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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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## 518 7. References

519

520 [1] Vasanthakumar MA, Upjohn MM, Watson TL, Dwyer CM. 'All My Animals Are  
521 Equal, but None Can Survive without the Horse'. The Contribution of Working Equids to the  
522 Livelihoods of Women across Six Communities in the Chimaltenango Region of Guatemala.  
523 *Animals* 2021;11:1509. <https://doi.org/10.3390/ani11061509>.

524 [2] Halley JO. *Horse crazy: girls and the lives of horses*. Athens: The University of  
525 Georgia Press; 2019.

526 [3] IFCE - Observatoire économique et social du cheval (OESC). *Les chiffres sur les*  
527 *activités équestres*. 2018.

- 528 [4] Palmer E, Chavatte-Palmer P. Contribution of reproduction management and  
529 technologies to genetic progress in horse breeding. *Journal of Equine Veterinary Science*  
530 2020;103016. <https://doi.org/10.1016/j.jevs.2020.103016>.
- 531 [5] Silver M. Prenatal maturation, the timing of birth and how it may be regulated in  
532 domestic animals. *Experimental Physiology* 1990;75:285–307.  
533 <https://doi.org/10.1113/expphysiol.1990.sp003405>.
- 534 [6] Allen WR, Stewart F. Equine placentation. *Reproduction, Fertility, and Development*  
535 2001;13:623–34.
- 536 [7] Wade CM, Giulotto E, Sigurdsson S, Zoli M, Gnerre S, Imsland F, et al. Genome  
537 Sequence, Comparative Analysis, and Population Genetics of the Domestic Horse. *Science*  
538 2009;326:865–7. <https://doi.org/10.1126/science.1178158>.
- 539 [8] Kalbfleisch TS, Rice ES, DePriest MS, Walenz BP, Hestand MS, Vermeesch JR, et  
540 al. Improved reference genome for the domestic horse increases assembly contiguity and  
541 composition. *Commun Biol* 2018;1:197. <https://doi.org/10.1038/s42003-018-0199-z>.
- 542 [9] Rossdale PD, Allen WR, Butterfield RM. Professor Virginia Esther Osborne, 1917-  
543 2007. *Equine Veterinary Journal* 2008;40:83–83. <https://doi.org/10.1111/j.2042-3306.2008.tb01070.x>.
- 544 [10] Osborne V. Factors influencing foaling percentages in Australian mares. *J Reprod*  
545 *Fertil Suppl* 1975;23:477–83.
- 546 [11] Allen WR, Wilsher S. A Review of Implantation and Early Placentation in the Mare.  
547 *Placenta* 2009;30:1005–15. <https://doi.org/10.1016/j.placenta.2009.09.007>.
- 548 [12] Swegen A. Maternal recognition of pregnancy in the mare: does it exist and why do  
549 we care? *Reproduction* 2021;161:R139–55. <https://doi.org/10.1530/REP-20-0437>.
- 550 [13] Betteridge KJ, Waelchli RO. Equine embryo encapsulation: ephemeral, essential and  
551 enigmatic n.d.:3.
- 552 [14] Allen WR. Immunological aspects of the endometrial cup reaction and the effect of  
553 xenogeneic pregnancy in horses and donkeys. *J Reprod Fert* 1982;Suppl. 31:57–94.
- 554 [15] de Mestre A, Noronha L, Wagner B, Antczak DF. Split immunological tolerance to  
555 trophoblast. *Int J Dev Biol* 2010;54:445–55. <https://doi.org/10.1387/ijdb.082795ad>.
- 556 [16] de Mestre AM, Miller D, Roberson MS, Liford J, Chizmar LC, McLaughlin KE, et al.  
557 Glial Cells Missing Homologue 1 Is Induced in Differentiating Equine Chorionic Girdle  
558 Trophoblast Cells1. *Biology of Reproduction* 2009;80:227–34.  
559 <https://doi.org/10.1095/biolreprod.108.070920>.
- 560 [17] Antczak DF, Miller DC, Rice EJ, Danko CG. Functional Annotation of Genes
- 561

562 Expressed in the Developing Equine Placenta. *Journal of Equine Veterinary Science* 2018;66.  
563 <https://doi.org/10.1016/j.jevs.2018.05.066>.

564 [18] Antczak DF. Immunological memory and tolerance at the maternal-fetal interface:  
565 Implications for reproductive management of mares. *Theriogenology* 2020;150:432–6.  
566 <https://doi.org/10.1016/j.theriogenology.2020.02.043>.

567 [19] Noronha LE, Huggler KE, de Mestre AM, Miller DC, Antczak DF. Molecular  
568 evidence for natural killer-like cells in equine endometrial cups. *Placenta* 2012;33:379–86.  
569 <https://doi.org/10.1016/j.placenta.2012.01.018>.

570 [20] Antczak DF, Allen WR. Maternal immunological recognition of pregnancy in equids.  
571 *J Reprod Fertil Suppl* 1989;37:69–78.

572 [21] Morales-Prieto DM, Ospina-Prieto S, Schmidt A, Chaiwangyen W, Markert UR.  
573 Elsevier Trophoblast Research Award Lecture: Origin, evolution and future of placenta  
574 miRNAs. *Placenta* 2014;35:S39–45. <https://doi.org/10.1016/j.placenta.2013.11.017>.

575 [22] Dini et al. - 2018 - Kinetics of the chromosome 14 microRNA cluster ort.pdf n.d.

576 [23] Dini P, Daels P, Loux SC, Esteller-Vico A, Carossino M, Scoggin KE, et al. Kinetics  
577 of the chromosome 14 microRNA cluster ortholog and its potential role during placental  
578 development in the pregnant mare. *BMC Genomics* 2018;19. [https://doi.org/10.1186/s12864-](https://doi.org/10.1186/s12864-018-5341-2)  
579 [018-5341-2](https://doi.org/10.1186/s12864-018-5341-2).

580 [24] Loux SC, Fernandes CB, Dini P, Wang K, Wu X, Baxter D, et al. Small RNA (sRNA)  
581 expression in the chorioallantois, endometrium and serum of mares following experimental  
582 induction of placentitis. *Reprod Fertil Dev* 2019;31:1144. <https://doi.org/10.1071/RD18400>.

583 [25] Dini P, El-Sheikh Ali H, Carossino M, C. Loux S, Esteller-Vico A, E. Scoggin K, et  
584 al. Expression Profile of the Chromosome 14 MicroRNA Cluster (C14MC) Ortholog in  
585 Equine Maternal Circulation throughout Pregnancy and Its Potential Implications. *IJMS*  
586 2019;20:6285. <https://doi.org/10.3390/ijms20246285>.

587 [26] Watanabe N, Hatano J, Asahina K, Iwasaki T, Hayakawa S. Molecular cloning and  
588 histological localization of LH-like substances in a bottlenose dolphin (*Tursiops truncatus*)  
589 placenta. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*  
590 *Physiology* 2007;146:105–18. <https://doi.org/10.1016/j.cbpa.2006.09.011>.

591 [27] Cole HH, Hart GH. The potency of blood serum of mares in progressive stages of  
592 pregnancy in effecting the sexual maturity of the immature rat. *American Journal of*  
593 *Physiology-Legacy Content* 1930;93:57–68. <https://doi.org/10.1152/ajplegacy.1930.93.1.57>.

594 [28] Stewart F, Allen WR. Biological functions and receptor binding activities of equine  
595 chorionic gonadotrophins. *Reproduction* 1981;62:527–36.

596 <https://doi.org/10.1530/jrf.0.0620527>.

597 [29] Hinrichs K. Assisted reproductive techniques in mares. *Reprod Dom Anim*  
598 2018;53:4–13. <https://doi.org/10.1111/rda.13259>.

599 [30] Lazzari G. Laboratory Production of Equine Embryos. *Journal of Equine Veterinary*  
600 *Science* 2020;10.

601 [31] Benammar A, Derisoud E, Vialard F, Palmer E, Ayoubi JM, Poulain M, et al. The  
602 Mare: A Pertinent Model for Human Assisted Reproductive Technologies? 2021:32.

603 [32] Roser JF, Meyers-Brown G. Enhancing Fertility in Mares: Recombinant Equine  
604 Gonadotropins. *Journal of Equine Veterinary Science* 2019;76:6–13.  
605 <https://doi.org/10.1016/j.jevs.2019.03.004>.

606 [33] Aleksic D, Blaschke L, Mißbach S, Hänske J, Weiß W, Handler J, et al. Convergent  
607 evolution of pregnancy-specific glycoproteins in human and horse. *Reproduction* 2016:171–  
608 84. <https://doi.org/10.1530/REP-16-0236>.

609 [34] Francioli ALR, da-Silva-Nunes-Barreto R, Carvalho RC, de-Oliveira FD, de-  
610 Carvalho AF, Ferraz AC, et al. Characteristics of the Equine Placenta at First Trimester. *Int J*  
611 *Morphol* 2020;38:1018–25. <https://doi.org/10.4067/S0717-95022020000401018>.

612 [35] Wooding FBP, Burton G. Implantation, Maternofetal Exchange and Vascular  
613 Relationships. *Comparative Placentation; Structures, Functions and Evolution*, Berlin  
614 Heidelberg: Springer-Verlag; 2008, p. 47–80.

615 [36] Samuel CA, Allen WR, Steven DH. Ultrastructural development of the equine  
616 placenta. *Journal of Reproduction and Fertility Supplement* 1975;23:575–8.

617 [37] Loux SC, Ball BA. The proteome of fetal fluids in mares with experimentally-induced  
618 placentitis. *Placenta* 2018;64:71–8. <https://doi.org/10.1016/j.placenta.2018.03.004>.

619 [38] MacDonald AA, Chavatte P, Fowden AL. Scanning electron microscopy of the  
620 microcotyledonary placenta of the horse (*Equus caballus*) in the latter half of gestation.  
621 *Placenta* 2000;21:565–74.

622 [39] Steven DH, Samuel CA. Anatomy of the placental barrier in the mare. *J Reprod Fert*  
623 1975;Suppl. 23:579–82.

624 [40] Palaiologou E, Goggin P, Chatelet DS, Ribeiro de Souza R, Chiu W, Ashley B, et al.  
625 Serial block-face scanning electron microscopy reveals novel intercellular connections in  
626 human term placental microvasculature. *J Anat* 2020;237:241–9.  
627 <https://doi.org/10.1111/joa.13191>.

628 [41] Lewis RM, Pearson-Farr JE. Multiscale three-dimensional imaging of the placenta.  
629 *Placenta* 2020;102:55–60. <https://doi.org/10.1016/j.placenta.2020.01.016>.

630 [42] Desforges M, Sibley CP. Placental nutrient supply and fetal growth.  
631 INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY 2010;54:377–90.  
632 <https://doi.org/10.1387/ijdb.082765md>.

633 [43] Gibson C, de Ruijter-Villani M, Rietveld J, Stout TAE. Expression of glucose  
634 transporters in the endometrium and early conceptus membranes of the horse. *Placenta*  
635 2018;68:23–32. <https://doi.org/10.1016/j.placenta.2018.06.308>.

636 [44] Wooding FB, Morgan G, Fowden AL, Allen WR. Separate sites and mechanisms for  
637 placental transport of calcium, iron and glucose in the equine placenta. *Placenta*  
638 2000;21:635–45. <https://doi.org/10.1053/plac.2000.0550>.

639 [45] Pere MC. Materno-foetal exchanges and utilisation of nutrients by the foetus:  
640 comparison between species. *Reproduction Nutrition Development* 2003;43:1–15.

641 [46] Fowden AL, Taylor PM, White KL, Forhead AJ. Ontogenic and nutritionally induced  
642 changes in fetal metabolism in the horse. *The Journal of Physiology* 2000;528:209–19.

643 [47] Fowden AL, Forhead AJ, White KL, Taylor PM. Equine Uteroplacental Metabolism  
644 at Mid- and Late Gestation. *Experimental Physiology* 2000;85:539–45.

645 [48] Silver M, Fowden AL, Taylor PM, Knox J, Hill CM. Blood amino acids in the  
646 pregnant mare and fetus: the effects of maternal fasting and intrafetal insulin. *Experimental*  
647 *Physiology* 1994;79:423–33. <https://doi.org/10.1113/expphysiol.1994.sp003777>.

648 [49] Stammers JP, Hull D, Silver M, Fowden AL, Ousey J, Rosedale PD. Release of lipid  
649 from the equine placenta during in vitro incubation. *Placenta* 1994;15:857–72.  
650 [https://doi.org/10.1016/S0143-4004\(05\)80187-0](https://doi.org/10.1016/S0143-4004(05)80187-0).

651 [50] Stammers JP, Silver M, Fowden AL. Effects of nutrition on uterine and umbilical  
652 venous plasma lipids in chronically catheterised mares in late gestation. *Equine Veterinary*  
653 *Journal* 2010;20:37–40. <https://doi.org/10.1111/j.2042-3306.1988.tb04634.x>.

654 [51] Chavatte-Palmer P, Tarrade A. Placentation in different mammalian species. *Annales*  
655 *d'Endocrinologie* 2016;77:67–74. <https://doi.org/10.1016/j.ando.2016.04.006>.

656 [52] Conley AJ. Review of the reproductive endocrinology of the pregnant and parturient  
657 mare. *Theriogenology* 2016;86:355–65.  
658 <https://doi.org/10.1016/j.theriogenology.2016.04.049>.

659 [53] Conley AJ, Ball BA. Steroids in the establishment and maintenance of pregnancy and  
660 at parturition in the mare. *Reproduction* 2019;158:R197–208. [https://doi.org/10.1530/REP-](https://doi.org/10.1530/REP-19-0179)  
661 [19-0179](https://doi.org/10.1530/REP-19-0179).

662 [54] Holtan DW, Houghton E, Silver M, Fowden AL, Ousey J, Rosedale PD. Plasma  
663 progestagens in the mare, fetus and newborn foal. *J Reprod Fertil Suppl* 1991;44:517–28.

- 664 [55] Rossdale PD, Ousey JC, Cottril CM, Chavatte PC, Allen WR, McGladdery AJ.  
665 Effects of placental pathology on maternal plasma progestagens and mammary secretion  
666 calcium concentrations and on neonatal adrenocortical function in the horse. *Journal of*  
667 *Reproduction and Fertility Supplement* 1991;44:579–90.
- 668 [56] Ousey JC, Houghton E, Grainger L, Rossdale PD, Fowden AL. Progestagen profiles  
669 during the last trimester of gestation in Thoroughbred mares with normal or compromised  
670 pregnancies. *Theriogenology* 2005;63:1844–56.  
671 <https://doi.org/10.1016/j.theriogenology.2004.08.010>.
- 672 [57] Legacki EL, Corbin CJ, Ball BA, Wynn M, Loux S, Stanley SD, et al. Progesterin  
673 withdrawal at parturition in the mare. *Reproduction* 2016;152:323–31.  
674 <https://doi.org/10.1530/REP-16-0227>.
- 675 [58] Jewgenow K, Meyer HHD. Comparative Binding Affinity Study of Progestins to the  
676 Cytosol Progesterin Receptor of Endometrium in Different Mammals. *General and*  
677 *Comparative Endocrinology* 1998;110:118–24. <https://doi.org/10.1006/gcen.1997.7054>.
- 678 [59] Chavatte-Palmer P, Duchamp G, Palmer E, Ousey JC, Rossdale PD, Lombès M.  
679 Progesterone, oestrogen and glucocorticoid receptors in the uterus and mammary glands of  
680 mares from mid- to late gestation. *J Reprod Fertil Suppl* 2000:661–72.
- 681 [60] Ousey JC, Freestone N, Fowden AL, Mason WT, Rossdale PD. The effects of  
682 oxytocin and progestagens on myometrial contractility in vitro during equine pregnancy. *J*  
683 *Reprod Fertil Suppl* 2000:681–91.
- 684 [61] Löfgren M, Holst J, Bäckström T. Effects in vitro of progesterone and two 5 $\alpha$ -reduced  
685 progestins, 5 $\alpha$ -pregnane-3,20-dione and 5 $\alpha$ -pregnane-3 $\alpha$ -ol-20-one, on contracting human  
686 myometrium at term. *Acta Obstet Gynecol Scand* 1992;71:28–33.  
687 <https://doi.org/10.3109/00016349209007943>.
- 688 [62] Putnam CD, Brann DW, Kolbeck RC, Mahesh VB. Inhibition of Uterine Contractility  
689 by Progesterone and Progesterone Metabolites: Mediation by Progesterone and Gamma  
690 Amino Butyric AcidA Receptor Systems. *Biology of Reproduction* 1991;45:266–72.  
691 <https://doi.org/10.1095/biolreprod45.2.266>.
- 692 [63] El-Sheikh Ali H, Legacki E, Loux SC, Esteller-Vico A, Scoggin KE, Conley AJ, et al.  
693 Equine Placentitis Is Associated with a Downregulation in Myometrial Progesterone  
694 Signaling. *Journal of Equine Veterinary Science* 2018;66.  
695 <https://doi.org/10.1016/j.jevs.2018.05.115>.
- 696 [64] Macpherson ML. Diagnosis and Treatment of Equine Placentitis. *Veterinary Clinics*  
697 *of North America: Equine Practice* 2006;22:763–76.



698 <https://doi.org/10.1016/j.cveq.2006.08.005>.

699 [65] Mckinnon AO, Lescun TB, Walker JH, Vasey JR, Allen WR. The inability of some  
700 synthetic progestagens to maintain pregnancy in the mare. *Equine Veterinary Journal*  
701 2000;32:83–5. <https://doi.org/10.2746/042516400777612035>.

702 [66] Neuhauser S, Palm F, Ambuehl F, Aurich C. Effects of Altrenogest Treatment of  
703 Mares in Late Pregnancy on Parturition and on Neonatal Viability of their Foals. *Exp Clin*  
704 *Endocrinol Diabetes* 2008;116:423–8. <https://doi.org/10.1055/s-2008-1065367>.

705 [67] Neuhauser S, Palm F, Ambuehl F, Möstl E, Schwendenwein I, Aurich C. Effect of  
706 altrenogest-treatment of mares in late gestation on adrenocortical function, blood count and  
707 plasma electrolytes in their foals. *Equine Veterinary Journal* 2009;41:572–7.  
708 <https://doi.org/10.2746/042516409X394481>.

709 [68] Fedorka CE, Ball BA, Walker OF, Conley AJ, Corbin CJ, Lu KG, et al. Alteration of  
710 the mare’s immune system by the synthetic progestin, altrenogest. *Am J Reprod Immunol*  
711 2019;82. <https://doi.org/10.1111/aji.13145>.

712 [69] Barreto RSN, Romagnolli P, Mess AM, Rigoglio NN, Sasahara THC, Simões LS, et  
713 al. Reproductive system development in male and female horse embryos and fetuses:  
714 Gonadal hyperplasia revisited. *Theriogenology* 2018;108:118–26.  
715 <https://doi.org/10.1016/j.theriogenology.2017.11.036>.

716 [70] González-Angulo A, Hernández-Jáuregui P, Martínez-Zedilo G. Fine structure of the  
717 gonads of the horse and its functional implications. *J Reprod Fertil Suppl* 1975:563–7.

718 [71] Hay MF, Allen WR. An ultrastructural and histochemical study of the interstitial cells  
719 in the gonads of the fetal horse. *J Reprod Fertil Suppl* 1975:557–61.

720 [72] Raeside JI. DHA in the fetal gonads in the horse. *J Reprod Fertil* 1976;Suppl. 46:423–  
721 5.

722 [73] Raeside JI. A Brief Account of the Discovery of the Fetal/Placental Unit for Estrogen  
723 Production in Equine and Human Pregnancies: Relation to Human Medicine. *Yale J Biol*  
724 *Med* 2017;90:449–61.

725 [74] Legacki EL, Ball BA, Corbin CJ, Loux SC, Scoggin KE, Stanley SD, et al. Equine  
726 fetal adrenal, gonadal and placental steroidogenesis. *Reproduction* 2017;154:445–54.  
727 <https://doi.org/10.1530/REP-17-0239>.

728 [75] Haluska GJ, Currie WB. Variation in plasma concentrations of oestradiol-17beta and  
729 their relationship to those of progesterone, 13,14-dihydro-15-keto-prostaglandin F-alpha and  
730 oxytocin across pregnancy and at parturition in pony mares n.d.:12.

731 [76] Pashen RL, Allen WR. The role of the fetal gonads and placenta in steroid production,

732 maintenance of pregnancy and parturition in the mare. *J Reprod Fertil Suppl* 1979;499–509.

733 [77] Pashen RL. Maternal and foetal endocrinology during late pregnancy and parturition  
734 in the mare. *Equine Veterinary Journal* 1984;16:233–8. [https://doi.org/10.1111/j.2042-](https://doi.org/10.1111/j.2042-3306.1984.tb01918.x)  
735 [3306.1984.tb01918.x](https://doi.org/10.1111/j.2042-3306.1984.tb01918.x).

736 [78] Loux SC, Dini P, El-Sheikh Ali H, Kalbfleisch T, Ball BA. Characterization of the  
737 placental transcriptome through mid to late gestation in the mare. *PLoS ONE*  
738 2019;14:e0224497. <https://doi.org/10.1371/journal.pone.0224497>.

739 [79] Wood EJ, Chin-Inmanu K, Jia H, Lipovich L. Sense-antisense gene pairs: sequence,  
740 transcription, and structure are not conserved between human and mouse. *Front Genet*  
741 2013;4. <https://doi.org/10.3389/fgene.2013.00183>.

742 [80] Dini, Norris, Ali, Loux, Carossino, Esteller-Vico, et al. Landscape of Overlapping  
743 Gene Expression in the Equine Placenta. *Genes* 2019;10:503.  
744 <https://doi.org/10.3390/genes10070503>.

745 [81] Ferguson-Smith AC. Genomic imprinting: the emergence of an epigenetic paradigm.  
746 *Nat Rev Genet* 2011;12:565–75. <https://doi.org/10.1038/nrg3032>.

747 [82] Reik W, Constancia M, Fowden A, Anderson N, Dean W, Ferguson-Smith A, et al.  
748 Regulation of supply and demand for maternal nutrients in mammals by imprinted genes.  
749 *Journal of Physiology-London* 2003;547:35–44.

750 [83] Chess A. Monoallelic Gene Expression in Mammals. *Annu Rev Genet* 2016;50:317–  
751 27. <https://doi.org/10.1146/annurev-genet-120215-035120>.

752 [84] Dini P, Kalbfleisch T, Uribe-Salazar JM, Carossino M, Ali HE-S, Loux SC, et al.  
753 Parental bias in expression and interaction of genes in the equine placenta. *Proc Natl Acad*  
754 *Sci USA* 2021;118:e2006474118. <https://doi.org/10.1073/pnas.2006474118>.

755 [85] Wolf JB, Hager R. A Maternal–Offspring Coadaptation Theory for the Evolution of  
756 Genomic Imprinting. *PLoS Biol* 2006;4:e380. <https://doi.org/10.1371/journal.pbio.0040380>.

757 [86] LeBlanc M. Ascending Placentitis in the Mare: An Update: Mare Placentitis.  
758 *Reproduction in Domestic Animals* 2010;45:28–34. [https://doi.org/10.1111/j.1439-](https://doi.org/10.1111/j.1439-0531.2010.01633.x)  
759 [0531.2010.01633.x](https://doi.org/10.1111/j.1439-0531.2010.01633.x).

760 [87] Bucca S. Diagnosis of the Compromised Equine Pregnancy. *Veterinary Clinics of*  
761 *North America: Equine Practice* 2006;22:749–61. <https://doi.org/10.1016/j.cveq.2006.07.006>.

762 [88] Sprayberry KA, Lu KG. Managing Reproduction Emergencies in the Field.  
763 *Veterinary Clinics of North America: Equine Practice* 2021;37:339–66.  
764 <https://doi.org/10.1016/j.cveq.2021.04.007>.

765 [89] Canisso IF, Loux SC, Lima FS. Biomarkers for placental disease in mares.

766 Theriogenology 2020;150:302–7. <https://doi.org/10.1016/j.theriogenology.2020.01.073>.

767 [90] Renaudin CD, Gillis CL, Tarantal AF, Coleman DA. Evaluation of equine fetal  
768 growth from day 100 of gestation to parturition by ultrasonography. *J Reprod Fert* 2000;56  
769 suppl. *Equine Reproduction*:651–60.

770 [91] Morris S, Kelleman AA, Stawicki RJ, Hansen PJ, Sheerin PC, Sheerin BR, et al.  
771 Transrectal ultrasonography and plasma progestin profiles identifies fetoplacental  
772 compromise in mares with experimentally induced placentitis. *Theriogenology* 2007;67:681–  
773 91. <https://doi.org/10.1016/j.theriogenology.2006.05.021>.

774 [92] El-Sheikh Ali H, Scoggin K, Linhares Boakari Y, Dini P, Loux S, Fedorka C, et al.  
775 Kinetics of placenta-specific 8 (PLAC8) in equine placenta during pregnancy and placentitis.  
776 *Theriogenology* 2021;160:81–9. <https://doi.org/10.1016/j.theriogenology.2020.10.041>.

777 [93] Cawdell-Smith AJ, Todhunter KH, Perkins NR, Bryden WL. Exposure of mares to  
778 processionary caterpillars *Ochrogaster lunifer* in early pregnancy: An additional dimension to  
779 equine amnionitis and fetal loss: Caterpillars cause abortion in early pregnancy. *Equine*  
780 *Veterinary Journal* 2013;45:755–60. <https://doi.org/10.1111/evj.12044>.

781 [94] Bailey CS, Macpherson ML, Pozor MA, Troedsson MHT, Benson S, Giguere S, et al.  
782 Treatment efficacy of trimethoprim sulfamethoxazole, pentoxifylline and altrenogest in  
783 experimentally induced equine placentitis. *Theriogenology* 2010;74:402–12.  
784 <https://doi.org/10.1016/j.theriogenology.2010.02.023>.

785 [95] Peugnet P, Robles M, Wimel L, Tarrade A, Chavatte-Palmer P. Management of the  
786 pregnant mare and long-term consequences on the offspring. *Theriogenology* 2016;86:99–  
787 109. <https://doi.org/10.1016/j.theriogenology.2016.01.028>.

788 [96] Fowden AL, Jellyman JK, Valenzuela OA, Forhead AJ. Nutritional Programming of  
789 Intrauterine Development: A Concept Applicable to the Horse? *Journal of Equine Veterinary*  
790 *Science* 2013;33:295–304. <https://doi.org/10.1016/j.jevs.2013.03.005>.

791 [97] Rossdale PD, Ousey JC. Fetal programming for athletic performance in the horse:  
792 potential effects of IUGR. *Equine Veterinary Education* 2003;14:98–111.

793 [98] Allen WR, Wilsher S, Tiplady C, Butterfield RM. The influence of maternal size on  
794 pre- and postnatal growth in the horse: III Postnatal growth. *Reproduction* 2004;127:67–77.  
795 <https://doi.org/10.1530/rep.1.00024>.

796 [99] Chavatte-Palmer P, Peugnet P, Robles M. Developmental programming in equine  
797 species: relevance for the horse industry. *Animal Frontiers* 2017;7:48–54.  
798 <https://doi.org/10.2527/af.2017-0128>.

799 [100] Chavatte-Palmer P, Robles M. Developmental programming: Can nutrition of the

800 mare influence the foal's health? Programação de desenvolvimento embrionário: A nutrição  
801 da égua pode influenciar a saúde do potro? vol. 43, Gramado, Brazil: 2019, p. 168–83.

802 [101] Robles M, Peugnet P, Dubois C, Piumi F, Jouneau L, Bouchez O, et al. Placental  
803 function and structure at term is altered in broodmares fed with cereals from mid-gestation.  
804 *Placenta* 2018;64:44–52. <https://doi.org/10.1016/j.placenta.2018.02.003>.

805 [102] Sessions-Bresnahan DR, Heuberger AL, Carnevale EM. Obesity in mares promotes  
806 uterine inflammation and alters embryo lipid fingerprints and homeostasis. *Biology of*  
807 *Reproduction* 2018;99:761–72. <https://doi.org/10.1093/biolre/ioy107>.

808 [103] Safi-Stibler S, Gabory A. Epigenetics and the Developmental Origins of Health and  
809 Disease: Parental environment signalling to the epigenome, critical time windows and  
810 sculpting the adult phenotype. *Seminars in Cell & Developmental Biology* 2020;97:172–80.  
811 <https://doi.org/10.1016/j.semcdb.2019.09.008>.

812 [104] Fowden AL, Giussani DA, Forhead AJ. Physiological development of the equine  
813 fetus during late gestation. *Equine Vet J* 2019;evj.13206. <https://doi.org/10.1111/evj.13206>.

814 [105] Gabory A, Roseboom TJ, Moore T, Moore LG, Junien C. Placental contribution to the  
815 origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. *Biol*  
816 *Sex Differ* 2013;4:5. <https://doi.org/10.1186/2042-6410-4-5>.

817 [106] Sandersen C, Vander Heyden L, Detilleux J, Lejeune JP, Serteyn D. Osteochondrosis  
818 in foals. *The Veterinary Record* 2013;172:456–7. <https://doi.org/10.1136/vr.f2579>.

819 [107] Metzger J, Distl O. Genetics of Equine Orthopedic Disease. *Veterinary Clinics of*  
820 *North America: Equine Practice* 2020;36:289–301.  
821 <https://doi.org/10.1016/j.cveq.2020.03.008>.

822 [108] Henson FM, Davenport C, Butler L, Moran I, Shingleton WD, Jeffcott LB, et al.  
823 Effects of insulin and insulin-like growth factors I and II on the growth of equine fetal and  
824 neonatal chondrocytes. *Equine Vet J* 1997;29:441–7. <https://doi.org/10.1111/j.2042-3306.1997.tb03156.x>.

825

826 [109] Lecocq M, Girard CA, Fogarty U, Beauchamp G, Richard H, Laverty S. Cartilage  
827 matrix changes in the developing epiphysis: Early events on the pathway to equine  
828 osteochondrosis? *Equine Veterinary Journal* 2008;40:442–54.  
829 <https://doi.org/10.2746/042516408X297453>.

830 [110] Carlsten J, Sandgren B, Dalin G. Development of osteochondrosis in the tarsocrural  
831 joint and osteochondral fragments in the fetlock joints of Standardbred trotters. I. A  
832 radiological survey. *Equine Veterinary Journal* 1993;25:42–7. <https://doi.org/10.1111/j.2042-3306.1993.tb04853.x>.

833

834 [111] Allen WR, Wilsher S, Turnbull C, Stewart F, Ousey J, Rossdale PD, et al. Influence  
835 of maternal size on placental, fetal and postnatal growth in the horse. I. Development in  
836 utero. *Reproduction* 2002;123:445–53.

837 [112] Walton A, Hammond J. The maternal effects on growth and conformation in shire  
838 horse-shetland pony crosses. *Proc R Soc Lond B* 1938;125:311–35.  
839 <https://doi.org/10.1098/rspb.1938.0029>.

840 [113] Forhead A, Ousey J, Allen W, Fowden A. Postnatal insulin secretion and sensitivity  
841 after manipulation of fetal growth by embryo transfer in the horse. *Journal of Endocrinology*  
842 2004;181:459–67. <https://doi.org/10.1677/joe.0.1810459>.

843 [114] Giussani DA, Forhead AJ, Gardner DS, Fletcher AJW, Allen WR. Postnatal  
844 cardiovascular function after manipulation of fetal growth by embryo transfer in the horse. *J*  
845 *Physiol* 2003;547:67–76.

846 [115] Ousey JC, Rossdale PD, Fowden AL, Palmer L, Turnbull C, Allen WR. Effects of  
847 manipulating intrauterine growth on post natal adrenocortical development and other  
848 parameters of maturity in neonatal foals. *Equine Veterinary Journal* 2004;36:616–21.

849 [116] Peugnet P, Wimel L, Duchamp G, Sandersen C, Camous S, Guillaume D, et al.  
850 Enhanced or Reduced Fetal Growth Induced by Embryo Transfer into Smaller or Larger  
851 Breeds Alters Post-Natal Growth and Metabolism in Pre-Weaning Horses. *PLoS ONE*  
852 2014;9:e102044. <https://doi.org/10.1371/journal.pone.0102044>.

853 [117] Peugnet P, Wimel L, Duchamp G, Sandersen C, Camous S, Guillaume D, et al.  
854 Enhanced or Reduced Fetal Growth Induced by Embryo Transfer Into Smaller or Larger  
855 Breeds Alters Postnatal Growth and Metabolism in Weaned Horses. *Journal of Equine*  
856 *Veterinary Science* 2017;48:143-153.e2. <https://doi.org/10.1016/j.jevs.2016.03.016>.

857 [118] Peugnet P, Tarrade A, Sandersen C, Dahirel M, Guillaume D, Wimel L, et al. Effects  
858 of embryo transfer in a larger breed on postnatal growth and glucose metabolism in horses.  
859 *Reprod Fertil Dev* 2013;25:228 (abstract). <https://doi.org/10.1071/RDv25n1abs>.

860 [119] Banach MA, Evans JW. Effects of inadequate energy during gestation and lactation  
861 on the oestrus cycle and conception rates of mares and of their foals weights. *Proc. 7th. Eq.*  
862 *Nutr. Physiol. Symp.*, 1981, p. 97–100.

863 [120] Henneke DR, Potter GD, Kreider JL. Body condition during pregnancy and lactation  
864 and reproductive efficiency of mares. *Theriogenology* 1984;21:897–909.

865 [121] Robles M, Gautier C, Mendoza L, Peugnet P, Dubois C, Dahirel M, et al. Maternal  
866 Nutrition during Pregnancy Affects Testicular and Bone Development, Glucose Metabolism  
867 and Response to Overnutrition in Weaned Horses Up to Two Years. *PLoS ONE*

868 2017;12:e0169295. <https://doi.org/10.1371/journal.pone.0169295>.

869 [122] Wilsher S, Allen WR. Effects of a *Streptococcus equi* infection-mediated nutritional  
870 insult during mid-gestation in primiparous Thoroughbred fillies. Part 1: Placental and fetal  
871 development. *Equine Veterinary Journal* 2006;38:549–57.  
872 <https://doi.org/10.2746/042516406X156497>.

873 [123] Vander Heyden L, Lejeune J-P, Caudron I, Detilleux J, Sandersen C, Chavatte P, et  
874 al. Association of breeding conditions with prevalence of osteochondrosis in foals. *Veterinary  
875 Record* 2013;172:68–68.

876 [124] Peugnet P, Robles M, Mendoza L, Wimel L, Dubois C, Dahirel M, et al. Effects of  
877 Moderate Amounts of Barley in Late Pregnancy on Growth, Glucose Metabolism and  
878 Osteoarticular Status of Pre-Weaning Horses. *PLOS ONE* 2015;10:e0122596.  
879 <https://doi.org/10.1371/journal.pone.0122596>.

880 [125] Kubiak JR, Evans JW, Potter GD, Harms PG, Jenkins WL. Parturition in the  
881 multiparous mare fed to obesity. *Journal of Equine Veterinary Science* 1988;8:135–40.  
882 [https://doi.org/10.1016/S0737-0806\(88\)80035-2](https://doi.org/10.1016/S0737-0806(88)80035-2).

883 [126] Caure S, Lebreton P. Ostéochondrose chez le trotteur au sevrage et corrélation avec  
884 divers paramètres. *Pratique Vétérinaire Équine* 2004;36:47–57.

885 [127] Robles M, Nouveau E, Gautier C, Mendoza L, Dubois C, Dahirel M, et al. Maternal  
886 obesity increases insulin resistance, low-grade inflammation and osteochondrosis lesions in  
887 foals and yearlings until 18 months of age. *PLoS ONE* 2018;13:e0190309.  
888 <https://doi.org/10.1371/journal.pone.0190309>.

889 [128] Bradbery AN, Coverdale JA, Hammer CJ, Dunlap KA, Leatherwood JL, Satterfield  
890 MC. Effect of maternal overnutrition on predisposition to insulin resistance in the foal: Foal  
891 skeletal muscle development and insulin signaling. *Domestic Animal Endocrinology*  
892 2021;77:106648. <https://doi.org/10.1016/j.domaniend.2021.106648>.

893 [129] Bradbery AN, Coverdale JA, Hartz CJ, Millican AA, Goehring MS, Fikes KK, et al.  
894 Effect of maternal overnutrition on predisposition to insulin resistance in the foal: Maternal  
895 parameters and foal pancreas histoarchitecture. *Animal Reproduction Science*  
896 2021;227:106720. <https://doi.org/10.1016/j.anireprosci.2021.106720>.

897 [130] Derisoud E, Auclair-Ronzaud J, Palmer E, Robles M, Chavatte-Palmer P. Female age  
898 and parity in horses: how and why does it matter? *Reproduction Fertility Development* 2021:in  
899 press.

900 [131] Pool-Anderson K, Raub RH, Warren JA. Maternal influences on growth and  
901 development of full-sibling foals<sup>2</sup>. *Journal of Animal Science* 1994;72:1661–6.

902 <https://doi.org/10.2527/1994.7271661x>.

903 [132] Cymbaluk NF, Laarveld B. The ontogeny of serum insulin-like growth factor-I  
904 concentration in foals: Effects of dam parity, diet, and age at weaning. *Domestic Animal*  
905 *Endocrinology* 1996;13:197–209. [https://doi.org/10.1016/0739-7240\(96\)00014-8](https://doi.org/10.1016/0739-7240(96)00014-8).

906 [133] Fernandes CB, Meirelles MG, Silva JQ, Serrano ECR, Veras MM, Alonso MA, et al.  
907 Influence of maternal parity on placental characteristics and foal weight, height and thorax  
908 perimeter at birth. *J Equine Vet Sci* 2012;32:418–9.

909 [134] Fernandes CB, Meirelles MG, Guimaraes CF, Nichi M, Affonso FJ, Fonte JS, et al.  
910 Which paternal, maternal and placental parameters influence foal size and vitality? *Journal of*  
911 *Equine Veterinary Science* 2014;34:225–7. <https://doi.org/10.1016/j.jevs.2013.10.161>.

912 [135] Meirelles MG, Veras MM, Alonso MA, de Fátima Guimarães C, Nichi M, Fernandes  
913 CB. Influence of Maternal Age and Parity on Placental Structure and Foal Characteristics  
914 From Birth up to 2 Years of Age. *Journal of Equine Veterinary Science* 2017;56:68–79.  
915 <https://doi.org/10.1016/j.jevs.2017.03.226>.

916 [136] Robles M, Dubois C, Gautier C, Dahirel M, Guenon I, Bouraima-Lelong H, et al.  
917 Maternal parity affects placental development, growth and metabolism of foals until 1 year  
918 and a half. *Theriogenology* 2018;108:321–30.  
919 <https://doi.org/10.1016/j.theriogenology.2017.12.019>.

920 [137] Aurich J, Köhne M, Wulf M, Nagel C, Beythien E, Gautier C, et al. Effects of dietary  
921 L-arginine supplementation to early pregnant mares on conceptus diameter-Preliminary  
922 findings. *Reproduction in Domestic Animals* 2019. <https://doi.org/10.1111/rda.13422>.

923 [138] Robles M, Couturier-Tarrade A, Derisoud E, Geeverding A, Dubois C, Dahirel M, et  
924 al. Effects of dietary arginine supplementation in pregnant mares on maternal metabolism,  
925 placental structure and function and foal growth. *Scientific Reports* 2019;9:6461.  
926 <https://doi.org/10.1038/s41598-019-42941-0>.

927 [139] Hintz HF. Factors affecting growth in the horse, 1979, p. 21–6.

928 [140] Lepeule J, Bareille N, Robert C, Ezanno P, Valette JP, Jacquet S, et al. Association of  
929 growth, feeding practices and exercise conditions with the prevalence of Developmental  
930 Orthopaedic Disease in limbs of French foals at weaning. *Preventive Veterinary Medicine*  
931 2009;89:167–77. <https://doi.org/10.1016/j.prevetmed.2009.02.018>.

932 [141] Beythien E, Aurich C, Wulf M, Aurich J. Effects of season on placental, foetal and  
933 neonatal development in horses. *Theriogenology* 2017;97:98–103.  
934 <https://doi.org/10.1016/j.theriogenology.2017.04.027>.

935 [142] Pagan JD. The incidence of developmental orthopedic disease (DOD) on a Kentucky

936 Thoroughbred farm. *Advances in Equine Nutrition*, vol. 1. JD Pagan, Versailles, KY, USA:  
937 Kentucky Equine Research; 1998, p. 469–76.

938 [143] Robles M, Hammer C, Staniar B, Chavatte-Palmer P. Nutrition of Broodmares.  
939 *Veterinary Clinics of North America: Equine Practice* 2021;37:177–205.  
940 <https://doi.org/10.1016/j.cveq.2021.01.001>.

941 [144] Duranthon V, Chavatte-Palmer P. Long term effects of ART: What do animals tell  
942 us? *Molecular Reproduction and Development* 2018;85:348–68.  
943 <https://doi.org/10.1002/mrd.22970>.

944 [145] Hart R, Norman RJ. The longer-term health outcomes for children born as a result of  
945 IVF treatment: Part I--General health outcomes. *Human Reproduction Update* 2013;19:232–  
946 43. <https://doi.org/10.1093/humupd/dms062>.

947 [146] Hart R, Norman RJ. The longer-term health outcomes for children born as a result of  
948 IVF treatment. Part II--Mental health and development outcomes. *Human Reproduction*  
949 *Update* 2013;19:244–50. <https://doi.org/10.1093/humupd/dmt002>.

950 [147] Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, et  
951 al. Origins of lifetime health around the time of conception: causes and consequences. *The*  
952 *Lancet* 2018;391:1842–52. [https://doi.org/10.1016/S0140-6736\(18\)30312-X](https://doi.org/10.1016/S0140-6736(18)30312-X).

953 [148] Sun C, Velazquez MA, Fleming TP. DOHaD and the Periconceptional Period, a  
954 Critical Window in Time. *The Epigenome and Developmental Origins of Health and Disease*,  
955 Elsevier; 2016, p. 33–47. <https://doi.org/10.1016/B978-0-12-801383-0.00003-7>.

956 [149] Valenzuela OA, Couturier-Tarrade A, Choi Y-H, Aubrière M-C, Ritthaler J,  
957 Chavatte-Palmer P, et al. Impact of equine assisted reproductive technologies (standard  
958 embryo transfer or intracytoplasmic sperm injection (ICSI) with in vitro culture and embryo  
959 transfer) on placenta and foal morphometry and placental gene expression. *Reproduction,*  
960 *Fertility and Development* 2018;30:371. <https://doi.org/10.1071/RD16536>.

961 [150] Pazinato FM, Curcio B da R, Fernandes CG, Santos CA, Feijó LS, Varela AS, et al.  
962 Histomorphometry of the placental vasculature and microcotyledons in Thoroughbred mares  
963 with chronic laminitis. *Theriogenology* 2017;91:77–81.  
964 <https://doi.org/10.1016/j.theriogenology.2016.12.009>.

965 [151] Yang Z, Shi J, Xie J, Wang Y, Sun J, Liu T, et al. Large-scale generation of functional  
966 mRNA-encapsulating exosomes via cellular nanoporation. *Nat Biomed Eng* 2020;4:69–83.  
967 <https://doi.org/10.1038/s41551-019-0485-1>.

968 [152] Soubry A. POHaD: why we should study future fathers. *Environ Epigenet*  
969 2018;4:dvy007. <https://doi.org/10.1093/eep/dvy007>.



970 [153] Ginther OJ. Equine Pregnancy: Physical Interactions Between the Uterus and  
971 Conceptus, 1998.

972 [154] Noronha LE, Antczak DF. Maternal Immune Responses to Trophoblast: The  
973 Contribution of the Horse to Pregnancy Immunology: MATERNAL IMMUNE RESPONSES  
974 TO EQUINE TROPHOBLAST. American Journal of Reproductive Immunology  
975 2010;64:231–44. <https://doi.org/10.1111/j.1600-0897.2010.00895.x>.

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977

978

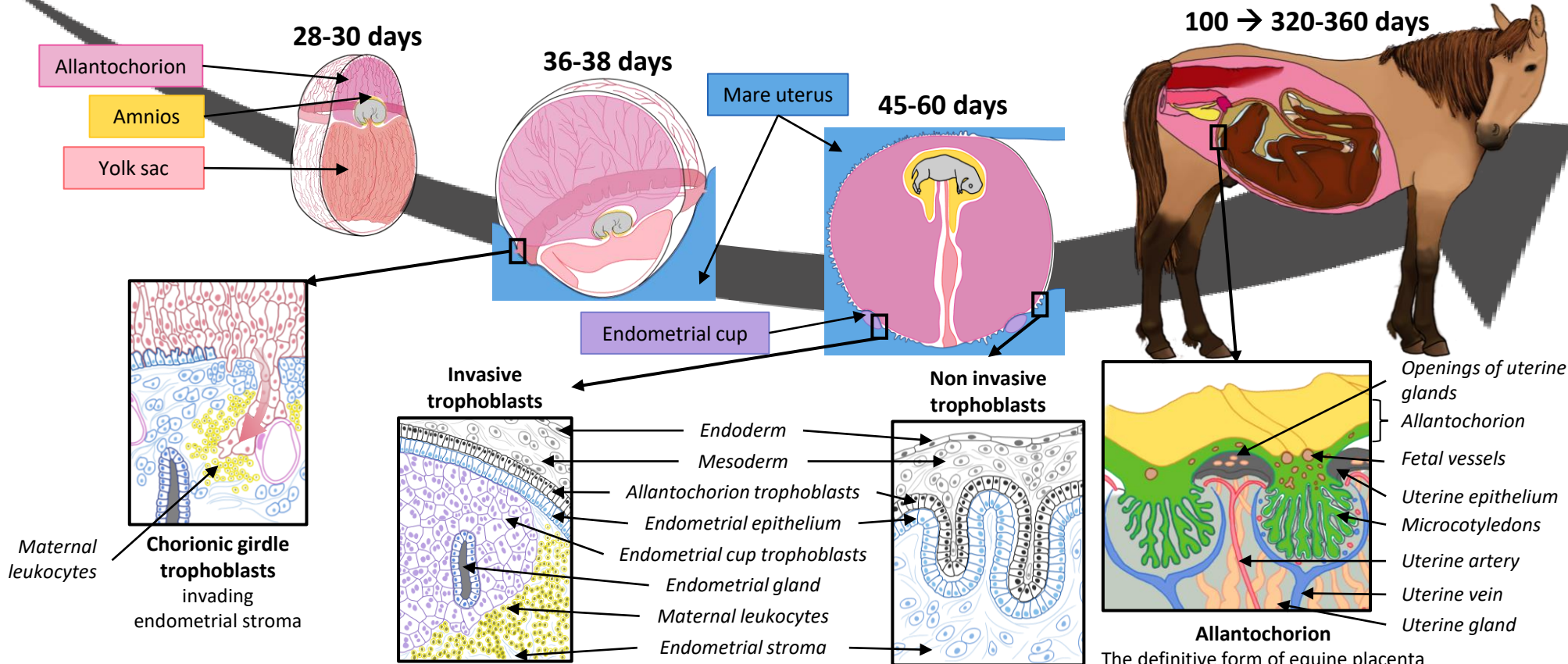
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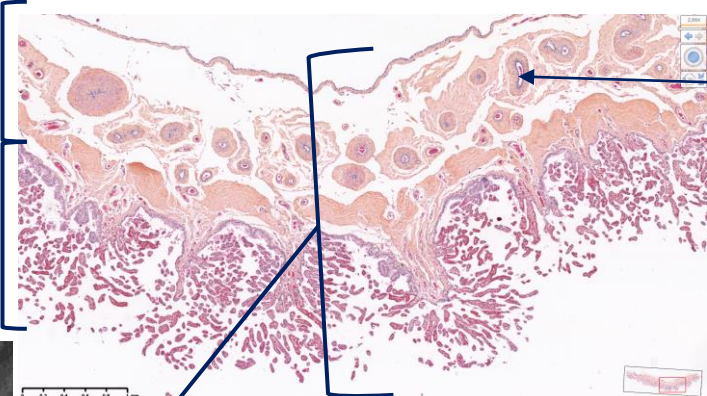
Fetal age	28 days	36 days	60 days	105 days
<b>Teguments</b>	Translucid skin + hoof started	Development of dermis vessels	Skin thicker and less transparent + hoof apparatus formed	Hair on the rostrum
<b>Bones</b>	Visible somite and cartilage + ribs in formation		Started ossification + visible ribs and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



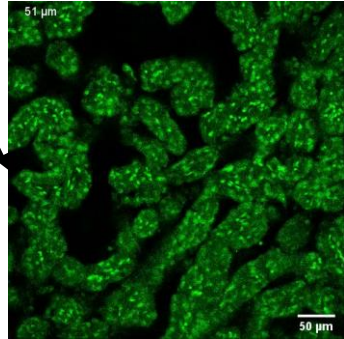
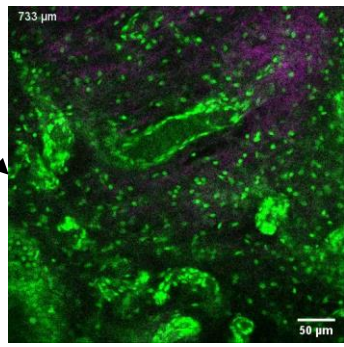
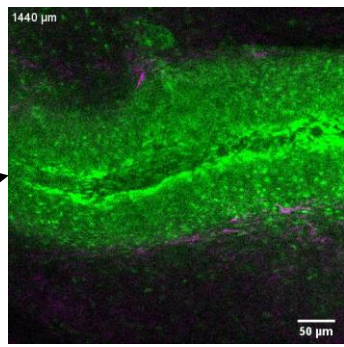
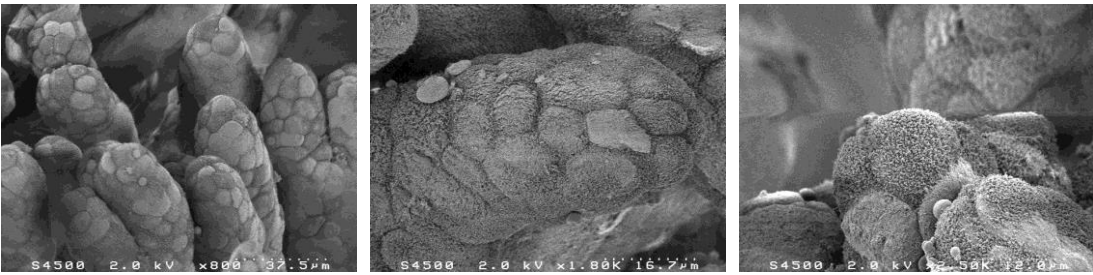
Allantoic region

Microcotyledonary region

Fetal vessels



Chorioallantois



Fetal vessels

Openings of uterine glands

Uterine epithelium

Microcotyledon

Uterine artery

Uterine vein

Endometrial glands

