U.S. FOOD & DRUG ADMINISTRATION

Animal Study Considerations for Artificial Womb Technology Devices

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Outline

Animal Studies

- When to conduct an animal study
- Animal study considerations
- Safety and proof-of-principle endpoints

Animal Models

- General considerations
- Sheep model for AWT

Comparative Anatomy and Physiology

- Organ stage of development and maturation
- Human and sheep fetal circulation
- Fetal animal model challenges in AWT device studies

Non-Clinical Device Evaluation

Risk Analysis

- Compile device and procedure-associated risks
- Device evaluation strategy to identify testing to address device attributes necessary for successful device performance

Bench Tests

- Engineering
- Simulated use
- Biocompatibility

Finding suitable alternative that may be performed in lieu of animal studies (the 3Rs: replacement, reduction & refinement)

FDA Modernization Act 2.0, NIH Revitalization Act 1993, Animal Welfare Act Regulations 9 CFR 2.31 (d)(1)(ii),2.32 (c)(2) and (5)(ii)

Pilot Animal Studies

- Determine useful animal model(s) [*9 CFR 2.31(e)*]
- Target assessment of device function & attributes needed for the device to work as intended
- Modify the device design and procedures based on pilot study results

Final Animal Studies

- GLP [*21 CFR §58*]
- Large animal models typically used
- Further assessment of device function & attributes needed for device to work as intended

Pre-Submission meetings with FDA is helpful to reach consensus on non-clinical testing needed to support a clinical study

Animal Study Considerations

- Select appropriate & feasible animal model(s) to provide relevant information that is applicable human physiology and pathology
  - Species-specific strengths & limitations
- High quality study design & execution needed
- Help determine if the device will work as intended: Proof of principle
- Help establish basic safety & can be used to evaluate device safety and performance to support FIH

Animal study data useful to support FDA approval of an IDE to conduct a clinical study of a significant risk device

For more information: Guidance for Industry and FDA Staff: General Considerations for Animal Studies Intended to Evaluate Medical Devices (2023)

Animal Study Design for AWT Devices

- Select relevant study endpoints
- Adequate sample size
- Select feasible and informative study duration
  - Clinical duration of use, risks, historical precedent, exploratory data
- Assessments
  - Safety, device performance, human factors
  - Physiologic assessments and clinical pathology
  - Gross and histopathology evaluation [*21 CFR 58.130(d)*]

Animal Study Insights: AWT Proof-of-Principle & Safety

- AWT proof-of-principle
  - Hemodynamic support
  - Critical organ system development and maturation
- Safety
  - Hemocompatibility: Hemolysis, hemorrhage, thrombosis
  - Clinical chemistry stability
  - Tissue injury
  - Organ dysfunction

Animal Model Considerations for AWT Devices

- Relevant anatomy, physiology, & pathophysiology
- Feasibility of cannulation
- Device size
- To accommodate animal growth
- To allow movement with or without restraints

Fetal animal models (more than one may be needed for evaluate different attributes of the AWT)

- Lamb
- Piglet
- Non-human primate

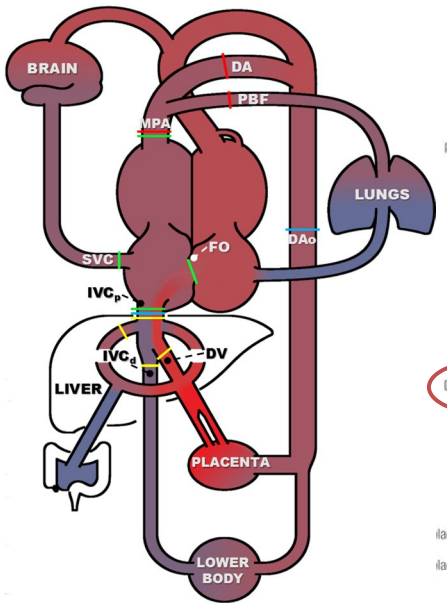
Determine appropriate animal model(s) through research and pilot studies considering the study objectives and model strengths and limitations

Fetal Lamb Model for AWT Devices

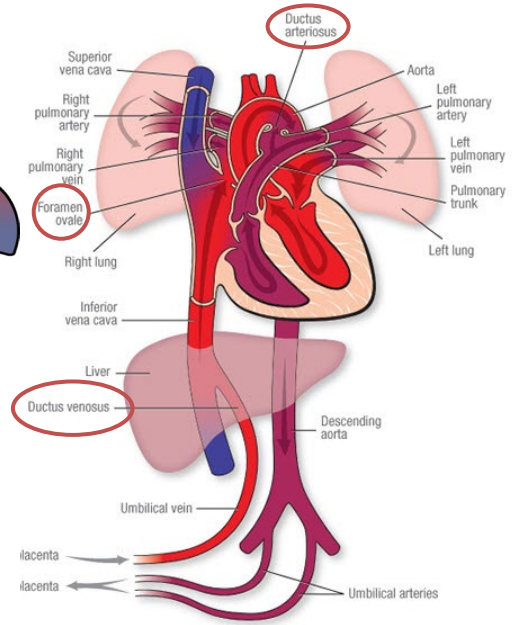
- Relevance to humans
- Body weight and surface area (for some breeds)
- Organ sizes
  - Heart and aorta
  - Blood vessel diameters
- Responses to shear stress and blood-contacting materials

Comparison of Lamb and Human Fetoplacental Circulation

Fetal Lamb



Human Fetus



Fetal blood volume reported in literature

Preparation	Fetal Blood Volume, ml/kg: Chromium red cells	RISA or Evans blue	Cr-RBC + RISA	Reference
Acute sheep		148*		Barcroft & Kennedy (4)
Acute sheep	106*	156	135	Creasey et al. (8)
Acute sheep	116			Broughton-Pipkin & Kirkpatrick (6)
Previable human		162		Morris et al. (16)
Acute sheep		168		Caton et al. (7)
Chronic sheep		150		Caton et al. (7)
Chronic sheep		156		Toubas et al. (24)
Average	111	157	135	
Chronic sheep	110	125	121	This study

Table 1. Organ blood flows as percentages of combined ventricular output (CVO) in the lamb and human fetus at midterm (0.5 gestation) and term gestation

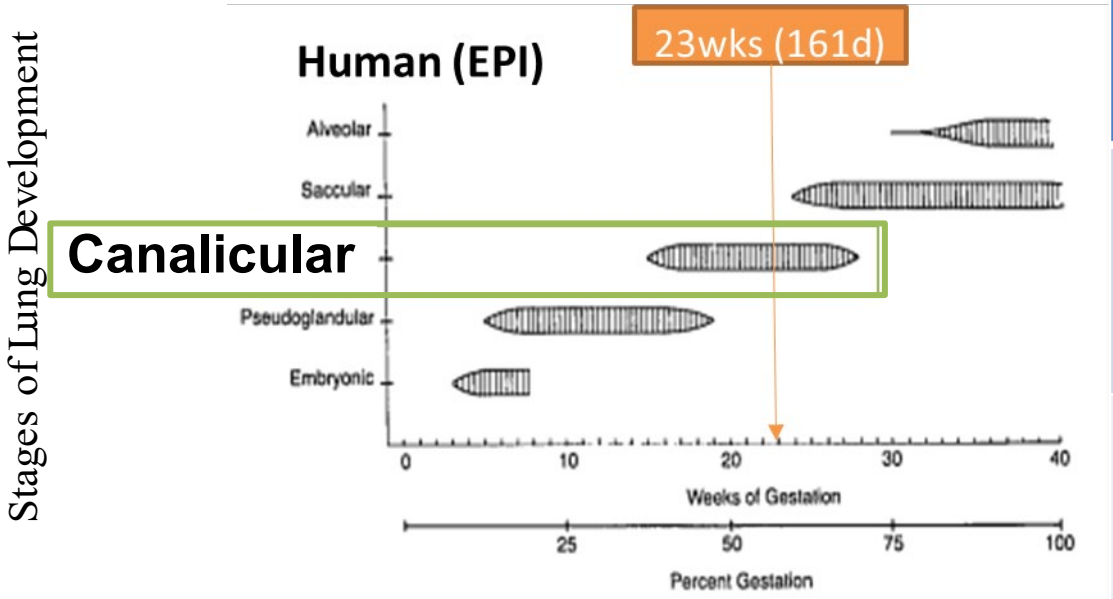
Blood flow	Lamb		Human	
	0.5 gestation	Term	0.5 gestation	Term
Umbilical–placental (% CVO)	45	40	35	20
Pulmonary (% CVO)	4	8	15	25
Cerebral (% CVO)	2	3	8	35

Table 2. Gestational changes in umbilical–placental blood flow, fetal hemoglobin concentrations, oxygen content of umbilical venous (UV) blood, oxygen delivery to the fetus, glucose concentration of UV blood, and glucose delivery to the lamb and human fetus at midterm (0.5 gestation) and term gestation

	Lamb		Human	
	0.5 gestation	Term	0.5 gestation	Term
Umbilical/placental flow (% CVO)	45	40	35	20
Hemoglobin concentration (g/dl)	8	10	9	16
Oxygen content UV (ml/dl)	8.6	10.8	9.7	17.2
Oxygen delivery (ml/min/kg)	17	19	15	15
Glucose concentration UV (mg/dl)	25	20	70	60
Glucose delivery (mg/min/kg)	50	36	110	54

Schrauben, E. M Journal of Cardiovascular Magnetic Resonance, 21(1), 1-11.; https://www.heart.org/en/health-topics/congenital-heart-defects/symptoms--diagnosis-of-congenital-heart-defects/fetal-circulation; Lumbers, E. R. (2000). Fetal renal circulation. In Advances in Organ Biology (Vol. 9, pp. 275-299): Elsevier.; Brace, R. A. (1983). Blood volume and its measurement in the chronically catheterized sheep fetus. American Journal of Physiology-Heart and Circulatory Physiology, 244(4), H487-H494. ; Rudolph, A. M. (2018). Pediatric Research, 84(3), 348-351.

Pulmonary Development



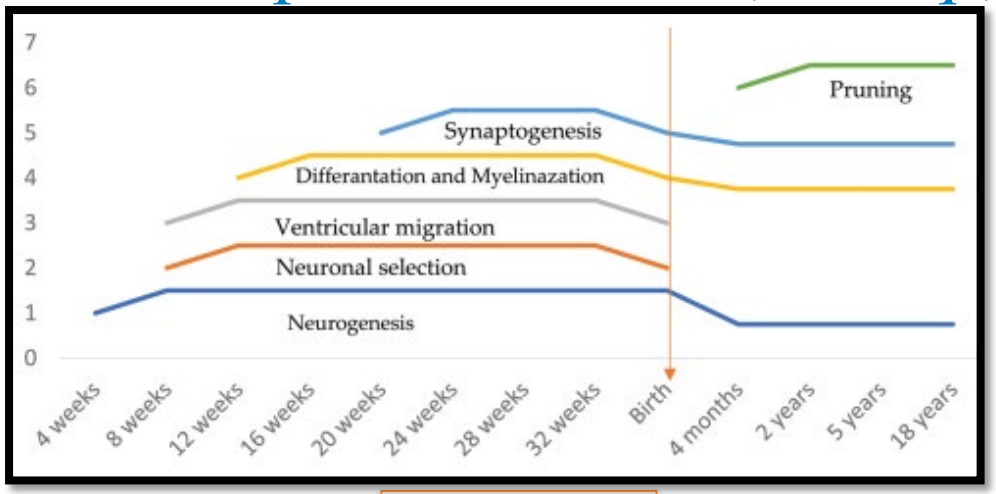
	EPI	Lamb	Macaque	Baboon
Mid-canalicular phase (days)	161	100	90	125
Total Gestation (days)	280	145	165	179

- 23-week GA EPI is in the middle of canalicular phase
- The timing of the mid-canalicular phase in animals as a function of total gestational time may not fully align with EPIs

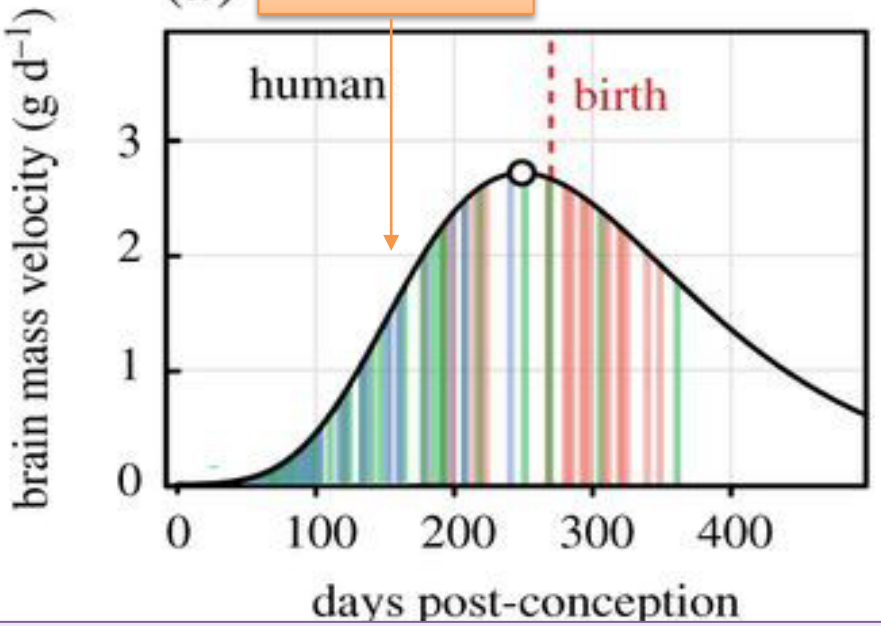
EPI – Extremely Premature Infant

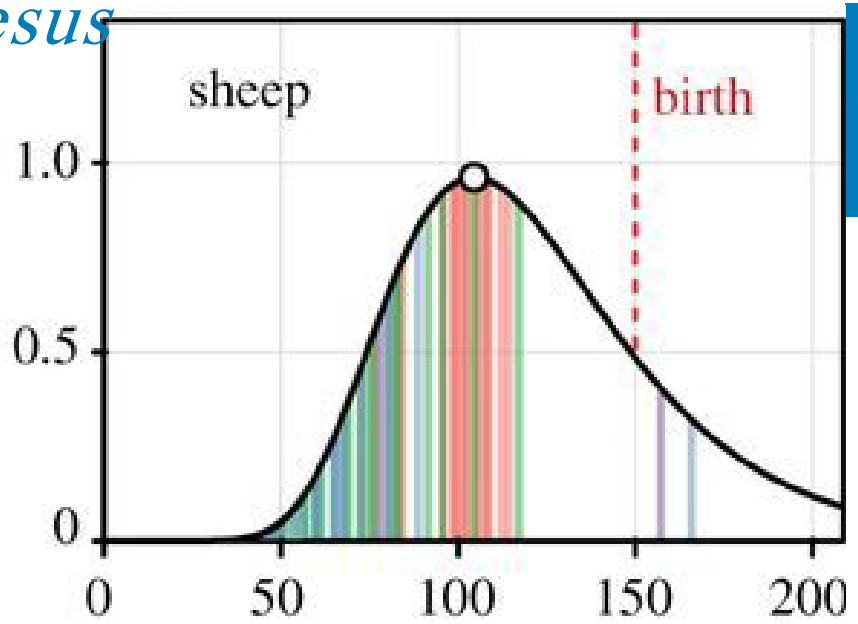
Pringle, Kevin C. MB, ChB. Human Fetal Lung Development and Related Animal Models. Clinical Obstetrics and Gynecology 29(3):p 502-513, September 1986; Schittny JC. Development of the lung. Cell Tissue Res. 2017 Mar;367(3):427-444.

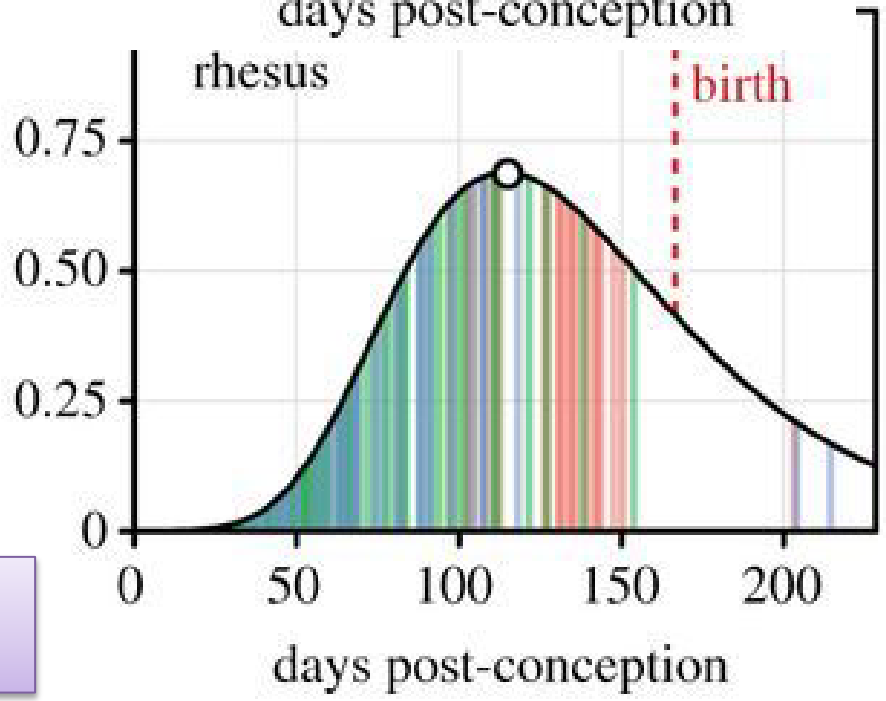
Brain Development *Human, Sheep, and Rhesus*



(a) **23wks(161d)**







Sheep and rhesus fetus at about 75 days or younger is the estimated equivalent of a 23-wk old human fetus stage of brain development

Minimal variation in eutherian brain growth rates during fetal neurogenesis, Volume: 284, Issue: 1854; Uytun, M. C. (2018). Development period of prefrontal cortex. In Prefrontal Cortex. IntechOpen.

Interspecies Comparison of Renal Development

Completion of Nephrogenesis (Data from Brown *et al.*; Zoetis and Hurtt)

Human	35–36 weeks; before birth
Monkey	before birth
Pig	postnatal week 3
Dog	postnatal week 2
Rat	postnatal day 11–15
Mouse	before birth
Sheep	before birth

Nephrogenesis in precocial species (human, sheep and NHPs) is completed before birth.

Lumbers, E. R. (2000). Fetal renal circulation. In Advances in Organ Biology (Vol. 9, pp. 275-299): Elsevier.
Zoetis, T. and Hurtt, M.E. (2003), Species comparison of anatomical and functional renal development. Birth Defects Research Part B: Developmental and Reproductive Toxicology, 68: 111-12
Seely JC. A brief review of kidney development, maturation, developmental abnormalities, and drug toxicity: juvenile animal relevancy. J Toxicol Pathol. 2017 Apr;30(2):125-133.

Strengths & Limitations of the Fetal Lamb Model for Evaluating AWT Devices

Strengths

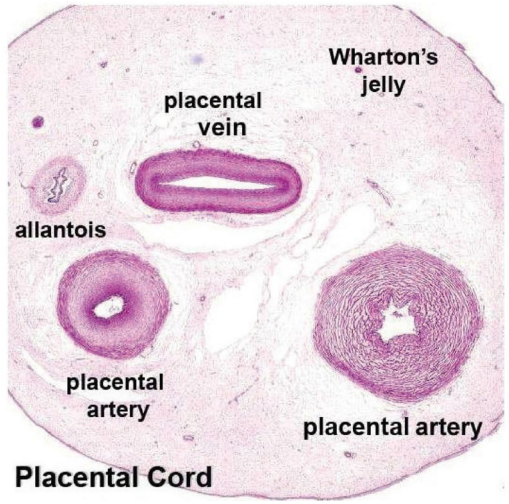
- Historical use as research animal model
- Reliable data
- Singleton, small sheep breeds (Merino, Welsh Mountain) may be equivalent in mid- and late gestation body weight and blood volume to human fetus

Limitations

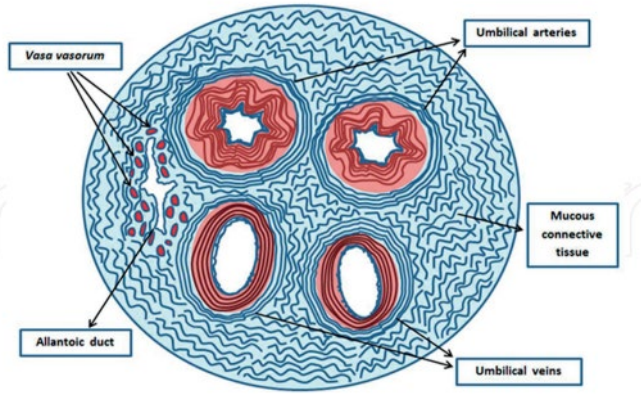
- Common laboratory sheep breeds (Suffolk, Dorset) larger than the 23-weeks human infant at the equivalent canalicular stage of lung development
- Shorter umbilical cord comprised of two arteries and two veins vs. two arteries and one vein in humans
- Growth rate approximately twice the rate of human fetal growth

Fetal Piglet for Umbilical Vessel Cannulation

- Fetal piglets may be more advantageous to fetal lamb for simulating cannulation of umbilical arteries and vein in EPI:
  - Comparable body size to EPI
  - Vascular anatomy similar to humans of 1 UV, 2 UA within umbilical cord
  - Similar umbilical vessel diameters in relation to body size



Human fetus (same as fetal piglet)



Fetal lamb

Spurway, J., Logan, P., & Pak, S. (2012). The development, structure and blood flow within the umbilical cord with particular reference to the venous system. Australasian Journal of Ultrasound in Medicine, 15(3), 97-102.
Arpi, L.M. (2018). Histology of Umbilical Cord in Mammals. Histology.

Strengths & Limitations of the Fetal NHP Model for Evaluating AWT Devices

Strengths

- Genetic, anatomical, and physiological similarities to humans
- Hemochorial placentation
- Human-like physical features

Limitations

- Olive baboon (*Papio anubis*)
  - Preterm baboon fetus, equivalent to canalicular stage of human lung development, is <500 g
  - Smaller umbilical vessels for cannulation
  - Smaller left ventricle and lower cardiac output to overcome ECMO circuit resistance
  - Circuit volume larger relative to the total blood volume of the fetus
- Rhesus macaque (*Macaca mulatta*), Pig-tailed macaque (*Macaca nemestrina*)
  - Preterm fetus equivalent to canalicular stage of human lung development is too small to cannulate
- Cynomolgus macaque (Macaca fascicularis)
  - Preterm fetus equivalent to canalicular stage of human lung development is too small to cannulate

Conclusions

Animal Study Design for Artificial Womb Technology (AWT)

- A comprehensive risk analysis and finding suitable alternatives (3Rs) are used to determine whether an animal study is necessary to evaluate the device
- Animal studies conducted as per GLP (21 CFR §58) are based on sound and valid conclusions from previous well-designed bench and pilot studies to ensure clinically translatable data
- Safety and proof-of-principle endpoints specific for AWT animal studies

Animal Model Considerations for AWT

- Size, including vessel size for cannulation, growth rate & organ maturation are critical
- Mid-gestation or late gestation fetus is an appropriate animal model

Comparative Anatomy and Physiology

- There are similarities and differences in human and lamb fetal circulation, and pulmonary, brain and renal development of human, lamb and macaque
- Fetal lamb and NHP animal model have strengths and limitations, piglet advantages in AWT device studies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