1–2% isoflurane for the X-ray examination.

X-Ray Examination of the Newborn

The X-ray imaging was performed using a veterinary digital radiograph machine (Sedecal, Buffalo Grove, Ill., USA). The images were taken in lateral and AP positions, using a 60 kVp and

3.2 mA for both. The images were analyzed after being placed into an IDEXX-PACS Imaging system (IDEXX Laboratories, Inc., Minnesota, Minn., USA). The tracheal width (diameter) was measured at the level of the thoracic inlet just above the carina and at the mid-distance between the above-mentioned points [20]. The combined (average tracheal) width was calculated. Additionally, the maximal diameter of the trachea was measured at a site of the trachea within the upper chest cavity.

Ultrasound Examinations

Ultrasound examinations were performed as described previously [21, 22] for fetal morphometry and Doppler evaluation of the pulsatility indices (PI) of fetal middle cerebral artery (MCA), ductus venosus, ductus arteriosus, pulmonary artery, uterine artery, umbilical artery, using a Samsung A30 machine (Samsung, Samsung Medison Co., Ltd., Ridgefield Park, N.J., USA).

Statistical Analyses

Statistical analyses were performed using the ANOVA and Kruskal-Wallis tests. We applied Friedman analysis to calculate changes in the variables over time to compare mean maternal biochemical, CBC parameters and stress axis hormones between first and second surgeries and between FETO and S-FETO groups. Significance was set at $p < 0.05$. Data are presented as mean $\pm$ SEM, unless indicated differently.

Results

Surgery Characteristics

There was no difference in duration of the FETO and S-FETO surgeries, neither at the first, nor at the second time point (2.61 ± 0.89 and 3.6 ± 0.14 vs. 2.12 ± 0.37 and 2.29 ± 0.23 h, respectively). The duration of the second surgery was significantly reduced compared to the first operation in the S-FETO group, associated with only the

Fig. 3. Placenta and fetus of the animal who delivered post-term infant. **a–c** Notice the well-developed fetus, area of necrosis and yellow discoloration of placenta. **d** The length of the trachea from the vocal cords to carina.

tracheal visualization as compared to balloon placement in these animals.

The total volume of saline solution used for the amnioinfusion to improve visualization during the procedure was 396.3 ± 201 and 740.0 ± 239.0 ml during the balloon placement surgery in FETO and S-FETO groups, respectively. During the second set of surgeries (balloon retrieval), one animal had amniorrhexis at the beginning of the procedure. Due to amniorrhexis, the amount of the saline solution used for the visualization and irrigation of the amniotic cavity in this baboon (2,900 ml) exceeded the amount infused during the second procedure in the rest of the animals in both groups (range 270–1,050 ml).

Maternal and Fetal Outcome

The average pregnancy duration in the FETO group did not differ from the animals in the S-FETO group (90.0 ± 1 vs. $98.4 \pm 6\%$). One animal in the S-FETO group went over term (200 dGA), which resulted in stillbirth (fig. 3). Neonatal weights did not differ between the two groups (830.0 ± 129 vs. 723.7 ± 65 g, respectively). One preterm infant in the S-FETO group was delivered at 153 dGA (5 days after the first sham operation). It was hand reared and developed normally (fig. 4d–f).

In one animal from the S-FETO group, which underwent insertion, but not the inflation of the balloon, the re-

siduals of the balloon were detected in the trachea during X-ray evaluation and was successfully removed using a conventional laryngoscope and an endoscopic extractor (fig. 4a–c).

Ultrasound Parameters

The ultrasound indices of fetal growth and blood flow are presented in table 2. There were no differences in maternal and fetal blood flow indices prior to and after the endoscopic interventions in both groups.

CBC and Biochemical Parameters

Maternal blood biochemical parameters did not differ between the two groups with the exception of the concentrations of alkaline phosphatase, which was increased in the FETO group compared to S-FETO group prior to the first and second surgeries (114 ± 12 vs. 71.7 ± 5 U/l and 119.3 ± 13.0 vs. 78 ± 9.6 U/l).

ACTH and β -Endorphins

The ACTH concentrations did not differ between the FETO and S-FETO groups prior to the first (30.7 ± 19 and 23.2 ± 15 pg/ml, respectively) and second procedures (33.7 ± 5 and 22.2 ± 4 pg/ml, respectively). The β -endorphin concentration did not differ in the FETO as compared to the S-FETO groups prior to the first procedure ($0.560 \pm$

Fig. 4. Residuals of the balloon in the S-FETO animal prior to removal (a), after removal (b), and gross photo (c). d Preterm infant born in the S-FETO group at delivery (153 dGA). e Same infant at equivalent of 163 dGA (left), compared to the healthy newborn at 163 dGA (right), and at f equivalent of 171 dGA.

0.025 and 0.752 ± 0.109 ng/ml, respectively) and to the second procedure (0.787 ± 0.013 and 0.939 ± 0.033 ng/ml, respectively; data are presented as mean $\pm$ SD).

X-Ray Evaluation

The maximal tracheal width was 7.02 ± 0.6 mm in the FETO group versus 5.46 ± 0.6 mm in the S-FETO group (fig. 5).

Discussion

NHP have been proven to be an ideal model to study fetal and postnatal lung maturation [14]; however, in NHP the development of fetal interventions with fetal survival has been practically nonexistent due to the high risk of preterm deliveries and complications [23–25]. Despite the fact that the natural occurrence of such pregnancy-related complications as stillbirths and placenta abruption cases in Old World NHP (baboons and macaques) are similar to humans [26–28], the differences

Table 2. Ultrasound indices of fetal growth and blood flow prior to surgeries in pregnant baboons (*Papio* spp.)

	FETO (n = 3)	S-FETO (n = 4)
Biparietal diameter, cm	5.1 ± 0.1	4.3 ± 0.4
Head circumference, cm	19.3 ± 0.6	16.6 ± 1.4
Abdominal circumference, cm	16.8 ± 0.7	13.4 ± 1.4
Femur length, cm	4.2 ± 0.1	3.5 ± 0.4
Amniotic fluid index, cm	5.3 ± 2.2	4.4 ± 1.3
Fetal heart rate, bpm	148.4 ± 2.4	148.0 ± 7.9
PI*		
MCA	1.67 ± 0.1	1.4 ± 0.3
Ductus venosus	0.99 ± 0.4	0.37 (n = 1)
Ductus arteriosus	2.64 ± 0.4	2.36 ± 0.02
Pulmonary artery	2.55 ± 0.4	2.41 ± 0.4
Uterine artery	1.38 ± 0.3	1.61 ± 0.39
Umbilical artery	1.39 ± 0.1	1.52 ± 0.1
Fetal lung measurements		
Area, cm ²		
Left	1.89 ± 0.4	1.37 ± 0.3
Right	2.75 ± 0.15	1.51 ± 0.2
Circumference, cm		
Left	6.0 ± 0.11	5.44 ± 0.8
Right	6.87 ± 0.7	5.46 ± 0.8

* PI = Pulsatility index.

Fig. 5. Representative radiographs of the tracheal width in the newborn after FETO procedure (a) compared to the S-FETO (sham operation) animal (b).

between NHP and humans including shallow trophoblast invasion, decreased amount of amniotic fluid in the third trimester, the mechanism of labor [13], and myometrial thickness [27], might be the reason behind the reported increased complication rate and technical difficulties in NHP. Interestingly, van der Wildt et al. [25] found the absence of myometrial electrical activity in rhesus monkeys after simple fetoscopic manipulations.

To ensure the decrease in fetal mortality and decrease incidence of preterm labor in NHP, ‘the precise adjustment of maternal anesthesia and tocolytics are required’ [29, 30]. Nakagawa et al. [31] reported the fetal loss in monkeys which underwent fetal surgeries to be between 6.7 and 50%, where the risk of complications was dependent on the degree of surgical manipulations [25, 32]. Feitz et al. [23, 24] were able to establish the macaque model for endoscopic fetal manipulation of the urogenital tract, while Mattison and King [33] reported the percentage of fetal loss in macaques as high as 52.4% with major complications including maternal hemorrhage into the amniotic fluid, inability to enter the amniotic cavity, and abortion.

Amniorrhexis is another important complication of FETO and subsequent early preterm delivery with the risk reported to be more than 35% in humans [34]. This

risk appears to decrease with the experience of the operator; however, it could not be totally eliminated [34]. In our study, there was only one case that was complicated by amniorrhexis. In this case, the surgery was performed at 163 dGA (it was a second surgery in the S-FETO group). This gestational age is defined as ‘at term’ in baboons [28]. The animal was close to delivery as indicated by vaginal discharge, position of fetal head and cervical opening at the ultrasound evaluation.

Tracheomegaly has been described in human fetuses undergoing FETO [20, 35–37]. In humans this effect has been linked to the coexistence of the hypoplastic bronchial tree [38] and the effect of the occlusion itself [36, 39]. In our experiments, the duration of the occlusion (3–4 weeks or 15.4% of the duration of gestation) was comparable to what was reported in humans (6–22.5% of the duration of gestation) [20]. The tracheal diameter in the baboon fetuses was within the lower range documented for the preterm human fetuses [20] and the dimensions of the balloon used in our experiments were the same as published for human fetuses (7 mm in width and 20 mm in length). In humans, the tracheomegaly could persist up to 5 years postnatally [36, 40]. The observation of the same complication in the baboons opens the pioneering

opportunity not reported in other animal models to unveil the mechanisms and develop the prevention and treatment strategies in case of this complication.

The procedure itself did not influence the maternal long-term HPA axis in our study. This observation is important since this pathway is closely involved in the mechanisms of parturition as well as in the process of lung maturation [41].

The PI of ductus venosus blood flow in the reported experiments was higher than those reported by us for the baboons at the same gestational age [42], but similar to those published for human fetuses [31]. The uterine artery PI was higher than estimated by some authors [43], but within the range reported by others [22]. These discrepancies could be due to the differences in the animal position, type of the anesthesia, and animal housing (indoor vs. outdoor). The PI of umbilical artery and MCA were within the range published for baboons [22, 43].

The application of the baboon model opens a new venue for the development of the adjuvant treatment of CDH. For example, the tracheal occlusion increased leptin expression in the experimental animal model [44]; however, leptin treatment in mice and sheep failed to stimulate lung development [45]. The latest could be due to the fact that leptin metabolism in pregnancy differs between human and ru-

minants or rodents. The leptin lung endocrinology in baboons is similar to humans [46], and leptin therapy might be beneficial in this model. The same might be true for other regulatory mechanisms and novel therapeutic targets.

In summary, the use of the closest to human animal models to study fetal pathophysiology and development of techniques of fetal intervention is imperative for the success of fetal care. Thus, the description in the present study of the feasibility of repeated fetal interventions in baboons (*Papio* spp.) opens new horizons to the understanding and treatment of fetal anomalies.

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