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Chapter 16

# Animal Models of Fetal Medicine and Obstetrics

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**Abstract**

Animal models remain essential to understand the fundamental mechanisms occurring in fetal medicine and obstetric diseases, such as intrauterine growth restriction, pre-eclampsia and gestational diabetes. These vary regarding the employed method used for induction of the disease, and differ in relation to the animal characteristics (size, number of fetuses, placenta barrier type, etc.). While none of these exactly mirrors the human condition, different pregnant animal models (mice, rats, guinea pigs, chinchillas, rabbits, sheep and pigs) are here described with respect to advantages and limitations. The ability to employ noninvasively diagnostics varies among species, specifically for ultrasound and clinical magnetic resonance imaging procedures. Management of feeding, handling, care and anesthesia are particularly important factors in the pregnant animal.

**Keywords:** animal models, fetal medicine, diagnostics and imaging, handling of pregnant animals

## 1. Introduction

Obstetrics deals with pregnancy, childbirth and the post-natal period, whereas gestation (from Latin: "to carry") is the time between conception and birth. Gestation is typical for mammals, where an embryo/fetus develops in the uterus.

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Research in the pregnant human is problematic and may pose significant ethical restrictions as the well-being of the mother and her unborn baby is critically important. Thus, the use of animal models provides a way to gain insight into the improved understanding of the human pregnancy. Animal models remain essential to understand the fundamental mechanisms underlying the onset of obstetric diseases, and to discover improved methods for prevention, diagnosis and treatment. However, the translatability between animals and humans should be carefully considered. In obstetric research, several factors may contribute in the selection of the most appropriate animal model, concerning the mother, fetus and placenta. In addition, the timing of the study during the gestation needs to be considered, as pregnancy is a dynamic process.

The aim of this chapter is to review the advantages and limitations of relevant animals, including mouse, rat, chinchilla, guinea pig, sheep and pig models, and their use in studying fetal growth disorders (intrauterine growth restriction, IUGR), preeclampsia and diabetes in pregnancy. Furthermore, available imaging modalities for studying pregnant animals including fetal and placental characteristics are presented. Finally, ethical and welfare considerations are described, as well as how physiological effects of pregnancy pose special requirements to the management of feeding, handling, care and anesthesia.

## 2. Considerations

Knowledge of the different characteristics of the animal and its gestation is a prerequisite in order to select the most suitable animal model, interpret experimental findings and reach appropriate translational conclusions. In obstetric research, in particular, it is necessary to consider fetal/neonatal characteristics and the physiological changes during the gestation period. Several species have been used to study the normal pregnancy and related pathological conditions [1]. First, the human gestation is described for comparison.

## 3. Human pregnancy and fetal development

The human gestation is about 280 days and is divided into three trimesters, each of which is marked by specific fetal developments and embryonic changes. The first trimester is from gestation week (GW) 1–12, including the conception, second trimester is GW 13–28 and third trimester is GW 29–40. In other mammals, the gestation is defined as the time between conception/fertilization and birth, which for comparison is 266 days in humans. A single fetus is carried in 97–98% of all human pregnancies. The human newborn is extremely dependent on the mother and has immature motoric skills which traditionally placed the neurodevelopment of the human newborn as *altrical* (from Latin: "to nurse"), referring to the undeveloped motoric system. However, the advanced development of the human brain at birth rather places the human newborn as *precocial*, meaning well-developed at birth [2]. The human brain at birth is more advanced than all other animal models used in research [3].

The placenta is the interface between the maternal and fetal circulation, facilitating an exchange of oxygen, nutrients, waste products and other molecules, for example, certain drugs. The fetal trophoblast cells form the external component of the placenta, the *chorionic plate*. The nomenclature of the placenta barrier refers to the degree of erosion of the maternal tissue in the uterine cavity and the interface between the maternal and fetal circulation. The placental interface differs greatly between species (**Figure 1**). Humans have *hemomonochorial* placenta barrier because the maternal blood (*hemo-*) is in direct contact with only one layer of trophoblasts (*–mono*) in the chorion plate (*–chorial*). Thus, the human placenta is implanted completely within the uterus with a deep invasion of the trophoblasts and erosion of the uterine epithelium [4]. In rodents (mice and rats), the placenta barrier is *hemotrichorail* with three layers of trophoblasts dividing the maternal blood from the fetal capillaries in the chorionic plate [5]. Another nomenclature used for the microscopic structure of the placenta exchange area refers to the villous or labyrinth type. The human placenta is of the villous type where chorionic vessels branch out with few interconnections. In a placenta of the labyrinth type, the fetal vessels, the trophoblasts and the maternal blood space branch out and are interconnected in a complex labyrinthine pattern [5].

### 3.1. Gestation length

A short gestation time (or rapid reproduction) is sometimes considered an advantage to obtain a high experimental productivity or for economic reasons. However, if repeated procedures are required during the gestation time, a longer gestation period is usually preferred. A longer interval between the experiments allows for longer restitution and thereby, reduces the induced stress response in the animals. In addition, surgical manipulation might be difficult to employ in animals with a short gestation. During a long gestation time, the response of environmental or physiological influences on the fetal development could also become more pronounced.

### 3.2. Number and size of fetuses

Occurrence of a single fetus in uterus is obviously preferred for individual fetal monitoring. A small number of fetuses will often correlate with bigger fetal size [6]. A bigger fetus makes it possible to receive a higher spatial resolution and sensitivity using non-invasive diagnostic tools, for example, clinical magnetic resonance imaging (clinical MRI) and computerized axial tomography (CT or CAT). Furthermore, surgical procedures are easier to perform. However, larger litter sizes provide a higher sampling size per gestation, and thus, the number of animals used can be reduced in accordance with the "3 R's" (see Section 9).

### 3.3. Placentation

Many differences exist in relation to placentation in the different animal models, such as the development and changes of the placenta during the time of gestation, blood flow, transfer of oxygen, nutrients and waste products, metabolic, endocrine and immunologic function [4].

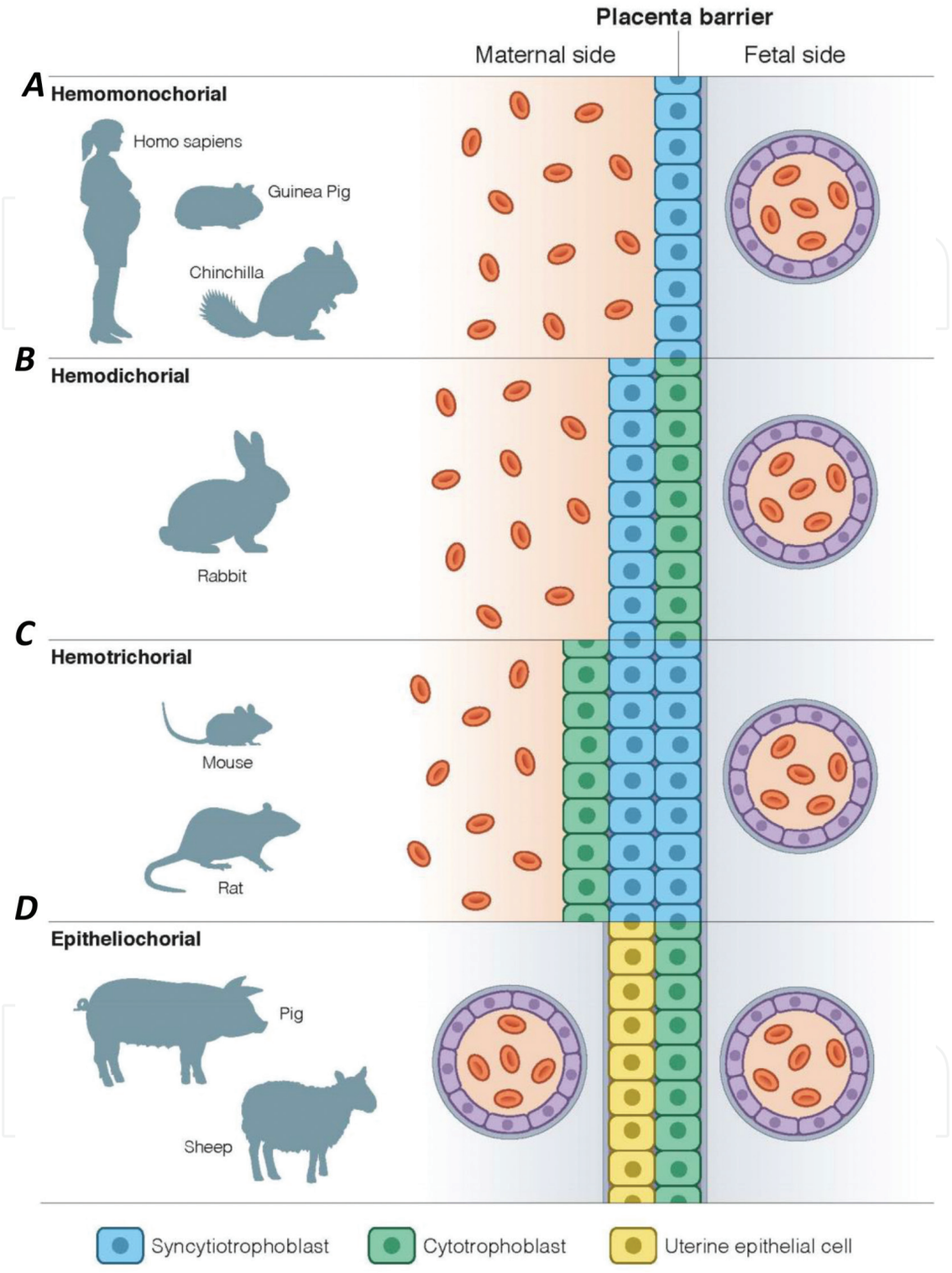



**Figure 1.** Schematic presentation of different placenta barriers as seen in a microscope. (A) Hemomonochorial placenta barrier as seen in, for example, human, guinea pigs and chinchillas. Only one layer of syncytiotrophoblasts separates the maternal blood space from the fetal capillaries. (B) Hemodichorial placentabarrier as seen in the rabbit. One layer of syncytiotrophoblasts and one layer of cytotrophoblasts separate the maternal blood space from the fetal capillaries. (C) Hemotrichorial placenta barrier as seen in, for example, mice and rats. Three layers of trophoblast cells separate the maternal blood space from the fetal capillaries. (D) Epitheliochorial placenta barrier as seen in, for example, sheep. One layer of uterine epithelium cells and one layer of trophoblast cells separate maternal and fetal capillaries. Furthermore, in all three cases, maternal and fetal blood is separated by connective tissue and basal laminae.

In relation to drug transfer, it seems obvious to use animal models to study the passage across placenta and potential teratogenic or toxic effects, but unfortunately the transplacental transfer and the placental metabolic demand vary greatly among species [7]. Drugs may transfer across placenta by passive transport, active transport or facilitated transport, whereas lipid-soluble molecules with a small molecular size can cross the placenta by passive diffusion. In that regards, the guinea pig seems as a more human translatable model compared to, for example, the sheep, because the guinea pig has a thin *hemomonochorial* placenta barrier compared to the thicker *epitheliochorial* placenta barrier in the sheep [7]. For hydrophilic molecules, the passive diffusion is negligible, and the transport capacity varies widely between species depending on the transport proteins located in the trophoblast cells. For example, the antidiabetic drug metformin, a hydrophilic molecule, is in humans transported across the placenta by organic cation transporters (OCTs). When studied in an animal model, it is highly relevant to identify the specific OCT transporters in the pregnant animal to verify the expression of the transport proteins [8]; otherwise the translatability has little value.

## 4. Animal models

**Table 1** shows the average gestation length, number of fetuses, maternal weight, neonate weight and the placental barrier type in human and relevant species.

**Basal gestation parameters of the laboratory animals**

| Animal species *Latin* | Gestation length (days) | Number of fetuses | Maternal pre-pregnancy weight (g) | Neonate weight (g) | Placenta barrier type |
|---|---|---|---|---|---|
| Human *Homo sapiens* | 266 | 1 | 5900 | 3183 | Hemomonochorial villous |
| Mouse *Mus musculus* | 20 | 5–6 | 19 | 1 | Hemotrichorial labyrinth |
| Rat *Rattus norvegicus* | 22 | 9 | 283 | 6 | Hemotrichorial labyrinth |
| Guinea pig *Cavia porcellus* | 67 | 3–4 | 728 | 80 | Hemomonochorial labyrinth |
| Chinchilla *Chinchilla lanigera* | 113 | 1–2 | 480 | 40 | Hemomonochorial labyrinth |
| Rabbit *Oryctolagus cuniculus* | 30 | 5 | 1591 | 39 | Hemodichorial labyrinth |
| Sheep *Ovis aries* | 153 | 1–2 | 39.100 | 2376 | Epitheliochorial |
| Pig *Sus scrofa* | 115 | 5–14* | 84.000* | 400–1900[a] | Epitheliochorial |

Data are acquired from the PanTheria database [82].*

Dependent of the breed of pig (domestic pig or mini-pig) [20].

**Table 1.** Basal gestation parameters in pregnant animal models.

### 4.1. Mice

Mice and rats are the most used species in research. Practical advantages include relatively low costs, an easy maintenance and a long tradition in scientific research. Mice have an important advantage in many genetic manipulated models along with inbreed strains. The mouse has a short gestation period of around 20 days, and it carries a litter size of 5–6, which allows for quick data collection. The placenta is of the *hemotrichorial* labyrinth type [5]. The newborn mice are neurodevelopmental immature with closed eyes. Because of the large litter size, it is difficult to measure and follow individual fetal and placental progress. In addition, the small size makes surgical procedures difficult.

### 4.2. Rats

Rats have a long tradition as research models because the intrinsic properties, like the physiology and macro- and microanatomy, are well-known [9]. Rats pose some of the same advantages and disadvantages as mice; a short gestation period (around 22 days), large litter size (around 9 fetuses) and placental structure of a *hemotrichorial* labyrinth type (**Figure 1**). The considerable larger size of rats compared to mice makes them more suitable for surgical procedures and diagnostic imaging. Unfortunately, the genetic manipulation is much less developed in rats than in mice, but this may become more pronounced in the future [10].

### 4.3. Guinea pigs

The guinea pig has a gestation length of around 67 days and gives birth to 3–4 precocious offspring with a well-developed nervous system at birth [2]. These characteristics make newborn guinea pigs suitable for research in fetal development. The placenta barrier is *hemomonochorial* (**Figure 1**), and it is histologically comparable with the human placenta barrier. In fact, the guinea pig is a well-established model to study placentation, and suggested to become one of the most important animal model for new placental studies in obstetric research [4]. They are affordable and easy to maintain in research environments. Intravenous approaches can be more complicated than for mice and rats due to the lack of a long tail.

### 4.4. Chinchillas

The chinchilla is not a traditional animal model in obstetric and fetal medicine. The chinchilla has mainly been used to study diseases of the ear due to similarities with human anatomy and function [11]. However, several characteristics of the gestation make the chinchilla a suitable model to imitate human pregnancy. Like the guinea pig, the chinchilla gives birth to 1–2 precocious offspring and has a *hemomonochorial* placenta barrier. Chinchillas have the longest gestational period (around 113 days) of any rodent, which is advantageous in longitudinal studies. The chinchilla has recently been used to study the placenta metabolism using hyperpolarized magnetic resonance imaging (MRI) [12]. Genomic and RNA sequencing information are available in this species [13]. Chinchillas are relatively cheap and easy to maintain in a research environment. However, the chinchilla has so far not been used to investigate intrauterine growth restriction (IUGR), preeclampsia or diabetic pregnancy.

### 4.5. Rabbits

The rabbit is known for its rapid reproduction with a short gestation of only 30 days and a litter size of around five cubs. Because coitus induces the ovulation, it is possible to time the gestation and obtain a precise age of the fetuses, which is of practical experimental advantage. In particular, the rabbit has been used to study reproduction and early embryogenesis [14]. The larger size of rabbits compared to rodents facilitates various diagnostic techniques, such as ultrasound imaging, allowing structural information about the fetal size and hemodynamic characteristics [15], and even fetal and placental vasculature and hemodynamics can be studied by Doppler ultrasonography [16]. The rabbit has a *hemodichorial* placenta barrier of the labyrinth type (**Figure 1**). The thickness of the trophoblast cells alternates, resulting in thick and thin areas of the barrier [5].

### 4.6. Sheep

The sheep has a gestation length of 153 days and gives birth to 1–2 neurodevelopmentally matured lambs with about the same weight as a human newborn [17]. Therefore, the sheep is a translatable model for investigating fetal physiology. However, the placenta structure is very distinct from the human placenta. The placenta barrier is of the *epitheliochorial* type where the uterus remains intact without invasion of the trophoblast cell. Thus, the fetal and maternal blood are divided by an intact uterine epithelium (**Figure 1**). The missing trophoblast invasion and no erosion of the uterine epithelium lead to a description of the placenta as "superficial" [5]. Sheep are easy to handle, and pregnant sheep tolerate invasive procedures [17].

### 4.7. Pigs

The anatomical and physiological similarities to humans make the pig an excellent animal model in, for example, research of metabolic, cardiovascular, infectious diseases, xenotransplantation and neurological disorders. Surgical and anesthetic procedures are well established in the pig [18], and the genome is today fully sequenced in parallel with the existence of an important homology between the human and pig genome [19]. However, regarding the gestation, the pig has some important differences from the human pregnancy. The pig, like sheep, has an *epitheliochorial* placenta barrier (**Figure 1**), where the uterine epithelium remains intact during the entire gestation period [5]. Depending of the type of pig, it gives birth to 5–14 piglets. The domestic pig has a litter size of 10–14 and a birthweight of 1.3–1.9 kg, whereas breeds of minipigs, like the Yacatan and Göttingen, has a litter size of 5–8 with a birthweight of 0.4–1.0 kg [20]. They have the same gestation length of around 115 days. Piglets are well-established models in fetal and neonatal research [21] and have been used, in particular, to study neonatal physiology in response to physical activity and nutrition [22].

## 5. IUGR models

Intrauterine growth restriction or retardation (IUGR) occurs when a fetus does not reach its genetic growth potential, mostly due to placental insufficiency with limited offer of oxygen

and energy; caused by multiple factors, that is, smoking, preeclampsia or multiple pregnancy. IUGR affects up to 8% of all human pregnancies and may lead to serious complications in the newborn. Similarly, IUGR also initiates late-onset diseases, such as diabetes and cardiovascular diseases. The most commonly used IUGR animal model is the rat, but pigs, guinea pigs, mice, rabbits and sheep have also been studied for this purpose (**Table 2**). Six different methods have been reported to obtain an IUGR animal model: (1) diet-induced IUGR, (2) heat-induced IUGR, (3) IUGR induced by artery ligation, (4) hypoxia-induced IUGR, (5) embolization-induced IUGR and (6) glucocorticoid-induced IUGR. The most frequently used methods are the diet and ligation approach.

### 5.1. Diet-restriction IUGR

Diet-induced IUGR has mainly been performed using either calorie restriction or low-protein diet. Calorie restriction is often provided via a 50% restriction diet as notably programs insulin resistance and hypertension [23]. This approach has been adopted by López-Tello, demonstrating a diet-induced IUGR rabbit model, where animals were offered 50% of daily global nutrition, allowing investigations of the early changes in fetoplacental hemodynamics [24]. Interestingly, they found that neonates from this group were significantly smaller than those in the control group, which were offered food *ad libitum* throughout the pregnancy, and that the IUGR-induced animals showed asymmetrical growth and brain sparing. Furthermore, the restriction diet provided a significant altered blood flow perfusion. Hawkins et al. investigated the impact of maternal malnutrition in early gestation on the ovine blood pressure and cardiovascular reflexes, also by reducing maternal global nutrition, but in this study only by 15% in the first 70 days of gestation [25]. This study showed that even mild maternal undernutrition altered fetal cardiovascular development and produced a low blood pressure. However this redcution was not sufficient to induce IUGR.

Low-protein diet in fetal programming features different compositions of macronutrients. The Southampton diet (SH) and the Hope farm diet (HF) is often used in fetal programming (**Table 3**). The main difference between these two diets is the amount of starch, simple sugars (sucrose and glucose) and lipids (corn oil and soy oil) vary, whereas SH has high starch

| **Animals** | **Methods** | | | | | |
|---|---|---|---|---|---|---|
| | **Diet induced** | **Artery ligation** | **Heat induced** | **Embolization** | **Hypoxia** | **Glucocorticoid** |
| Rats | [83] | [84] | | | [31] | [85] |
| Mice | [86] | [27] | | | [30] | |
| Guinea pigs | [87] | [28] | | | | |
| Rabbits | [24] | [88] | | | | |
| Sheep | [25] | | [32] | [33] | [89] | [90] |
| Pigs | [91] | | | | | |

**Table 2.** IUGR models (number refers to reference list).

| Diet | Protein | Fibre | Starch | Simple sugars | Lipid | Methionine |
|---|---|---|---|---|---|---|
| **Southampton diet 9%** | Casein 9% | 5% | 48.5% | Sucrose 23.4% | Corn oil 10% | 0.5% |
| **Southampton diet 12%** | Casein 12% | 5% | 46.5% | Sucrose 24.3% | Corn oil 10% | 0.5% |
| **Hope Farm diet** | Casein 9% | 5% | 8% | Glucose 66,7% | Soy oil 4,3% | 0.2% |

**Table 3.** Different low-protein diets used for fetal programming.

content (42–51%), corn oil (10%) and sucrose (21–24%) and HF has low starch (8%), soy oil (4.3%) and glucose (53–67%). They also differ in their impact on the offspring; the SH has shown to program hypertension whereas HF programs insulin resistance [26].

### 5.2. Ligation-induced IUGR

IUGR induced by artery ligation is frequently used in animal research. Ligation intends to reduce blood flow and thereby oxygen and nutrition to the fetus. This approach has been introduced in relevant animals (**Table 4**). Notice that all the listed animals have bicornated uteruses while humans have a simple pyramid-shaped uterus [1]. These animals have two large horns and each have their own blood supply, allowing animal to act as both control (one horn) and case (another horn). Ligation is performed on the uterine vessel and can be performed either unilaterally (on only one of the horns) or bilaterally.

The timing and the site of ligation is of important matter in the ligation-induced IUGR model. Ligating at the distal portion of the uterine vessel implies a complete blockage of the iliac artery and the uterine blood supply is then solely dependent on the ovarian artery. Conversely, when ligating at the central portion of the uterine vessel, the blood supply comes from both the ovarian and iliac artery, resulting in a less affected uterine blood delivery.

| Animals | Methods | | | | |
|---|---|---|---|---|---|
| | Artery ligation | NO reduction | RAS-related models | Immunological | Transgenic models |
| Rats | [37] | [40] | [92] | [47] | |
| Mice | | [42] | [43] | [48] | [50] |
| Guinea pigs | [93] | | [94] | | |
| Rabbits | [95] | | | | |
| Sheep | [96] | | | | |
| Pigs | | | | [97] | |

**Table 4.** Preeclampsia models (number refers to reference list).

Janot et al. demonstrated that ligating on the central portion of the uterine vessel was necessary to maintain a viable pregnancy, by establishing IUGR models in mice with ligation at either positions [27]. Mice ligated at the distal portion had a 100% abortion rate and a 50% mortality rate. In contrast, mice ligated at the central portion had an abortion rate of 75% (but still inducing a characteristic IUGR profile) and no maternal mortality. Herrera et al. used an ameroid occlusion to ligate the uterine artery bilateral in guinea pigs at day 35 of gestation [28] (**Figure 2A**). The occlusion led to an increased placental vascular resistance associated with a decreased fetal and placental weight, and the study also showed asymmetrical growth of the fetal organs.

### 5.3. Hypoxia-induced IUGR

Hypoxia has been shown to affect the size of the offspring pathologically and functionally [29]. Hypoperfusion of placenta increases the amount of reactive oxygen species, causing oxidative stress and a reduced vasodilation. Ligation, as described earlier, also causes hypoperfusion of placenta creating hypoxia, but in this section hypoxia will be refered to as reduced environmental oxygen saturation. Rueda-Clausen et al. studied the impact of hypoxia on IUGR and preeclampsia in mice [30]. Mice were mated and randomly assigned to either cases or controls. Cases were placed in a sealed chamber for 3 days with an oxygen concentration of 10.5% ± 0.3% (normal oxygen content is 20% in atmospheric air) and then placed in clean cages. This prolonged lack of oxygen significantly induced IUGR, but the pub survival was down to approximatly 10%. Tapanaien et al. found that rat dams having an oxygen concentration of 13–14% induced IUGR with a birthweight of 24% lower than controls (20% oxygen), but without significant fetal death, suggesting that an oxygen concentrations of 13–14% may become beneficial for inducing of IUGR [31].

### 5.4. Additional methods for IUGR

#### *5.4.1. Hyperthermia*

Galan et al. initiated a study by exposing five pregnant ewes to hyperthermic conditions for 80 days, initiated from the 40th gestation day [32]. The ewes were exposed to 40°C during the day and 35°C during the night. The study established an interesting IUGR model with some similarities with the human IUGR (asymmetrical growth, hypoxia and hypoglycaemia). Even though this method successfully induced IUGR, a more widespread use of this hyperthermic-based IUGR model could become difficult due to animal ethical restrictions, and this method has only been reported in sheep.

#### *5.4.2. Embolization*

Duncan et al. induced IUGR by injecting microspheres of 15–30 μm into the umbilical-placental vascular bed from day 120 of gestation in a sheep model [33]. This procedure reduced the fetal oxygen saturation to 50%, resulting in significantly reduced growth and significant altered pH, $SaO_2$ and $pO_2$.

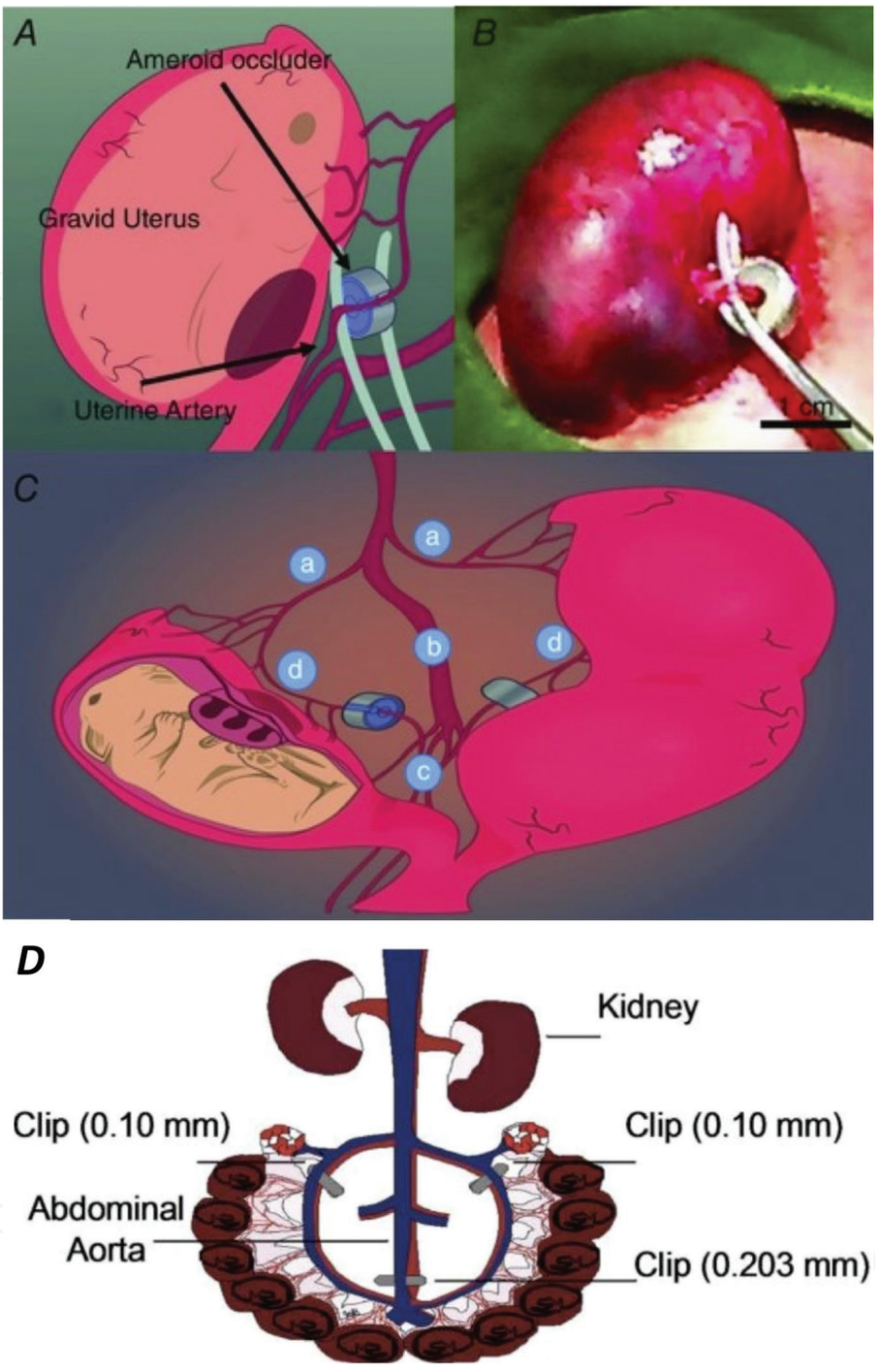



**Figure 2.** Ameroid occludder placement (reproduced from ref. [28]). Schematic representation (A and C) and photograph (B) of the placement site of the ameroid constrictors in the uterine artery of a pregnant guinea pig at 35 days of gestation. C shows the maternal artery supply to the uterus in gunea pigs; a, ovarian arteries; b, aorta; c, uterine arteries; d arcade arteries. D shows induction of reduced uterine perfusion pressure (RUPP) model in pregnant rats (reproduced from ref. [37]). In the rat RUPP model, laparotomy is performed through an abdominal incision on day 14 of gestation. A silver clip with a 0.203-mm internal diameter is placed around the aorta right above the iliac bifurcation, and silver clips with 0.1 mm internal diameter were placed around the left and right uterine arcade at the ovarian artery before the first segmental artery. Uterine perfusion pressure in the gravid rat is reduced by ~40%. Blood pressure is measured via a carotid arterial catheter.

*5.4.3. Glucocorticoid*

Exposure to glucocorticoid during pregnancy has long been known to be associated with a low birthweight and concomitant adult diseases. When investigating the effect of glucocorticoid, it is necessary to distinguish between natural cortisol and synthetic glucocorticoid. Previous studies have shown that dexamethasone induces hypertension in rodents whereas cortisone acetate and betamethasone do not [23]. Additionally, different species and sex may react differently to glucocorticoid exposure. When looking at long-gestation mammals, the timing of glucocorticoid exposure is essential. Exposure to glucocorticoid in the pregnant ewe in the early gestation has shown to induce hypertension in adulthood of the offspring, whereas exposure late in gestation promoted insulin resistance rather than hypertension in the offspring. Glucocorticoid exposure can be administered subcutaneously, through maternal drinking water or intraperitoneal injections [23].

## 6. Preeclampsia models

Human preeclampsia is a multiorgan disorder with onset after the 20th week of gestation. The dignostic criteria includes a blood pressure that exceeds 140 mmHg (systolic) and 90 mmHg (diastolic) and simultaneous dection of proteinuria. The condition can lead to kidney failure, liver rupture, stroke, eclampsia with seizures and HELLP syndrome [34]. The definitive pathogenesis of preeclampsia is yet to be found but may be associated with oxidative stress, angiogenic factors, an immunological response between mother and placenta or superficial placentation [35]. Appropriate animal models of preeclampsia must meet the following criteria (**Table 4**): they should initiate hypertension, proteinuria and endothelial dysfunction, and furthermore, resolve after delivery of the placenta [34]. Preeclampsia is presumably caused by reduced uterine blood flow due to abnormal trophoblastic invasion in spiral arteries. This has implicated the need of an animal model of reduced uterine perfusion pressure to study the mechanisms within preeclampsia. In 1940, one of the first studies describing this correlation was performed [36], demonstrating pregnancy-mediated hypertension in dogs following partial ligation of the infrarenal abdominal aorta. This ligation procedure has subsequently been performed in rabbits, monkeys, sheep, primates, guinea pigs and rabbits [37]. One of the best-described studies was performed in baboons [38], showing that hypertension occurred in parallel with renal changes due to uteroplacental ligation, supporting the view that hypoxia/ischemia participates in the potential mechanisms underlying the pathogenesis of preeclampsia. Rodents are also reported as important ligation-induced models of preeclampsia. Preeclampsia in rats has been established by clipping around aorta, above the iliac arteries, and at both uterine arteries, at day 14 of gestation (**Figure 2D**), providing characteristic pathological conditions, including hypertension, proteinuria and renal impairment [37].

### 6.1. Additional methods

*6.1.1. Nitrogen oxide reduction*

Another approach to stimulate the conditions of preeclampsia is to manipulate genes thought to influence the pathogenesis. NO production is reduced in preeclampsia [39], and several

studies have been performed to mimic this pathogenesis [40],[41]. A study by Molnár et al. inhibited the NO synthase in pregnant rats [40], resulting in hypertension, proteinuria, thrombocytopenia and IUGR; all characteristic findings that were considered consistent with preeclampsia. However, one study in eNOS knockout mice showed, controversially, a decreased blood pressure [42].

*6.1.2. RAS-related models*

Women with preeclampsia have general elevated levels of autoantibodies ($AT_1$-AA) that bind and activate the angiotensin II type 1a receptor, mediating augmented blood pressure. To imitate this pathogenesis, Zhou et al. successfully injected purified $AA_1$-AA into pregnant mice, resulting in hypertension, proteinuria, placental abnormalities, glomerular endotheliosis and small fetus size [43]. This study also showed that co-injection of losartan ($AT_1$-antagonist) prevented these conditions. However, losartan in human pregnancies is contraindicated due to teratogenicity.

*6.1.3. Anti-angiogenic factors*

In pregnancy, VEGF plays an important role in angiogenesis, while placental growth factor (PIGF) plays an important role in placentation. Preeclampsia in women, however, shows elevated levels of sFlt-1, a VEGF receptor binding and inactivating both VEGF and PIGF. sFlt-1 has been introduced to both mice and rats by a adenoviral vector, demonstrating preeclampsia characteristics (increased BP, proteinuria and glomerular endotheliosis) [44-46] . A limitation of this method is that the reported results were not specific to pregnancy and were dose dependent [44].

*6.1.4. Immunological methods*

A host of immunological mediators, thought to be a part of the pathogenesis in preeclampsia, have been studied in animal models, including TNF-$\alpha$ (tumor necrosis factor), IL-6 and anti-IL-10, and all mediators provoked elevated blood pressures [47] [45] [46]. A study by Zenclussen et al. injected T-helper-1-like-cells into mice, causing increased blood pressure, proteinuria and glomerular fibrosis [48]. This method is interesting as it exhibits the inflammatory pathway, but they are considered unlikely to participate in the primary events of preeclampsia.

*6.1.5. Transgenic models*

It is well-known that a genetic predisposition exists in relation to preeclampsia [34]. Transgenic mice models can be generated to study the influence of relevant genes. The APOL1 gene encodes apolipoprotein L1, and variants of the gene (APOL1-G1 and -G2) are associated with kidney disease [49]. As the gene is only found in humans and some primates, transgenic mice models were developed to study the gene variants *in vivo*. Beckerman et al. found an association between the APOL1 gene and a preeclampsia phenotype that occurred during the second half of pregnancy with significant blood pressure elevation, loss of litters and maternal death from eclampsia [50]. Mice with the G2 gene variant were affected more severely. Importantly, also wild type mice carrying transgenic litters developed eclampsia, which is consistent with the known influence from the fetal genotype and the placenta.

There are several other ways to induce preeclampsia, including adriamycin-induced, chatechol-O-methyltransferase-deficient and BPH/5 mice strain. However, these methods have only been used in mice and will not be discussed further [51].

## 7. Diabetic pregnancy

Diabetes in pregnancy is divided in two groups, pre-gestational diabetes mellitus (PGDM) and gestational diabetes mellitus (GDM). In PGDM, the pregnant woman suffers from diabetes acquired prior to onset of pregnancy. PGDM is subdivided in type 1 (insulin deficiency) or type 2 (insulin resistance). Type 1 diabetes (DM1) is caused by an autoimmune reaction against the insulin producing pancreatic β cells [52]. DM1 is often diagnosed in early childhood, and DM1 patients will require exogenous insulin. Type 2 diabetes (DM2) is the most common, less severe type of diabetes. In DM2, the skeletal muscle and adipose tissue are insensitive to insulin, and the β cells fail to compensate.

In normal pregnancy, maternal tissues become progressively insensitive to insulin. This effect is likely caused by hormones from the placenta. In order to maintain a euglycemic state, the woman must increase her insulin secretion by 200–250%. About 3–10% of the pregnant population is unable to produce an adequate insulin response to compensate this insulin resistance and they develop GDM. The choice of animal model should depend on the type of diabetic pregnancy that the research aim to study. Diabetes can be induced pre-gestationally or gestationally as either an insulin-resistant or insulin-deficient model and the following methods can be used to induce PGDM or GDM in animals: (1) surgical induced (partial pancreatectomy), (2) chemical induced (streptozotocin or alloxan), (3) diet induced and (4) genetic models.

### 7.1. Surgical-induced diabetic pregnancy by partial pancreatectomy

Partial pancreatectomy is provided by removal of up to 95% of pancreas prior to mating, leading to onset of PGDM with concomitant insulin deficiency [53]. This model was introduced in female rats in 1970 [54], but the model is hampered by several factors: surgical complexity, high post-surgical mortality, a long time between surgery and development of diabetes (2–3 months) and sequelae like digestive problems from the missing exocrine pancreas. However, partial pancreatectomy in sheep fetuses in late gestation has been used to study fetal insulin and glucose metabolism *in utero* [55].

### 7.2. Chemical-induced diabetic pregnancy

A widely used method for induction of experimental diabetes is chemical destruction of pancreatic β cells, resulting in insulin deficiency. This approach resembles a DM1 model, but it has been used to mimic GDM. Streptozotocin and alloxan are the most used drugs, especially in rats and mice. The amount of time required to induce diabetes and the phenotype (mild to server diabetes) depend on factors such as animal species, strain, dose and mode of administration (sc, iv, ip or im) [56]. In rats, streptozotocin has shown to cause ovarian dysfunction

[57], and untreated diabetes generally results in subfertility. For these reasons, streptozotocin is often administered on the day of mating in order not to interfere with a successful mating and where the risks of direct toxic effects on the embryo are little [56].

### 7.3. Diet-induced diabetic pregnancy

Obesity is a well-known risk factor for DM2 and GDM [58]. Feeding with high-fat diets and/or high concentrations of sucrose and fructose induces insulin resistance, and this approach is used to create animal models of DM2 and GDM in rats, mice and sheep (**Table 5**) [56] [59]. This method is cheap and accessible, but relatively more time-consuming than chemical induction. Holemans et al. fed female rats with a diabetogenic diet 4 weeks prior to mating and during gestation [59]. They found that diabetes was not present prior to mating, but was confirmed at gestation day 20, resembling a GDM model. Liang et al. used a similar protocol in mice, but diabetes was developed pre-gestational in this study [60]. In sheep, a 60 days of diabetogenic diet before mating resulted in insulin resistance and increased fetal adipose tissue and β cell mass in mid-gestation (gestation day 75) [61]. Another way to study hyperglycemia and hyperinsulinemia and the impact on the fetus is by continuous iv glucose infusion during gestation [62]. However, this method is considered too simple and lacks the complexity of a diabetic pregnancy.

### 7.4. Genetic models of diabetic pregnancy

Several genetic mice models of diabetic pregnancy exist. Genetic engineering and inbreeding are unfortunately impossible in several species [56]. The "non-obese diabetic" mice and "bio breeding" rats are inbreed strains spontaneously developing DM1. They are used to study fertility and fetal complications in DM1 diabetic pregnancy [53]. The "db/db" mouse is a classic DM2 model with a mutation in the leptin receptor gene (ObR) resulting in excessive appetite and hence obesity [63] [56]. These mice are infertile, but the heterozygote "db/+" mouse are fertile and develops insulin resistance during gestation, and they are therefore providing a model of GDM [64]. Newborns of "db/+" mice show complications related to GDM like

| | **Methods** | | | | |
|---|---|---|---|---|---|
| | **Partial pancreatectomy** | **Chemical** | | **Diet** | **Genetic** |
| | | **STZ** | **Alloxan** | | |
| Rats | [54] | [98] | [99] | [59] | [100] |
| Mice | | [101] | [102] | [60] | [64] |
| Guinea pigs | | [103] | [104] | | |
| Rabbits | | [105] | [106] | | |
| Sheep | [107] fetal surgery *in utero* | [108] | [109] | [61] | |
| Pigs | | [110] | [111] | | |

**Table 5.** Diabetic pregnancy models (number refers to reference list).

macrosomia regardless of fetal genotype. An important factor is that the diabetic phenotype of the "db/+" mouse is not present prior to gestation, making this model more transferable to GDM than many other models [56].

With genetic models of diabetic pregnancy, it is important to remember the genetic predisposition to diabetes in the fetus. Embryo transfer can be used to study the influence of maternal diabetes separately from the fetal genotype [65]. Many genes affecting β cell function in pregnancy can be mutated in mice to induce a diabetic phenotype [56].

## 8. Imaging diagnostics of the pregnant animal

Ultrasound imaging is widely used in small animal practice for the evaluation of the pregnancy and determination of number of fetuses, and it is also used to monitor abnormal pregnancies, such as poorly fetal development for gestational age and to identify pregnancies in which there is embryonic resorption or fetal abortion. In the placenta, ultrasound-based Doppler is the first-line technique for the evaluation of uteroplacental blood flow. The Doppler technology is based on analysis of the change in frequency or intensity of ultrasound waves when they are reflected by a moving target such as erythrocytes. Ultrasound exposure is considered harmless; and in fact, animal experiments subjected to fetal ultrasound imaging in various mammalian species showed no pathological effects for the embryo, no congenital malformations or adverse neurobehavioral effects [66]. The technique is often combined with simultaneous administration of a sonographic contrast agent, resulting in an enhanced gray scale or color Doppler signal, facilitating visualization of microvascular structures down to the microvascular perfusion. The mean diameter of micro-bubbles ranges from 2 to 10 μm, less than that of a red blood cell but sufficiently large to be trapped within the vascular space [67]. Thus, ultrasound imaging allows discrimination between fetal and maternal circulatory systems by imaging the intervillous space alone, and it could be used to diagnose the abnormalities of placental blood flow [68] (**Figure 3A**).

MRI is another non-invasive method for diagnostic information. MRI uses the body's natural magnetic properties to produce detailed images from any part of the body. For imaging purposes, the hydrogen nucleus is used because of its abundance in water and fat. What makes MRI so powerful is the exquisite soft tissue and anatomic details. MRI has been increasingly used for detailed visualization of the fetus *in utero* as well as placental structures. While small rodents have fetal sizes that are difficult to investigate with most MRI systems, recent development in very high-field MRI systems now allows visualization of fetal anatomical structures down to a mouse fetus. Wu et al. demonstrated how embryonic mice brain structures could be delineated *in vivo* at embryonic day 17 using an 11.7 T MRI system. *In utero*, 3D MRI has been extensively used in larger animals in clinically available MRI systems. As an example, high-resolution MRI of the inner ear structures of fetal sheep *in vivo* has been demonstrated [69]. Pregnant domestic pigs, on the other hand, are too large to fit in a standard MRI machine bore, precluding MRI as a diagnostic tool in this animal model. Non-brain investigations of the fetus have been increasingly performed using MRI.

Similar to the ultrasound-based contrast-enhanced method, an excellent soft tissue image contrast can be obtained by MRI contrast agents; usually a paramagnetic (gadolinium) molecule that alters

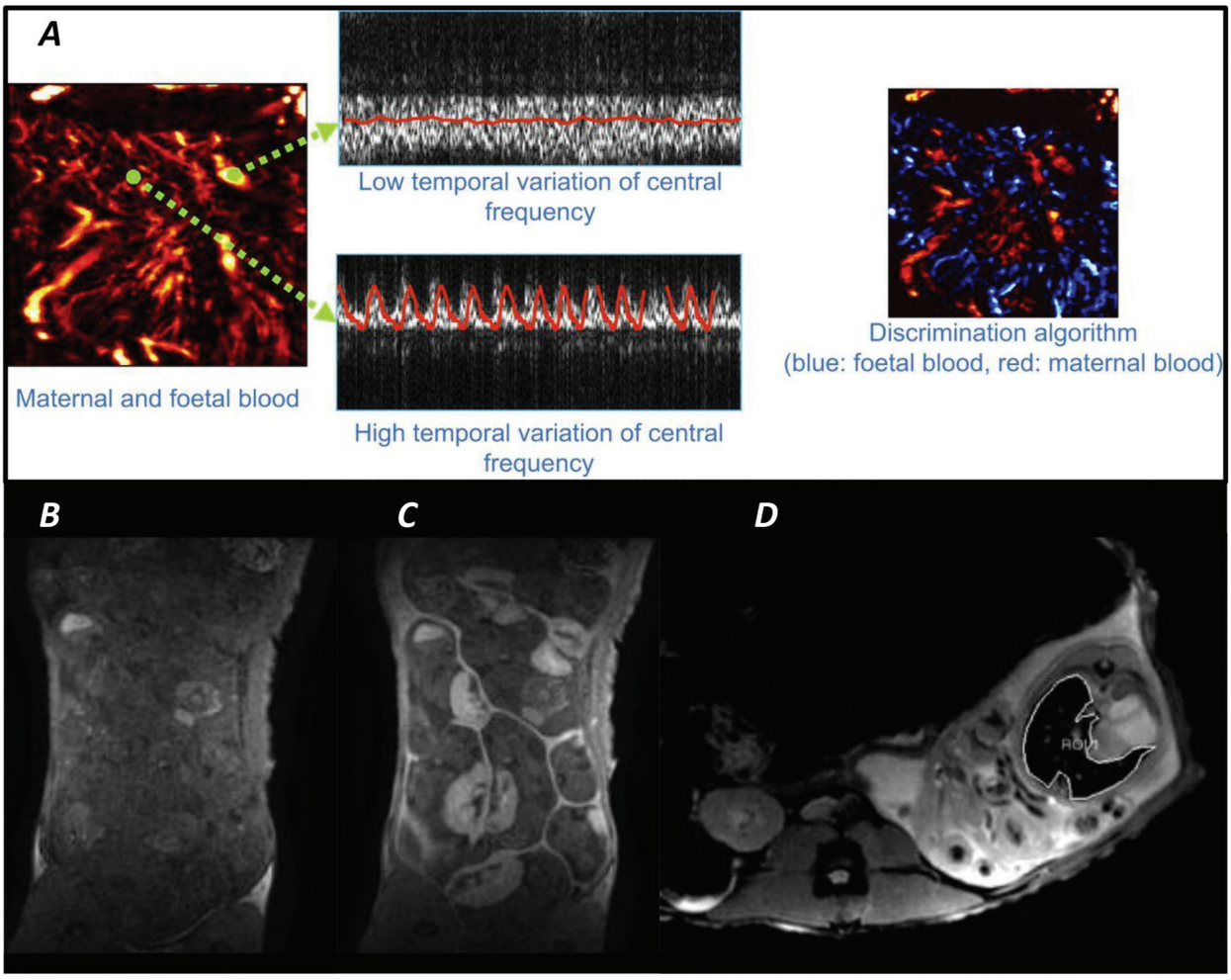



**Figure 3.** Placental blood flow mapping with discrimination of fetal and maternal circulation using ultrafast ultrasound Doppler in the rabbit model. With this technique, the pulsatility of each placental vessel is analyzed. Discrimination between maternal and fetal blood flows is performed using advanced analysis (A). Evaluation of placental blood flow with magnetic resonance imaging (MRI) in the rabbit model before (B) and after (C) injection of contrast product. Blood oxygen level-dependent MRI showing an axial view of the fetus with the fetal liver selected as the region of interest (D). (A–C are reprinted from ref. [66]; D is reprinted from ref. [71]).

the intrinsic T1-relaxation times of various soft tissues where the contrast agent accumulates. For example, Mourier et al. have evaluated placental blood flow with MRI in a rabbit model before and after injection of a paramagnetic contrast agent [68] (**Figure 3B–C**). Animal studies have shown that small-size gadolinium agents cross the placenta and are extracted by the fetal kidneys into the amniotic fluid [70] [71]. Mikkelsen et al [12] revealed a high signal of [1-13C]-pyruvate and its derivative [1-13C]-lactate in the chinchilla placenta using hyperpolarized MRI; a non-harmful imaging modality using non-ionizing endogenous substrates for interrogating accumulation and metabolic pathways. In parallel, Friesen-Waldner et al. examined noninvasively the fetoplacental metabolism and transport of pyruvate in guinea pigs using the same technique [72]. The relation between maternal oxygen challenge and fetal oxygenation has recently become possible to study using the blood oxygen-level dependent (BOLD) MRI sequence; a non-invasive technique for evaluating organ tissue oxygenation that requires no contrast exposure (**Figure 3D**). Studies in sheep fetuses have shown that changes in cotyledon and fetal BOLD MRI signals are closely related to changes in fetal oxygenation estimated by fetal arterial hemoglobin saturation [73]. MRI

has also shown promises in the fetal heart. As example, Yamamura et al. have demonstrated the applicability of MRI for future evaluation of fetuses with complex congenital heart defects [74].

Moreover, more sophisticated MRI sequences, such as diffusion-weighted imaging (DWI), MR spectroscopy and diffusion tensor imaging allow for visualization of inherent structural, metabolic, cellular and microvascular characteristics. While these techniques have potential applications in fetal imaging, the familiarity with fetal MRI is still limited within researchers working with animal pregnancies.

## 9. Animal ethics and handling

From an ethical viewpoint, animal experiments involving pregnant animals do not differ from other types of animal experimentation, but the ethical aspects of using fetuses for experiments should be considered. The 3Rs should be considered in all other types of animal experimentation [75], including the options for replacement, which is the first R. One obvious alternative option is to use the placenta from women who have just given birth [76]. The maternal and the fetal circulations are re-established with a pump system, and this option has been used to study the passage of chemical substances from mother to fetus [77]. In addition to the fact that this alternative can replace the use of live animals, it also has the advantage of avoiding problems related to species differences in the maternal-fetal barrier. However, it can only represent transport in the last part of the third trimester. The second R, reduction, should not only include the number of mothers, but also the number of fetuses. The studies should be conducted so that no more experimental animals are used than is necessary to obtain statistically safe results [75]. The third R, refinement, should importantly include the accommodation, feeding and care of the experimental animals based on the specific requirements of the pregnant animals.

### 9.1. Physiological effects of gestation

The physiology of pregnancy can create stress problems in animals which therefore pose special requirements for their handling, care and anesthesia. Most studies on this topic have been performed in ewes, but the same changes are expected in other species. When the size of the fetus increases, more energy and blood must flow to the uterus, and therefore maternal blood volume, cardiac output and contractility approach their maximum [78]. Additionally, the lungs should be able to deliver an increased amount of oxygen while the uterus presses on the diaphragm so that the thoracic cavity volume is decreased. As a result, respiratory rate increases, but at the same time the risk of hypoxia increases even during short-term apnea. The expanding uterus further delays gastric emptying and decreases the esophageal sphincter tone, which increases the risk of regurgitation and aspiration pneumonia during anesthesia. Special conditions apply to sheep and other ruminants, as the last part of the gestation period is characterized by very little space in the abdominal cavity, which may limit the volume of the fermenting compartment so that feed intake is limited. Furthermore, pregnancy may also prolong plasma half-lives of anesthetics and other drugs, and the high level of progesterone will have a sedative effect, and therefore anesthesia doses must be reduced to prevent overdosing [79]. The fasting period prior to anesthesia should be minimized to prevent metabolic disturbances.

### 9.2. Feeding pregnant animals

Many females will have a temporary loss of appetite in the first part of the pregnancy, and an increased appetite later. In general, the nutrition requirements are the same as for non-pregnant females. However, in the last part of the gestation period, the fetus growth will increase dramatically, and so will the nutrition requirements for the mother [80]. For the species with largest litters and heaviest fetuses, the need for energy will increase most. Pregnant rodents should typically be fed with a special breeding mix, which has a slightly higher content of proteins, vitamins and minerals. They are typical feed *ad libitum*, so the increased amount of feed is not observed, but for *restricted* fed animals, like pigs, the amount should be adjusted. Pregnant sows should be fed individually so that they maintain a normal weight and body mass, and their energy needs will specially increase during the last 4 weeks [81]. The composition of the sow feed does not need to be changed once it has been ensured that it contains sufficient amino acids; this is particularly important in young sows. Sheep are fed normal maintenance diet during the first 2/3 of the gestation period, and it should be ensured that they maintain a normal weight and body mass. In the last 1/3 of the pregnancy, the feed requirement increases, and as the space in the abdominal cavity is limited, it is important to feed them with a high quality feed that does not overload the rumen.

### 9.3. Handling and care of pregnant animals

Pregnancy poses special requirements for the handling of experimental animals. Generally, pregnant animals tolerate less stress than non-pregnant animals, and should be transported as little as possible during the first and last part of the pregnancy. At the beginning of pregnancy, the implantation process of ovarian eggs is sensitive to stress, and ultimately in pregnancy, the mothers are physiologically stressed and therefore have a low threshold of stress tolerance. Mice and rats are bred in monogamous (one male and one female) or polygamous mating systems (one male and two to six females). In guinea pigs, the polygamous mating system can be practiced with 1 male to 10 females. In polygamous systems, the females are removed from the male before they give birth. For pigs, the gilts will go into estrus after contact with a boar, and after mating, the pregnant sows are group housed. The sheep differs by being seasonally polyestrous. Ewes are typically paired in autumn so that they lamb in the spring. If the animals should give birth, they must have access to pre-birth material during the last days of gestation.

### 9.4. Anesthesia of pregnant animals

The anesthesia risk is higher in the pregnant than in non-pregnant animals due to physiological alterations described above [79]. In general, anesthetics can cross the blood-brain barrier and will usually cross placenta. Therefore, in some species, local anesthesia, such as epidural anesthesia, is preferred due to their minimal systemic effects; this applies especially to cows, sheep and other ruminants where general anesthesia furthermore can lead to tympanitis. In most other species, it is necessary to use general anesthesia. The choice of anesthetics depends on the animal species, but drugs generally have to be selected for their minimum effects on cardiac output, renal blood flow and fetus physiology [79]. Drugs with major depression effects on the fetus should be avoided. Inhalation drugs can be used, but as the degree of neonatal depression depends on the maternal anesthesia depth, higher doses should be avoided. Furthermore, they

can induce decreased uterine blood flow and fetal acidosis. Both sevoflurane, isoflurane and nitrous oxide are safe to use. Caution should be taken when using opioids as these are only slowly eliminated in the fetus. Xylazine and other alpha-2-agonists are also problematic as they have major depression effects on the fetus. During cesarean section, anticholinergic drugs should be given for inhibition of vagal tone during uterus traction, and ketamine can be used in combination with other drugs, such as thiopental, as long as ketamine is administrated in low doses. Propofol induces a rapid anesthetic phase and is rapidly cleared from the neonates blood circulation [79].

## 10. Conclusion

Because there is no animal model equal to the human situation, caution should be taken to extrapolate the results to human diseases. As one animal model may have advantages in one study, it may have disadvantages in others. The careful choice of model is therefore crucial. Mice and rats are currently the most used animal models to study pregnancy, including pregnancy-related diseases like IUGR, preeclampsia and diabetic pregnancy. However, larger animal models, like the guinea pig or sheep, have advantages making them more translational to human pregnancy. Introduction of new diagnostic techniques has facilitated (non-invasive) imaging of physiological, hemodynamic and metabolic measures, even in the smallest animal models. In parallel, the increasingly better management of feeding, handling, care and anesthesia of the pregnant animals reduce physiologically stress. These factors contribute to an increasingly translatability to the human pregnancy. The use of animal models provides a way to gain insight into the improved understanding of the human pregnancy, and there are today available pregnancy-related animal models that facilitate experimental studies that cannot be made in humans.

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