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# Pregnancy and placental development in horses: an update

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## Highlights

- ~~Many women scientists contributed to the understanding of equine foetoplacental development~~
- Next generation sequencing and imaging data provide new insight on equine placenta
- Mare nutrition and breeding management affect foal growth and health
- Research is needed to determine the critical periods of pregnancy on foal health

## **Abstract**

Horses have been domesticated by man and historical information mostly associates horses with men. Nowadays, however, horse riding is essentially practised by women. Women are also very much involved in equine sciences, with a large contribution to the understanding of foetoplacental development. While highlighting the work of female scientists, this review describes the recent advances in equine foetoplacental studies, focussing on data obtained by new generation sequencing and progress on the understanding of the role of placental progesterone metabolites throughout gestation. A second emphasis is made on foetal programming, a currently very active field, where the importance of maternal nutrition, mare management or the use of embryo technologies has been shown to induce long term effects in the offspring that might affect progeny's performance. Finally, new perspectives for the study of equine pregnancy are drawn, that will rely on new methodologies applied to molecular explorations and imaging.

## **Keywords**

equine; mare; allantochorion; gestation

## 1. Introduction

Horses have been domesticated by man and historical information mostly associates horses with men, although the working horse's contribution to the survival of women in primitive and current civilisations is essential [1].

The horse industry at large supports the development of horse riding, with reproduction at the heart of the production of horses, whatever the discipline. In addition, most animals must prove their sporting potential before they are allowed to breed [4]. With an 11 months pregnancy length, a late implantation, the existence of two different kinds of placentation and a very late foetal maturation [5,6], the development of the equine conceptus into a healthy newborn is a challenge both to the breeder and the veterinarian. Recently, with the sequencing of the equine genome [7,8] and high throughput gene expression studies becoming more affordable, large amounts of data have been providing new mechanistic insights on normal and pathological pregnancy.

The objective of this review is thus to sum up currently available data on horse placentation, pointing towards recent data and scientific challenges in this scientific area in particular in response to maternal environment. This review focuses on the placental period of pregnancy, *i.e.*, from implantation.

## 2. Implantation, endometrial cups and placental proteins

As early as 1975, the observation of discrepancies between early pregnancy diagnosis and actual pregnancy rates in Thoroughbred mares led to the hypothesis that the stress induced by management systems was important for early and late pregnancy loss [10], leading the way to modern understanding of maternal influence on foeto-placental and postnatal development.

The equine embryo enters the uterus at the late morula/early blastocyst stage, where its movements in the uterine cavity have been shown to be necessary for maternal recognition of pregnancy (for review [12]). The blastocyst remains free and the zona pellucida is replaced by a glycoprotein capsule that plays an important role in the cessation of embryo movement at 16-17 days of pregnancy [13]. Implantation begins at 35–40 days after ovulation, and therefore an important part of embryo organogenesis begins before its implantation.

Implantation is characterized by the co-existence of endometrial cups, an invasive trophoblast, stemming from the embryo's chorionic girdle, that will remain active until the 4th month of pregnancy, and the non-invasive allantochorion that will enable foeto-maternal exchanges until term. On Day 36 post-ovulation, binucleated cells from the chorionic girdle start invading the maternal endometrium to become the endometrial cups (Figure 1). Mature cups appear as grey structures with a depressed surface on the endometrium. A single layer of apparently normal trophoblast cells persists beneath the girdle, ensuring continuity of the chorion [14]. Their lifespan is 60-100 days, throughout which increasing numbers of leukocytes accumulate in the surrounding endometrial stroma, invade and appear to destroy the cups cells until, eventually, they are sloughed from the surface of the endometrium [14].

## 2.1. Gene expression in endometrial cups

In the past 10 years, insight into mechanisms involved in the development of the equine conceptus at the time of implantation and on the aetiology of late embryo losses has been gained, largely due to recent contributions relying on New Generation Sequencing (NGS) [15–18].

Many genes expressed by the endometrial cups are shared with the chorionic girdle but there is also a clear effect of the endometrial environment. On Day 45, shortly after implantation, immune cells (Tregs, CD4+ and CD8+ T cells) [15] and also NK cells are present in the microenvironment of the endometrial cups [19]. The high level of MHC Class I expression observed in the chorionic girdle decreases after the endometrial cups are established and increases again around the time of their death [20], believed to be due to programmed cell-death or lymphocyte mediated destruction.

The expression of the equine chromosome 24 miRNA cluster (C24MC), an ortholog of the human C14MC, reported to be involved in embryo and foetoplacental development [21], was explored by the group of Barry Ball, with the contribution of Shavahn Loux [22]. In the mare's serum, four miRNAs were enriched in mares at Day 25 of gestation compared to non-pregnant controls. Of these, four (eca-miR-409-3p, eca-miR-379-5p, eca-miR-1247-3p and eca-miR-134-5p) were significantly upregulated in the serum of Day 45 mares, compared to non-pregnant controls and three (eca-miR-127-5p, eca-miR-370-3p, and eca-miR-412-3p) were significantly upregulated in the serum of Day 45 mares, compared to 4 months pregnant mares [23], with a similar expression profile in chorioallantois [24]. Some of these could thus be used for pregnancy check when ultrasound diagnosis is not possible. Gene Ontology (GO) analysis indicated that these miRNAs are involved in the development of the nervous system and that of other organ development, in cell-cell signalling and Wnt signalling pathway. The expression of C24MC miRNAs was shown to decline gradually throughout gestation [23,25].

## 2.2. Endocrine secretion by endometrial cups

Endometrial cups are very active and very unique in terms of endocrine secretion. Equine Chorionic Gonadotropin (eCG), previously known as Pregnant Mare Serum Gonadotropin (PMSG), selectively binds to LH receptors in equids whereas it has a dual FSH and LH activity in other domestic mammalian species. Only in humans (hCG) and in the bottlenose dolphin [26] have other Chorionic Gonadotropins (CG) been described [27,28]. *In vivo*, the main effect of the human CG (hCG) in pregnant women is the regulation of steroidogenesis whereas the equine eCG is involved in the formation of secondary corpora lutea during gestation. In addition, their assay in different biological fluids is used for the diagnosis of pregnancy and also for the detection of foetoplacental abnormalities during pregnancy.

Because of their biological activity, both these glycoproteins have been and are still used in reproductive control programs in domestic animals and in assisted reproduction programs in humans and horses [29–31]. The horse eCG has been intensively used in the ruminant and pig industry as a FSH analogue, but its use is declining especially due to the ethical conditions associated with its collection from the blood of pregnant mares. In horses, hCG extracted from pregnant women's urine, is still widely used to induce ovulation in mares, although the use of GnRH analogues and recombinant equine gonadotropins [32] is increasing, especially since the latter do not induce the production of antibodies that decrease their activity. One interesting aspect for comparative placentology is the observation that invasive chorionic girdle cells and non-invasive binucleated cells from endometrial cups secrete pregnancy specific glycoproteins (PSGs) from the carcinoembryonic antigen-related cell adhesion molecules (CEACAM) gene family, similar to what is observed in haemochorial placentas from primates and rodents. Functional assays indicate that PSGs may control activity of T-cells located at the borders of endometrial cups and perhaps on peripheral T-cells [33].

### **3. The allantochorion in health and disease**

#### **3.1. Allantochorion structure**

The allantochorion is the definitive form of the equine placenta, resulting from the fusion of chorion and allantoic vascularisation [11,34,35]. Present from 40 days post ovulation, it is categorised as a diffuse, epitheliochorial placenta with microvillous structures ([6,36] for review). Its development is contemporary to the regression of the yolk sack (totally regressed by 56-75 days) and the transition from a vitelline to a chorioallantoic placenta. During this early stage, proteomic studies indicate that there is probably a crosstalk between the yolk sack and the chorioallantoic placenta for the transport of molecules to the foetus [34,37]. Once the yolk sack has regressed, it is the sole provider of nutrient exchange between the mare and the foetus, through both haemotrophic and histotrophic exchanges, representing roughly 90 and 10% of exchanges, respectively. Although the allantochorion structure is mature by 120-150 days of gestation, the main exchange structure, i.e., the microvillosity, increases in size throughout gestation [38].

Nevertheless, the 3D observation of the placenta using 3D-microtomography, micro CT-scan or light sheet imaging may yield further insight into both structure and function [40,41]. For example, vascular branching, transepithelial channels, pericytes can be visualized and analysed at different stages of gestation or in response to environment. An example of 4D imaging of the equine term placenta, zooming from above the microcotyledonary structure down to the allantois, is provided in Figure 2 and Supplementary data 1. Figure 2 also presents electron microscopic images of microcotyledons demonstrating the presence of ciliae at the surface of the trophoblast in term placenta, probably involved in increasing trophoblastic exchange surface and foeto-maternal exchanges, as also shown by others [34,38].

### 3.2. Transplacental transfer

The placenta is involved in nutrient transfers such as glucose, amino acid and fatty acid transport, as well as ion exchange [42]. Many glucose transporters (*SLC2A1-3*, *2A5*, *2A8*, *2A10*, *5A1* and *5A11*) are expressed in the trophoblast as early as day 14, with the *SLC2A1* protein (GLUT1) located on the apical membrane of the trophectoderm [43]. Subsequently, *SLC2A1* is expressed only in the basolateral membrane of the haemotrophic trophoblast, uterine epithelial cells, and in the membranes of foetal capillaries. It is absent from the histotrophic trophoblast. The GLUT3 transporter is expressed in the apical membrane (opposite the endometrial epithelium) of haemotrophic trophoblast cells [44]. In addition, the equine placenta is more permeable to glucose than bovine and ovine placentas but less so than the human placenta [45]. Foetal metabolism is indeed highly dependent on glucose from mid to late gestation [46]. Calcium transport via 9kDa calcium binding protein (9CBP) is performed from the uterine glands through the histotrophic trophoblast [44]. Haemotrophic and histotrophic trophoblasts may therefore have different roles in placental nutrient transport.

The placenta also has metabolic functions, for the provision of its own energy but also to synthesize additional nutrients for the foetus. It is able to produce lactate and fructose from maternal glucose [47] but also to use amino acids [48] and lipids [49,50] as metabolic substrates. Lactate, fructose and amino acids do not appear to be predominantly utilized by utero-placental tissues during mid- and late-gestation in horses [47]. The equine placenta is also permeable to fatty acids, used as an energy source, especially at the end of gestation [47].



### 3.3. Steroid secretion

The equine placenta is involved in the synthesis of steroid hormones and polypeptide hormones such as eCG and growth factors. In most species, progesterone is necessary to maintain pregnancy, either being secreted by the corpus luteum (CL) during the whole duration of pregnancy, or with a placental relay ([51] for review). In the horse, current knowledge on placental steroid secretion has been reviewed [52,53].

The CL of the mare is able to produce and secrete progesterone until Day 180-200 of gestation but thereafter, progesterone is undetectable in maternal blood [54]. Nevertheless, plasma concentrations of allantochorion-derived progesterone metabolites increase progressively from 70 days so that the luteo- placental shift in progestagen synthesis occurs around 110 days of pregnancy ([52] for review).

Overall plasma progestagen concentrations remain stable until about three weeks before birth, when they rise rapidly to reach a peak around 3 days before foaling and fall abruptly [55–57]. Among those progestagens, the progesterone metabolite 5- $\alpha$  dihydroprogesterone (DHP) could be the active compound ensuring myometrial quiescence based on its competitive binding to the equine progesterone receptor [58,59], although it was not able to prevent oxytocin-induced myometrial contractions in vitro [60]. Effects may be mediated by its own metabolite, allopregnanolone, which is an agonist of gamma-aminobutyric acid A receptors that has been shown to induce myometrial relaxation in human [61] and rat [62]. Indeed, the rapid reduction in maternal plasma progestagens before foaling has been related to the decline in the allantochorion expression of the *SRD5A1* and *AKR1C23* genes, that encode respectively for the 5 $\alpha$ -reductase and 20- $\alpha$ -hydroxysteroid dehydrogenase enzymes and are involved in progestagen synthesis [63].

In order to prevent abortion or premature delivery, mares are often treated with the progesterone analogue altrenogest, which successfully maintains pregnancy without preventing parturition to occur [64,65]. Although initial works indicated that altrenogest treatment might be deleterious for proper foetal maturation [66], subsequent work showed that foetal maturation was normal [67] and validated the use of altrenogest, although some doubts remain about its effects on the immune response of the mare [68].

Another remarkable characteristic of the mare is the high plasma concentrations of estrogens, in particular estrone sulfate, related to the production of precursors (DHEA and pregnenolone) by the hyperplastic foetal gonads [69–71], that are metabolized within the allantochorion as estrone, and subsequently sulfated in the endometrium [53,72,73]. Indeed, the presence of the *CYP19A1* transcript as well as aromatase activity have been recently described in the allantochorion [74]. The role of these estrogens, that decline at the end of gestation [75] together with the regression and degeneration of the foetal gonad interstitial cells [71], is still unknown. Although gonadectomy did not prevent mares to go to term, maternal estrone sulfate dropped and foals were dysmature [76,77], indicating that the foetal gonad may be playing a role in promoting foetal maturation prior to term.

#### 3.4. Gene expression in the allantochorion

Interestingly, the expression of only 29.1% of placental genes was found to change significantly with gestation age, whereas the expression of 47.4% of the endometrial genes changed during the same period. Mid-gestation (4-6 months) appeared to foster stable gene expression, changes in gene expression were observed at 10 months and leading to subsequent very distinct patterns of gene expression at 11 months, indicating that analyses of term placenta are very distinct from earlier gestational periods. The ten most abundant genes expressed in the allantochorion were related to endocrinology (i.e., relaxin, aromatase), immune response (i.e., serpin family A member 14, serine protease inhibitor, secretory leukocyte peptidase inhibitor, lipocalin 2) and transport (GM2 ganglioside activator, lipocalin 2, serpin family A member 14, uteroferrin; acid-resistant phosphatase 5, fatty acid binding protein 1) [78].

Overlapping genes (when two genes are located on the opposite genomic strands within the same locus, also known as sense-antisense gene pairs [79]), have been described in the equine allantochorion but their physiological relevance remains to be determined [80]. Imprinted genes, with allele-specific transcription, are particularly involved in foeto-placental development [81,82]. The number of imprinted genes is estimated to range to around 100 in mice and 50 in humans [83]. In the horse, 254 potentially imprinted genes (129 maternally and 125 paternally imprinted) were reported in the equine placenta, 23 originating from overlapping loci [84]. The parental conflict theory states that paternally inherited alleles increase foetal growth at the expense of the mother whereas maternally inherited genes control nutrient exchanges to optimize offspring fitness [85]. In the horse, maternally expressed genes seemed to reduce while paternally expressed genes attempted to extend gestation length [84].

### 3.5. Gene expression in placentitis

The diagnosis of placentitis in mares is currently based on clinical signs (vulval discharge, premature mammary secretions), transrectal ultrasonography, the monitoring of plasma progestagen concentrations (see above) and several other biomarkers (for review, see [86–89]). Placentitis can be associated with abortions and neonatal death. However, apart from ultrasound examination [87,90] and/or an abrupt decrease in maternal plasma progestagen concentrations [55,56,91], precise indications of foetal death are lacking. Thus, early markers of the disease and of foetal demise are still being explored. Experimentally induced placentitis at 280-300 days of gestation induced the upregulation of 12 small RNAs in the chorioallantois and 9 in the serum [24], that could potentially be used as biomarkers. Recently, increased expression of *PLAC8* was reported in the allantochorion of mares with nocardioform placentitis (an epizootic form of placentitis mostly observed in Kentucky and leading to abortions, neonatal death and foetal growth retardation), showing that there is a specific signature for this particular infection [92]. It is not known if placentitis caused by the ingestion of shedded caterpillar exoskeletons [93], that is very similar to nocardioform placentitis, is also associated with the same *PLAC8* gene expression patterns.

Treatments of placentitis, including antibiotics, progesterone receptor agonists (altrenogest), tocolytics and anti-inflammatory drugs have been reviewed elsewhere [64,86,94] and are not the object of this review.

#### **4. Foetal programming**

Birth of a healthy foal is preceded by physiological foetal maturation and proper synchronisation between mare and foetus. Premature foaling, even by a couple of days, may threaten the foal's survival due to immaturity. Indeed, the foetal adrenocortical secretion of cortisol, that leads to the final maturation of foetal organs, takes place very late in the horse [5]. In the present journal issue, foetal maturation as well as the induction of foaling in the mare are reviewed by Abby Fowden and Christina Nagel and are not developed here.

The concept of the Developmental Origins of Health and Disease (DOHaD) is now well established and has been demonstrated to be valid in the horse by [95–98]. Current information about foetal programming in the horse has been recently reviewed [95,99,100]. So far, in the horse, only a very limited number of developmental programming studies used NGS methods to evaluate the effect of maternal environment on placental function [101,102]. This is why most studies discussed in this part of the review used RT-qPCR to assess the placental gene expression.

##### **4.1. Critical periods**

Adverse effects of maternal environment on foetal and postnatal development have been shown to differ, depending on the gestational stage at which they were applied [103]. This implies the existence of critical periods of development that are directly correlated to the timeline of foetal organ development and maturation.

In the horse, at the time of implantation, the embryo has completed most of its organogenesis and critical periods of development may be inferred from organ development and maturation [103]. The differential timing of organ versus placental development is summarized in Figure 1. So far, information on effects of mare nutrition at specific times of gestation is scarce. In addition, sexual dimorphism in the placental and offspring response has been well documented in other species [105]. So far, to the authors' knowledge such a dimorphism has not been observed in Equidae.

It is also interesting to note that the presence of osteochondrosis, a juvenile osteochondral lesion affecting from 0% to 64% of foals depending on breeds [106,107], can be observed in foals as young as 2 days of age. During the last 2/3 of gestation, the foal epiphyseal cartilage undergoes significant changes in collagen structure and vascularization and the development of foetal chondrocytes in vitro is sensitive to hormones such as IGF and insulin [108,109], indicating that the development of osteochondrosis lesions can begin before birth [110]. This review, however, covers early and late gestation only as, to our knowledge, effects of maternal environment during mid-gestation have not been studied in the horse so far.

#### 4.2. Effect of size discrepancy between embryo and recipient mare breeds

Because the equine placenta is diffuse, its size is strongly correlated with the size of the uterus and therefore the mare [111]. Indeed, placentas of Shire x Shetland cross foals were lighter for foals born to Shetland mares than for foals born to Shire mares [112]. Using embryo transfer experiments in pony and Thoroughbred mares, Allen *et al.* showed that the placentas and foals of Welsh pony embryos transferred into Thoroughbred mares were heavier and larger than the placentas and foals of control ponies but lighter and smaller compared to those of control Thoroughbreds. Conversely, the placentas and foals of Thoroughbred embryos carried by Welsh ponies were lighter and smaller than those of control Thoroughbreds [111]. These results demonstrated the reciprocal influence of foetal genotype and maternal environment on the macroscopic development of the placenta and foal. Allen and colleagues also validated the hypothesis of foetal programming of neonatal carbohydrate homeostasis [113], cardiovascular [114] and adrenal cortical function [115] in foals. They also confirmed the hypothesis of developmental origins of growth until the age of 3 years [98], with mare lactation being an additional factor involved in the programming mechanism. Using the same type experimental protocol, Peugeot *et al.* showed that not only growth, but also insulin metabolism and plasma growth hormones were inversely affected in foals with reduced growth (increased thyroid hormones, high fasting glycemia, high insulin sensitivity, increased risk of developing juvenile osteoarticular lesions) and foals with enhanced growth (reduced thyroid hormones, low fasting glycemia, insulin resistance) were affected until one year and a half [116,117].

#### 4.3. Foetal programming through maternal nutrition

Maternal nutrition has been demonstrated to affect foal metabolism, onset of osteochondrosis and the maturation of reproductive organs. Placental adaptations through increased vascularization and nutrient transport, as well as modifications of the maternal metabolism through lipid mobilization and reduced insulin secretion are sufficient to compensate for moderate maternal undernutrition (70-80% of maternal requirement) [118–120]. Nevertheless, delayed testicular maturation, reduced insulin sensitivity and reduced cannon width have been observed in growing foals [121]. Severe undernutrition, however, affects foetal growth despite placental morphological adaptation [122].

Similar to moderate undernutrition, epidemiological and experimental studies show that feeding concentrates to pregnant mares increases the development of osteochondrosis lesions in growing foals [123,124]. Experimentally, maternal supplementation with carbohydrate concentrates does not affect foal birth weight [125]. Placental vascularisation, however, is altered, gene sets related to inflammation and vascularization are enriched whilst gene sets related to placental metabolism are downregulated [101]. Moreover, maternal postprandial insulin response is increased during gestation, implying a potential effect of maternal metabolism on foal osteoarticular development [124,126].

Maternal obesity, characterized by excess body condition score, decreased insulin sensitivity and chronic inflammation, was shown to decrease insulin sensitivity and increase systemic inflammation and incidence of osteochondrosis in foals, although foal growth was not affected until 18 months of age [127]. In addition, excessive maternal energy intake only in the third part of pregnancy was recently shown in newborn foals to alter the pancreatic architecture and the growth and differentiation of skeletal muscle together with the muscular expression of genes involved in insulin-signalling [128,129].

Altogether, these data confirm that maternal nutrition may alter the development of organs essential for athletic performance and, as seen in humans, impair health in ageing animals through the development of metabolic diseases such as the equine metabolic syndrome.

#### 4.3. Foetal programming through other environmental constraints

Effects of age and parity of mares on gestation and foal health have recently been reviewed [130]. In general, primiparous mares produce foals of reduced size and weight compared to multiparous mares. These foals remain smaller until at least one year of age and lighter until 4 months of age in most studies [131–136]. Robles *et al.* showed that this difference was associated with increased plasma triiodothyronine concentrations at birth in foals born to primiparous mares and decreased placental histotrophic exchange area. Foals from primiparous mares remained smaller than foals from multiparous mares until at least 18 months of age and had a delayed development of the carbohydrate homeostasis regulatory system until 12 months of age [136]. In addition, prepubertal males had delayed testicular maturation at 12 months [136]. Some of these effects could be possibly reduced using arginine supplementation in pregnant mares either in early [137] or late pregnancy [138] but more studies are needed to confirm these observations.

Both youngest and oldest dams were reported to produce lighter and smaller foals at birth and until 510 days of age [139]. In most studies, however, maternal age is not controlled for parity, thus inducing important bias. The risk of developing osteochondrosis lesions is increased in foals born to mares less than 10 years old and more than 15 years old [140].

Effects of season of birth have also been described on the neonatal development of horses, with reduced placental weight and postnatal growth in foals born in January and February (northern hemisphere) compared to foals born later in the season [141]. Foals born early (January to March) or late (after May) in the season have a higher risk of developing osteochondrosis lesions [140,142]. These effects may be also related to nutritional effects as diet may considerably differ between winter and spring months, possibly affecting foetal programming [143].

In terms of breeding management, the use of artificial reproduction techniques (ART) is increasing in the equine industry. There are currently concerns about the safety of the use of these technologies for the long term health of offspring, both in humans and animals ([31,144–148] for review). So far, information collected from *in vitro* produced foals are reassuring [149] but longer follow-up are needed to evaluate potential effects on sport and reproductive performance.



Finally, in horses, laminitis is a metabolic pathology associated with significant adiposity leading to tilting of the 3rd phalanx and thus lameness. This pathology is also associated with hypertension and changes in the vascular endothelium. Mares with laminitis in pregnancy were shown to have a reduced gestation length associated with decreased foal and placental weight at birth [150]. In addition, their placentas had thickened vascular wall, as well as increased fibrosis in the microcotyledons [150].

## **5. The role of women scientists in understanding the equine foetal**

Women scientists have largely been involved in the building up of knowledge on equine foeto-placental development. Virginia Osborne was a pioneer: as professor in anatomy at the university of Sydney at a time when women veterinarians specialised in horses were very few, she was the first to point out that breeding management of mares could play a role in pregnancy loss [9]. In terms of physiological events around implantation, morphological and hormonal events taking place in early pregnancy have been thoroughly reviewed by the late Twink Allen with his late colleague Francesca Stewart [6] and subsequently with Sandra Wilsher [11]. In addition, gene expression in the chorionic girdle, at the origin of the endometrial cups, has been characterized by the group of Doug Antczack and more recently Amanda de Mestre [15–18].

Further on into pregnancy, the ultrastructural organisation of the placental microcotyledons has been very thoroughly described by histology by Carole Samuel and, subsequently, Sandra Wilsher and Twink Allen and Maria Angelica Miglino's group [6,34,36,39]. The unique steroid profiles of the equine pregnant mare have been the focus of many researchers, out of which the female scientists Marian Silver, Jenny Ousey, Abby Fowden, Pascale Chavatte-Palmer, Shavahn Loux and Erin Legacki have been very active in deciphering of the role of progestogens and glucocorticoids.

More recently, using next generation sequencing tools, placental and endometrial gene expression from mid to late gestation have been recently explored using RNA sequencing by the group of Shavahn Loux and Barry Ball [78].

In terms of placental pathology, many female veterinary scientists (among whom Michelle Leblanc, Catherine Renaudin, Margo MacPherson, Judy Cawdell-Smith and Shavahn Loux) are at the origin of the current understanding of placentitis.

Marian Silver first showed that the foetal adrenocortical secretion of cortisol, that leads to the final maturation of foetal organs, takes place very late in the horse [5]. Physiological development of the equine foetus has been studied in depth by Marian Silver, Abby Fowden and Alison Forhead and the latter recently published a very thorough review on the subject [104]. Pioneer experiments of the developmental origins of health and disease in the horse were performed by Twink Allen, Jenny Ousey, Abby Fowden, the late Josie Coverdale, Pauline Peugeot, Christine Aurich and the authors of the present article.

## **6. Conclusion**

The data presented above illustrate the recent advances in the exploration of placental function in the horse. Access to new generation sequencing (NGS) provides an immense advantage for understanding the peculiarities of this species and developing new therapeutic treatments for placental pathologies. Nevertheless, all sequencing so far has been performed on the whole placenta and only single cell sequencing will be able to unravel the contribution of respective cell lines. NGS will also provide insight into the role of circulating microvesicles and microRNAs during pregnancy, possibly enabling the development of targeted therapies using exosomes [151]. Moreover, other avenues need to be explored such as better microscopic imaging to properly understand cellular interactions within this complex tissue. In terms of programming, it is urgent to more clearly explore critical periods in relation to horse management and to better define nutritional guidelines for the pregnant mares, but also for stallions, as paternal programming has also been demonstrated in other species [152]. Finally, as artificial reproduction techniques are increasingly used in horses, their effects on foetoplacental and postnatal development need to be further explored in a species where animals are bred for athletic performance and kept until old age. In that context, the horse may also be an interesting model for humans [31].

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Figure 1: Comparative development of placenta and foetus in the equine from 28 days of gestation. Inspired by [11,34,39,153,154].

Figure 2: Term placental structure from three different imaging methods. A. histology (transversal section stained with hematoxylin, eosin and saffron). B. scanning electron microscopy of microcotyledons (the membrane of haemotrophic trophoblastic cells, that cover the microcotyledons, is folded into microvilli. This leads to an increase in surface exchange between maternal and foetal tissues.). C. Light sheet microscopy (vessels fluoresce in green and stroma fluoresces in purple). D. Diagram of placental structure inspired by [39]

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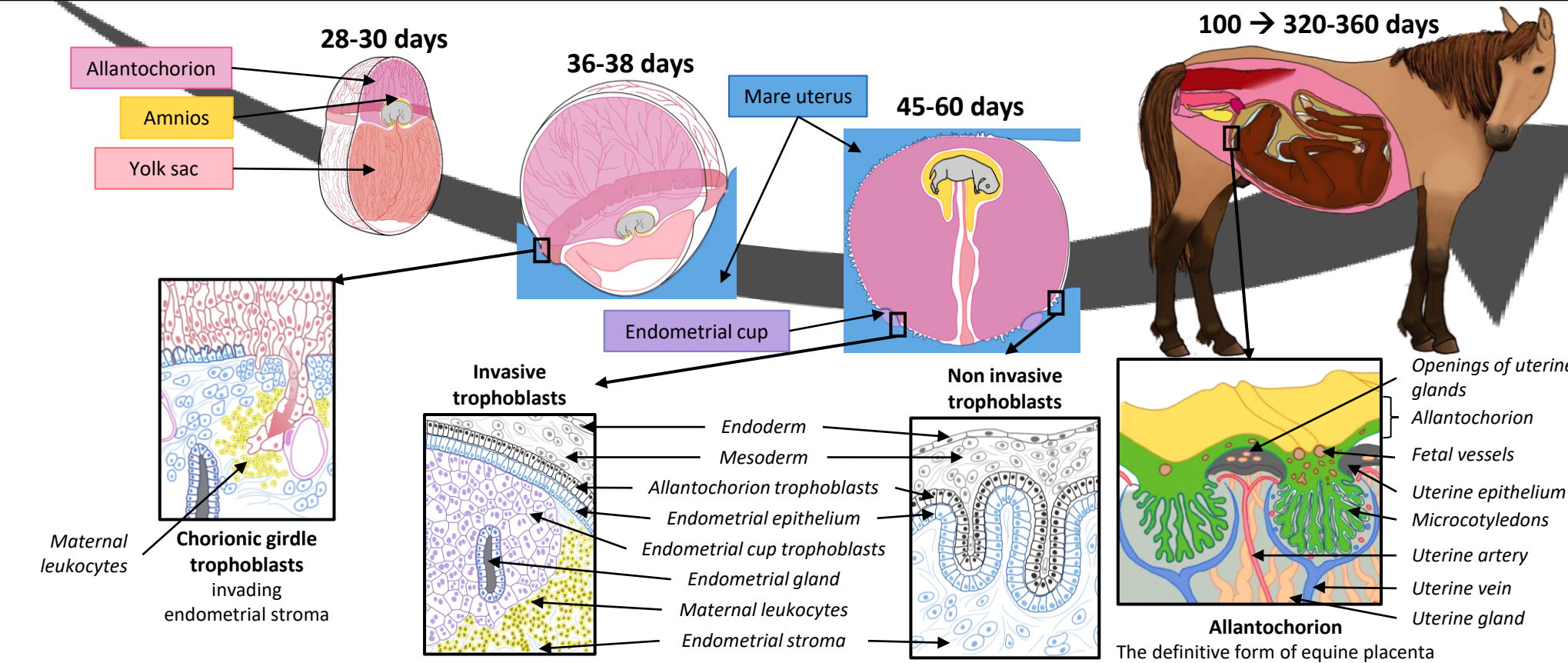
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Fetal age	28 days	36 days	60 days	105 days
<b>Teguments</b>	Translucid skin + hoof started	Development of dermis vessels	Skin thicker and less transparent + hoof apparatus formed	Hair on the rostrum
<b>Bones</b>	Visible somite and cartilage + ribs in formation		Started ossification + visible ribs and sternum with cartilage formation	Development of bones
<b>Muscles</b>	Mesenchymal tissue	Diaphragm development	Transition from mesenchyme to muscle	
<b>Central Nervous system</b>	Spinal cord present + development of encephalic vesicles		Formation of the brainstem and the definitive brain	
<b>Sensorial organs</b>	Weakly pigmented optical vesicles	Development of the otic vesicle (hearing)	Pigmented retina + eyelids almost closed + formation of external auricle + development of the olfactory epithelium	Present orbit vesicle + tactile hairs on the lips
<b>Respiratory system</b>		Nasal area delimited + visible long and trachea	Formation of system from nasal cavity to trachea	
<b>Circulatory system</b>	Vascularization of the dermis + heart divided in 2 chambers		Heart formed	
<b>Digestive system</b>	Voluminous liver present	Visible oral cavity + thoracic esophagus	Esophagus and tongue formed + Visible intestinal loops	
<b>Urinary system</b>			Visible kidneys	
<b>Reproductive system</b>	Genital tubercle formed		Early external reproductive organs	Formation of the mammary gland
<b>Appendices</b>	Tail development + bud of members	Members formed	Tail formed	



Allantoic region

Microcotyledonary region

Fetal vessels

Chorioallantois

Fetal vessels

Openings of uterine glands

Uterine epithelium

Microcotyledon

Uterine artery

Uterine vein

Endometrial glands

