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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

30 **Abstract**

31

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

45

46 **Keywords**

47 equine; mare; allantochorion; gestation

48        **1. Introduction**

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

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

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

66

67        **2. Implantation, endometrial cups and placental proteins**

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

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

80 Implantation is characterized by the co-existence of endometrial cups, an invasive  
81 trophoblast, stemming from the embryo's chorionic girdle, that will remain active until  
82 the 4th month of pregnancy, and the non-invasive allantochorion that will enable  
83 foeto-maternal exchanges until term. On Day 36 post-ovulation, binucleated cells  
84 from the chorionic girdle start invading the maternal endometrium to become the  
85 endometrial cups (Figure 1). Mature cups appear as grey structures with a  
86 depressed surface on the endometrium. A single layer of apparently normal  
87 trophoblast cells persists beneath the girdle, ensuring continuity of the chorion [14].  
88 Their lifespan is 60-100 days, throughout which increasing numbers of leukocytes  
89 accumulate in the surrounding endometrial stroma, invade and appear to destroy the  
90 cups cells until, eventually, they are sloughed from the surface of the endometrium  
91 [14].

92

### 93 2.1. Gene expression in endometrial cups

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

98 Many genes expressed by the endometrial cups are shared with the chorionic girdle  
99 but there is also a clear effect of the endometrial environment. On Day 45, shortly  
100 after implantation, immune cells (Tregs, CD4+ and CD8+ T cells) [15] and also NK  
101 cells are present in the microenvironment of the endometrial cups [19]. The high  
102 level of MHC Class I expression observed in the chorionic girdle decreases after the  
103 endometrial cups are established and increases again around the time of their death  
104 [20], believed to be due to programmed cell-death or lymphocyte mediated  
105 destruction.

106 The expression of the equine chromosome 24 miRNA cluster (C24MC), an ortholog  
107 of the human C14MC, reported to be involved in embryo and foetoplacental  
108 development [21], was explored by the group of Barry Ball, with the contribution of  
109 Shavahn Loux [22]. In the mare's serum, four miRNAs were enriched in mares at  
110 Day 25 of gestation compared to non-pregnant controls. Of these, four (eca-miR-  
111 409-3p, eca-miR-379-5p, eca-miR-1247-3p and eca-miR-134-5p) were significantly  
112 upregulated in the serum of Day 45 mares, compared to non-pregnant controls and  
113 three (eca-miR-127-5p, eca-miR-370-3p, and eca-miR-412-3p) were significantly  
114 upregulated in the serum of Day 45 mares, compared to 4 months pregnant mares  
115 [23], with a similar expression profile in chorioallantois [24]. Some of these could  
116 thus be used for pregnancy check when ultrasound diagnosis is not possible. Gene  
117 Ontology (GO) analysis indicated that these miRNAs are involved in the  
118 development of the nervous system and that of other organ development, in cell-cell  
119 signalling and Wnt signalling pathway. The expression of C24MC miRNAs was  
120 shown to decline gradually throughout gestation [23,25].

121

## 122 2.2. Endocrine secretion by endometrial cups

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

133

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

150

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

#### 152 3.1. Allantochorion structure

153 The allantochorion is the definitive form of the equine placenta, resulting from the  
154 fusion of chorion and allantoic vascularisation [11,34,35]. Present from 40 days post  
155 ovulation, it is categorised as a diffuse, epitheliochorial placenta with microvillous  
156 structures ([6,36] for review). Its development is contemporary to the regression of  
157 the yolk sack (totally regressed by 56-75 days) and the transition from a vitelline to a  
158 chorioallantoic placenta. During this early stage, proteomic studies indicate that there  
159 is probably a crosstalk between the yolk sack and the chorioallantoic placenta for the  
160 transport of molecules to the foetus [34,37]. Once the yolk sack has regressed, it is  
161 the sole provider of nutrient exchange between the mare and the foetus, through  
162 both haemotrophic and histotrophic exchanges, representing roughly 90 and 10% of  
163 exchanges, respectively.

164 Although the allantochorion structure is mature by 120-150 days of gestation, the  
165 main exchange structure, i.e., the microvillosity, increases in size throughout  
166 gestation [38].

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

177

### 178 3.2. Transplacental transfer

179 The placenta is involved in nutrient transfers such as glucose, amino acid and fatty  
180 acid transport, as well as ion exchange [42]. Many glucose transporters (*SLC2A1-3*,  
181 *2A5*, *2A8*, *2A10*, *5A1* and *5A11*) are expressed in the trophoblast as early as day 14,  
182 with the *SLC2A1* protein (GLUT1) located on the apical membrane of the  
183 trophoblast [43]. Subsequently, *SLC2A1* is expressed only in the basolateral  
184 membrane of the haemotrophic trophoblast, uterine epithelial cells, and in the  
185 membranes of foetal capillaries. It is absent from the histotrophic trophoblast. The  
186 GLUT3 transporter is expressed in the apical membrane (opposite the endometrial  
187 epithelium) of haemotrophic trophoblast cells [44]. In addition, the equine placenta is  
188 more permeable to glucose than bovine and ovine placentas but less so than the  
189 human placenta [45]. Foetal metabolism is indeed highly dependent on glucose from  
190 mid to late gestation [46]. Calcium transport via 9kDa calcium binding protein (9CBP)  
191 is performed from the uterine glands through the histotrophic trophoblast [44].  
192 Haemotrophic and histotrophic trophoblasts may therefore have different roles in  
193 placental nutrient transport.

194 The placenta also has metabolic functions, for the provision of its own energy but  
195 also to synthesize additional nutrients for the foetus. It is able to produce lactate and  
196 fructose from maternal glucose [47] but also to use amino acids [48] and lipids  
197 [49,50] as metabolic substrates. Lactate, fructose and amino acids do not appear to  
198 be predominantly utilized by utero-placental tissues during mid- and late-gestation in  
199 horses [47]. The equine placenta is also permeable to fatty acids, used as an energy  
200 source, especially at the end of gestation [47].



201

### 202 3.3. Steroid secretion

203 The equine placenta is involved in the synthesis of steroid hormones and  
204 polypeptide hormones such as eCG and growth factors. In most species,  
205 progesterone is necessary to maintain pregnancy, either being secreted by the  
206 corpus luteum (CL) during the whole duration of pregnancy, or with a placental relay  
207 ([51] for review). In the horse, current knowledge on placental steroid secretion has  
208 been reviewed [52,53].

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

214 Overall plasma progestagen concentrations remain stable until about three weeks  
215 before birth, when they rise rapidly to reach a peak around 3 days before foaling and  
216 fall abruptly [55–57]. Among those progestagens, the progesterone metabolite 5- $\alpha$   
217 dihydroprogesterone (DHP) could be the active compound ensuring myometrial  
218 quiescence based on its competitive binding to the equine progesterone receptor  
219 [58,59], although it was not able to prevent oxytocin-induced myometrial contractions  
220 in vitro [60]. Effects may be mediated by its own metabolite, allopregnanolone, which  
221 is an agonist of gamma-aminobutyric acid A receptors that has been shown to  
222 induce myometrial relaxation in human [61] and rat [62]. Indeed, the rapid reduction  
223 in maternal plasma progestagens before foaling has been related to the decline in  
224 the allantochorion expression of the *SRD5A1* and *AKR1C23* genes, that encode  
225 respectively for the 5 $\alpha$ -reductase and 20- $\alpha$ -hydroxysteroid dehydrogenase enzymes  
226 and are involved in progestagen synthesis [63].

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

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

245

#### 246 3.4. Gene expression in the allantochorion

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

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

272

273

### 274 3.5. Gene expression in placentitis

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

292 Treatments of placentitis, including antibiotics, progesterone receptor agonists  
293 (altrenogest), tocolytics and anti-inflammatory drugs have been reviewed elsewhere  
294 [64,86,94] and are not the object of this review.

295

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

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

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

312

##### 313 4.1. Critical periods

314 Adverse effects of maternal environment on foetal and postnatal development have  
315 been shown to differ, depending on the gestational stage at which they were applied  
316 [103]. This implies the existence of critical periods of development that are directly  
317 correlated to the timeline of foetal organ development and maturation.

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

326 It is also interesting to note that the presence of osteochondrosis, a juvenile  
327 osteochondral lesion affecting from 0% to 64% of foals depending on breeds  
328 [106,107], can be observed in foals as young as 2 days of age. During the last 2/3 of  
329 gestation, the foal epiphyseal cartilage undergoes significant changes in collagen  
330 structure and vascularization and the development of foetal chondrocytes in vitro is  
331 sensitive to hormones such as IGF and insulin [108,109], indicating that the  
332 development of osteochondrosis lesions can begin before birth [110].

333 This review, however, covers early and late gestation only as, to our knowledge,  
334 effects of maternal environment during mid-gestation have not been studied in the  
335 horse so far.

336

337 4.2. Effect of size discrepancy between embryo and recipient mare breeds

338 Because the equine placenta is diffuse, its size is strongly correlated with the size of  
339 the uterus and therefore the mare [111]. Indeed, placentas of Shire x Shetland cross  
340 foals were lighter for foals born to Shetland mares than for foals born to Shire mares  
341 [112]. Using embryo transfer experiments in pony and Thoroughbred mares, Allen *et*  
342 *al.* showed that the placentas and foals of Welsh pony embryos transferred into  
343 Thoroughbred mares were heavier and larger than the placentas and foals of control  
344 ponies but lighter and smaller compared to those of control Thoroughbreds.  
345 Conversely, the placentas and foals of Thoroughbred embryos carried by Welsh  
346 ponies were lighter and smaller than those of control Thoroughbreds [111]. These  
347 results demonstrated the reciprocal influence of foetal genotype and maternal  
348 environment on the macroscopic development of the placenta and foal. Allen and  
349 colleagues also validated the hypothesis of foetal programming of neonatal  
350 carbohydrate homeostasis [113], cardiovascular [114] and adrenal cortical function  
351 [115] in foals. They also confirmed the hypothesis of developmental origins of growth  
352 until the age of 3 years [98], with mare lactation being an additional factor involved in  
353 the programming mechanism. Using the same type experimental protocol, Peugeot  
354 *et al.* showed that not only growth, but also insulin metabolism and plasma growth  
355 hormones were inversely affected in foals with reduced growth (increased thyroid  
356 hormones, high fasting glycemia, high insulin sensitivity, increased risk of developing  
357 juvenile osteoarticular lesions) and foals with enhanced growth (reduced thyroid  
358 hormones, low fasting glycemia, insulin resistance) were affected until one year and  
359 a half [116,117].

#### 360 4.3. Foetal programming through maternal nutrition

361  
362 Maternal nutrition has been demonstrated to affect foal metabolism, onset of  
363 osteochondrosis and the maturation of reproductive organs. Placental adaptations  
364 through increased vascularization and nutrient transport, as well as modifications of  
365 the maternal metabolism through lipid mobilization and reduced insulin secretion are  
366 sufficient to compensate for moderate maternal undernutrition (70-80% of maternal  
367 requirement) [118–120]. Nevertheless, delayed testicular maturation, reduced insulin  
368 sensitivity and reduced cannon width have been observed in growing foals [121].  
369 Severe undernutrition, however, affects foetal growth despite placental  
370 morphological adaptation [122].

371 Similar to moderate undernutrition, epidemiological and experimental studies show  
372 that feeding concentrates to pregnant mares increases the development of  
373 osteochondrosis lesions in growing foals [123,124]. Experimentally, maternal  
374 supplementation with carbohydrate concentrates does not affect foal birth weight  
375 [125]. Placental vascularisation, however, is altered, gene sets related to  
376 inflammation and vascularization are enriched whilst gene sets related to placental  
377 metabolism are downregulated [101]. Moreover, maternal postprandial insulin  
378 response is increased during gestation, implying a potential effect of maternal  
379 metabolism on foal osteoarticular development [124,126].

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

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

392

393 4.3. Foetal programming through other environmental constraints

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

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

412 Effects of season of birth have also been described on the neonatal development of  
413 horses, with reduced placental weight and postnatal growth in foals born in January  
414 and February (northern hemisphere) compared to foals born later in the season  
415 [141]. Foals born early (January to March) or late (after May) in the season have a  
416 higher risk of developing osteochondrosis lesions [140,142]. These effects may be  
417 also related to nutritional effects as diet may considerably differ between winter and  
418 spring months, possibly affecting foetal programming [143].

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

425



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

433

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

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

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

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

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

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

468

## 469 **6. Conclusion**

470 The data presented above illustrate the recent advances in the exploration of  
471 placental function in the horse. Access to new generation sequencing (NGS)  
472 provides an immense advantage for understanding the peculiarities of this species  
473 and developing new therapeutic treatments for placental pathologies. Nevertheless,  
474 all sequencing so far has been performed on the whole placenta and only single cell  
475 sequencing will be able to unravel the contribution of respective cell lines. NGS will  
476 also provide insight into the role of circulating microvesicles and microRNAs during  
477 pregnancy, possibly enabling the development of targeted therapies using exosomes  
478 [151]. Moreover, other avenues need to be explored such as better microscopic  
479 imaging to properly understand cellular interactions within this complex tissue. In  
480 terms of programming, it is urgent to more clearly explore critical periods in relation  
481 to horse management and to better define nutritional guidelines for the pregnant  
482 mares, but also for stallions, as paternal programming has also been demonstrated  
483 in other species [152]. Finally, as artificial reproduction techniques are increasingly  
484 used in horses, their effects on foetoplacental and postnatal development need to be  
485 further explored in a species where animals are bred for athletic performance and  
486 kept until old age. In that context, the horse may also be an interesting model for  
487 humans [31].

488

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

506

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

514

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517

## 518 7. References

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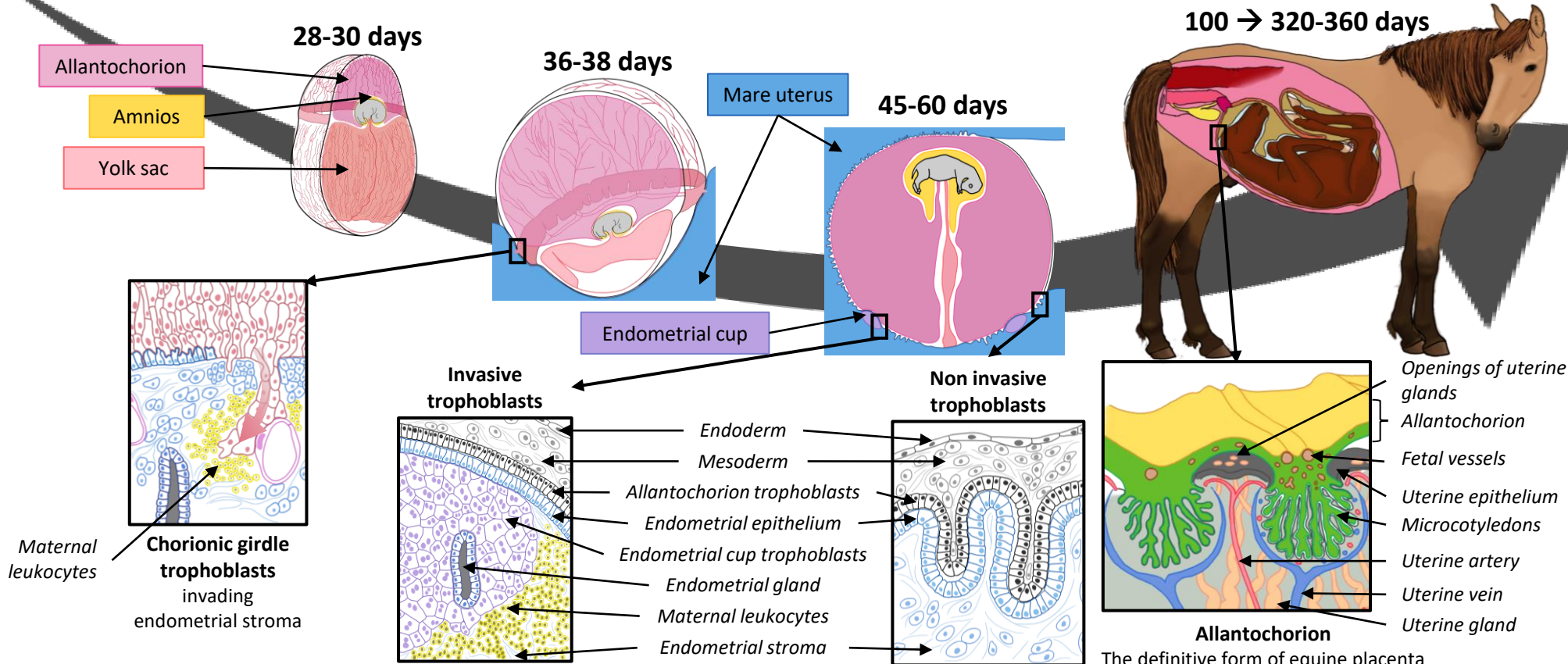
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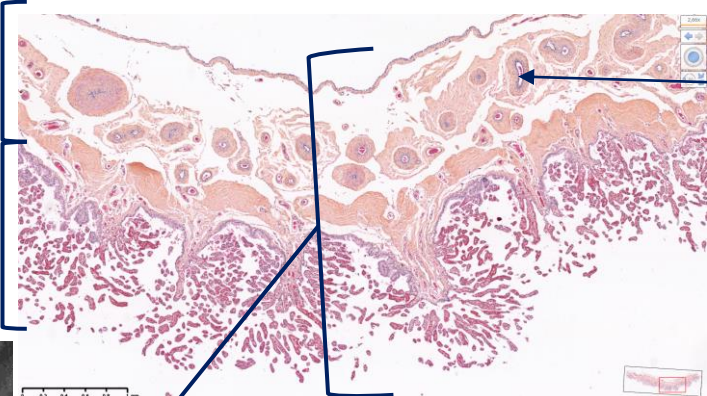
Fetal age	28 days	36 days	60 days	105 days
<b>Teguments</b>	Translucid skin + hoof started	Development of dermis vessels	Skin thicker and less transparent + hoof apparatus formed	Hair on the rostrum
<b>Bones</b>	Visible somite and cartilage + ribs in formation		Started ossification + visible ribs and 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 tubercule 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

