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# 1 Female age and parity in horses: how and why does it 2 matter?

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13 Running title: Effects of maternal age and parity in horses

14

15 Abstract:

16 Although puberty can occur as soon 14-15 months of age, depending on their breed and use, the  
17 reproductive career of mares may start up to advanced age. Once mares are used as broodmares, they  
18 will usually produce foals once a year until they become infertile, and their production can be  
19 enhanced and/or prolonged through embryo technologies. There is a general consensus that old mares  
20 become less fertile, but maternal age and parity are confounding factors as younger mares are usually  
21 nulliparous and older mares are multiparous in most studies. This review shows that age essentially  
22 affects cyclicity, folliculogenesis, oocyte and embryo quality as well as oviductal masses and uterine  
23 tract function. Maternal parity has a non-linear effect. Primiparity has a major influence on placental  
24 and foal development, with smaller foals in first gestation that remain smaller postnatally. After the  
25 first gestation, endometrial quality and uterine clearance capacities progressively decline with  
26 increasing parity, whilst placental and foal birthweight and milk production increase. These combined

27 effects should be carefully weighed when breeding mares, in particular when choosing and caring for  
28 recipients and their foals.

29

30

## 31 Introduction

32 Horses and ponies are mainly bred for athletic performance and leisure (mainly ponies and warmblood  
33 horses), some being also used for meat production (mainly draft horses). For sport and leisure, the  
34 length of a horse's athletic career varies depending on the discipline, but also on performance and  
35 health. For instance, Thoroughbreds are usually trained for flat races, being allowed to compete until  
36 4 years of age, while trotters compete from 2 to 7-8 years of age. In show jumping, dressage, however,  
37 horses are commonly competing past 15 years of age while for leisure, they may still be ridden after  
38 20. Although puberty can occur as soon 14-15 months of age if this age happens during the breeding  
39 season (Palmer and Driancourt 1983), the reproductive career of mares may start at different ages  
40 after completing their performance. In contrast, draft mares, similar to cattle, are usually bred at an  
41 early age.

42 Unlike in other farm animal production schemes, a horse breeder may have several reasons to produce  
43 foals from old mares: i) the mare previously produced valuable foals, ii) the mare's performances  
44 and/or genetic or sentimental value is important, iii) the mare is fertile. There are many old mares in  
45 breeding farms. In Canada and Finland, 37% and 53% of Standardbred mares, respectively, were  
46 reported to be older than 10 (Physick-Sheard 1995; Katila *et al.* 2010). In a retrospective study of 3373  
47 Thoroughbred broodmares in UK, 54% were older than 8 and 4% older than 18 (Allen *et al.* 2007). In  
48 Spain, a survey on 296 Thoroughbred mares showed that mean age at first foaling in was 6.88, that  
49 mares produced a mean of 5.47 foals, with a mean time from last foaling to death of 18 months and a  
50 total reproductive career of 10.93 years in average (Hevia *et al.* 1994). Moreover, in Finland, 63% of  
51 Finn horse mares are older than 10 at insemination (Katila *et al.* 2010).

52 Regardless of breed and for a long time, many studies have shown that mares' embryo recovery  
53 (Pascoe *et al.* 1985; Vogelsang and Vogelsang 1989; Ball *et al.* 1989; Carnevale, Griffin *et al.* 1993; Uliani  
54 *et al.* 2010; Panzani *et al.* 2014; Marinone *et al.* 2015; Cuervo-Arango *et al.* 2019b), fertility (Ball *et al.*  
55 1986; Woods *et al.* 1987; Barbacini *et al.* 1999; Morris and Allen 2002; Samper *et al.* 2002; Allen *et al.*  
56 2007; Bosh *et al.* 2009; Benhajali *et al.* 2010; Sharma *et al.* 2010; Hanlon *et al.* 2012; Haadem *et al.*  
57 2015; Lane *et al.* 2016; Kareskoski *et al.* 2019) or foaling rates (Hutton and Meacham 1968; Jeffcott *et*  
58 *al.* 1982; Gibbs and Davison 1992; McDowell *et al.* 1992; Baker *et al.* 1993; Brück *et al.* 1993; Morley  
59 and Townsend 1997; Vidament *et al.* 1997; Hemberg *et al.* 2004; Allen *et al.* 2007; Katila *et al.* 2010;  
60 Sharma *et al.* 2010; Nath *et al.* 2010; Haadem *et al.* 2015) decrease with maternal age, due to increased  
61 embryo and fetal mortality (Bain 1969; Platt 1973; Pascoe *et al.* 1985; Woods *et al.* 1985; Ball *et al.*  
62 1989; Chevalier-Clément 1989; Carnevale and Ginther 1992; Gibbs and Davison 1992; Baker *et al.* 1993;  
63 Brück *et al.* 1993; Morley and Townsend 1997; Morris and Allen 2002; Hemberg *et al.* 2004; Allen *et*

64 *al.* 2007; Bosh *et al.* 2009; Uliani *et al.* 2010; Sharma *et al.* 2010; Panzani *et al.* 2016; de Mestre *et al.*  
65 2019; Malheiros de Souza *et al.* 2020). The age of oocyte donors, however, does not appear to affect  
66 blastocyst production (Altermatt *et al.* 2009) nor gestation rate after oocyte transfer (Carnevale *et al.*  
67 2005). In addition, several studies did not observe any difference in mare fertility related to maternal  
68 age (McCue *et al.* 2009; Uliani *et al.* 2010; Avci *et al.* 2016; Pérez-Marín *et al.* 2017; Malheiros de Souza  
69 *et al.* 2020; Carluccio *et al.* 2020). These discrepancies on the effect of mare age on fertility could be  
70 due to the difficulty to define at what age a mare will experience reproductive aging. Indeed, there is  
71 a great variation in fertility decline depending on individuals, breeds, mare management, farms and  
72 thus, according to studies.

73 The analysis of 35,746 fertility records in mixed breeds in France showed that mares' fertility was the  
74 highest from 4 to 6 years of age and then decreased gradually to become almost zero at 25-26 (Langlois  
75 and Blouin 2004). This study also calculated that if a decline of 10% of her highest fertility was chosen  
76 as a cutoff value to stop a broodmare's breeding career, then mares older than 8 should not be bred  
77 anymore (Langlois and Blouin 2004). Similarly, a progressive decline in fertility with age was described  
78 in Thoroughbred mares, starting from 75% fertility at 4 years of age (Figure 1) (Jeffcott *et al.* 1982). In  
79 a Brazilian study, analysis of 11 years lactating Thoroughbreds' fertility data showed that the threshold  
80 for embryo mortality to remain above average was 10 years of age (Malheiros de Souza *et al.* 2020).  
81 Therefore, if fertility was the only criterion for keeping a broodmare, as in other farm animal breeding,  
82 mares older than 10 should be culled, which would also increase genetic progress (Palmer and  
83 Chavatte-Palmer 2020). Nevertheless, this is not the case as breeders will continue breeding as long as  
84 they can produce a foal from their favorite mare(s). Thus, treatments to improve older mares' fertility  
85 are part of routine practice for veterinarians.

86 As sport career is prioritized in Warmbloods, it is common to see nulliparous mares (mares that did  
87 not previously foal, also known as maiden mares) bred at old age. Indeed, nulliparous mares older than  
88 10 years of age have been shown to represent 20.5% of Finn horse and 15.5% of Standardbred bred  
89 mares in Finland (Katila *et al.* 2010). In farm animals and draft horses, maternal age is highly correlated  
90 with parity, as defined, for an individual mare, as the number of her pregnancies that reached a viable  
91 gestational age, stillbirths and live birth included. Indeed, younger animals have given birth to less  
92 offspring than older dams. In equine studies, this is mainly also true, especially when animals dedicated  
93 to equine research studies are used.

94 The effects of parity alone on fertility, embryo recovery, embryo/fetal mortality rates and term birth  
95 have been studied but results are controversial. Only one study reported a detrimental effect of mares'  
96 nulliparity on pregnancy rate and only when using frozen semen (35% pregnancy rate per cycle in 155

97 maiden mares *versus* 53% and 54% in 94 barren and 174 foaling mares, respectively) (Squires *et al.*  
98 2006). This detrimental effect seems to be due to older nulliparous mares as an important number of  
99 mares were older than 7, whereas in papers showing no effect, maiden mares were between 3 and 5  
100 years old (Brück *et al.* 1993). Similar observations on reduced fertility in maiden mares older than 8  
101 have also been reported by others (Samper *et al.* 2002). In terms of embryo development, while some  
102 did not find any effect of parity (Platt 1973; Chevalier-Clément 1989; Gibbs and Davison 1992;  
103 Vidament *et al.* 1997; Morris and Allen 2002; Langlois and Blouin 2004; Hanlon *et al.* 2012; Panzani *et*  
104 *al.* 2016), others showed that mares that did not previously foal are more productive with lower  
105 embryo and fetal mortality compared to multiparous mares (Baker *et al.* 1993; Brück *et al.* 1993;  
106 Barbacini *et al.* 1999; Hemberg *et al.* 2004; Nath *et al.* 2010; Hanlon *et al.* 2012; Carluccio *et al.* 2020).  
107 Moreover, when embryos collected from nulliparous mares were transferred in commercial embryo  
108 transfer programs, the pregnancy rate increased and the abortion rate decreased (Vogelsang and  
109 Vogelsang 1989) compared to embryos collected from other mares. In most of these latter studies,  
110 however, mainly young mares were used. In studies where donors were mostly old and nulliparous at  
111 the same time, however, fertility was decreased (Samper *et al.* 2002; Katila *et al.* 2010).

112 As described above, maternal age and parity in horses have been shown to affect reproductive  
113 efficiency. Reproductive efficiency is dependent of oocyte, embryo and uterine quality as well as  
114 maternal ability to carry out gestation. Those factors are known in other species to possibly also affect  
115 long term offspring health (Watkins *et al.* 2010; Duranthon and Chavatte-Palmer 2018).

116 In a first approach, it would seem logical that age would affect ovary and oocyte quality primarily, while  
117 parity could affect the reproductive tract and feto-placental development. Nevertheless, these effects  
118 need to be studied thoroughly. Thus, the aim of this review is first to focus on the known effects of  
119 mares' age and parity on ovarian environment, oocyte and embryo development and to summarize  
120 effects of maternal age and parity on gestation, placental and fetal development, as well as long-term  
121 effects on the offspring. Since almost no studies on the effects of maternal age used the same  
122 comparison groups, the authors decided to use the words "old" and "young" to represent the oldest  
123 or youngest groups in a study, respectively. The precise age categories for each study cited below are  
124 represented in the various summary tables.

125

## 126 **Ovary and the estrus cycle (Table 1)**

127 *Follicular reserve*

128 As for other mammals, mares are born with a set number of primordial follicles, that varies between  
129 individuals (Driancourt *et al.* 1983; Monniaux *et al.* 2014). Starting around 1 year of age, mares' oocytes  
130 are released from the follicle during ovulation or degraded by atresia (Ginther 1992). Therefore, the  
131 number of available follicles declines with aging (Carnevale, Bergfelt *et al.* 1993; Vanderwall *et al.* 1993;  
132 Ginther *et al.* 2009; Claes *et al.* 2015, 2017; Alves *et al.* 2017; Cuervo-Arango *et al.* 2019b; Goncalves  
133 *et al.* 2020). Even if "menopause" *per se* does not apply to horses, because mares do not menstruate,  
134 ovarian activity is reduced after 15 years of age (Wesson and Ginther 1981) and is not observed  
135 anymore in an average 17% pony and Saddlebred mares over 20 (Vanderwall *et al.* 1993; Carnevale *et*  
136 *al.* 1994). It has been suggested that at 25 years of age, mares have consumed all their available oocyte  
137 stock (Ginther 1992). For several mammals, antral follicle counts (AFC), related to ovarian reserve, are  
138 predicted by anti-Müllerian hormone (AMH) serum concentrations, a granulosa-cell-derived hormone  
139 (for review, Visser and Themmen 2005). For horses, the measurement of AMH serum concentration  
140 has been shown to predict mare fertility (Ball *et al.* 2019). While in old mares the correlation between  
141 AFC and AMH is strong, it appears weaker in young mares (Claes *et al.* 2015; Uliani *et al.* 2019; Korkmaz  
142 *et al.* 2020).

#### 143 *Estrus cycle and follicular waves*

144 Mares are seasonal breeders with a long day breeding season (Burkhardt 1947; Nishikawa 1959). The  
145 breeding period is shorter in pony mares younger than 5 (Wesson and Ginther 1981) and delayed in  
146 ponies over 15 years old (Wesson and Ginther 1981) and in mares at 20 years of age (Vanderwall *et al.*  
147 1993; Carnevale *et al.* 1997). During the reproductive season, mares' reproductive cycle lasts in  
148 average 21 days (Nishikawa 1959). Mares older than 20-year-old have been shown to have a longer  
149 interovulatory interval due to prolonged follicular growth but without changes in luteal phase length  
150 (Ginther *et al.* 1993, 2008, 2009; Carnevale, Bergfelt *et al.* 1993; Vanderwall *et al.* 1993; Carnevale *et*  
151 *al.* 1994; Marinone *et al.* 2015; Claes *et al.* 2017). One explanation for this prolonged follicular growth  
152 is that, at the time of luteolysis, older mares have smaller follicles (Wesson and Ginther 1981; Ginther  
153 *et al.* 1993; Carnevale *et al.* 1997). In addition, the follicular wave emerges later (Ginther *et al.* 1993)  
154 and with less follicles in old mares than in younger (Ginther *et al.* 1993, 2009). This is associated with  
155 a reduction in the ovulatory follicle growth in oldest mares (Carnevale, Bergfelt *et al.* 1993; Ginther *et*  
156 *al.* 2008; Altermatt *et al.* 2012).

#### 157 *Hormonal profiles prior to ovulation*

158 As demonstrated in other species, such as cows or women, age-related differences are associated with  
159 a higher follicle stimulating hormone (FSH) plasma concentration in older mares (Carnevale, Bergfelt  
160 *et al.* 1993; Ginther *et al.* 2008; Claes *et al.* 2017). Two studies have also shown that maximal luteinizing

161 hormone (LH) plasma concentration is reduced in mares older than 10 years of age (Ginther *et al.* 2008,  
162 2009) while another one only finds a difference when mares were older than 20 compared to younger  
163 mares (Carnevale, Bergfelt *et al.* 1993). In humans, the increased FSH plasma concentration and the  
164 less pronounced peak of LH plasma concentration, that is also observed in mares, is thought to be an  
165 early sign of reproductive aging (Carnevale 2008). Furthermore, FSH upregulation is responsible for  
166 the recruitment of more follicles per wave than required, which is accelerating the reduction of follicle  
167 number (Davis *et al.* 2015). Subsequently, the reduction in follicle number induces a decreased ovarian  
168 production of hormones such as estradiol or inhibin, leading to a reduced hypothalamic pituitary axis  
169 inhibitory feedback. The effect of mares' aging on estradiol plasma concentrations is controversial: one  
170 study reported that mares younger than 10 years have a higher maximum concentration of estradiol  
171 (Ginther *et al.* 2008) while others did not find any difference according to age (Ginther *et al.* 2009). In  
172 any case, improper feedback leads to a gradual dysregulation of circulating gonadotropins  
173 concentration.

#### 174 *Ovulation*

175 Hormonal perturbations are expected to alter the ovulation process, although studies show  
176 contradictory results. Mare's age was reported to be negatively correlated with follicular size prior to  
177 ovulation (Carnevale *et al.* 1997; Ginther *et al.* 2008; Davies Morel *et al.* 2010; Mata *et al.* 2013) but  
178 other studies did not demonstrate any association (Carnevale, Bergfelt *et al.* 1993; Ginther *et al.* 2009;  
179 Claes *et al.* 2017). In terms of ovulation, an almost 40 years old study based on veterinarian responses  
180 to a survey indicated that maternal age does not seem to influence multiple ovulation rate (Ginther *et*  
181 *al.* 1982). Nevertheless, more recent studies showed that multiple ovulation rates continuously  
182 increase until mares reach around 20 years of age (Davies Morel and O'Sullivan 2001; Morel *et al.*  
183 2005; Panzani *et al.* 2014; Marinone *et al.* 2015) and subsequently decrease (Morel *et al.* 2005; Panzani  
184 *et al.* 2014). Follicular vascularization, as measured by Doppler ultrasonography, has been shown to be  
185 greater in old compared to younger mares. This may be partly due to the altered LH secretion or  
186 follicular sensitivity and could therefore be the result of differences in the timing of follicular  
187 maturation between ages (Altermatt *et al.* 2012).

#### 188 *Induction of ovulation*

189 Reproductive cycle alterations in old mares also interfere with the response to induction of ovulation.  
190 Indeed, more mares over 16 years of age were shown to fail to ovulate 48 hours after human Chorionic  
191 Gonadotropin (hCG) injection compared to younger mares (Barbacini *et al.* 2000). This could actually  
192 be due to immunization against hCG in older mares. The ovulatory response to deslorelin acetate  
193 implant was shown to be reduced in mares older than 20 (Farquhar *et al.* 2000), but the data are



194 moderately convincing because ovulation rates were very similar to the other groups, with a limited  
195 number of mares.

#### 196 *Luteal phase*

197 After ovulation, the ruptured follicle develops as corpus luteum (CL) and starts producing progesterone  
198 (Stabenfeldt *et al.* 1972). Luteal phase plasma progesterone concentrations are increased in  
199 reproductively active old mares although the threshold age to observe this increase varies from one  
200 study to another (Carnevale, Bergfelt *et al.* 1993; Vanderwall *et al.* 1993; Ginther *et al.* 2008; Claes *et*  
201 *al.* 2017). Old mares' CL were shown to undergo structural remodeling (increased luteal volume that  
202 compensates a reduced luteal vascularization) enabling the maintenance of progesterone production  
203 (Camargo Ferreira *et al.* 2019).

204 **In summary, mare's age affects the ovarian cycle of the mare with altered cycle regularity and**  
205 **reduced number of ovulatory cycles in very old mares (>18-20), accompanied with reduced AMH**  
206 **concentrations, indicating a declining follicular reserve. Nevertheless, signs of reduced ovarian**  
207 **activity may appear as early as 15 years of age. Together with increasing FSH concentrations, age**  
208 **induces a higher incidence of multiple ovulations.**

209 **To the authors' knowledge, the effects of mare parity have not been studied.**

210

## 211 **Follicular organization, oocytes and embryos (Table 2)**

### 212 *Follicular organization*

213 In addition to the reduced number of follicles observed in old mares described above, these follicles  
214 are less likely to be morphologically regular in terms of shape and number of granulosa cell layers  
215 surrounding the oocyte (Alves *et al.* 2017). Mares' age has also been shown to affect the expression of  
216 genes involved in mitochondrial function, receptors of metabolic hormones, inflammatory processes,  
217 steroids and tissue remodeling in granulosa cells, all of which could impair fertility (Sessions-Bresnahan  
218 and Carnevale 2015).

219 Changes in gene expression in the granulosa cells may also impair follicular fluid composition. Although  
220 plasma concentrations of ovarian steroids are not affected by mares' age, insulin-like growth factor 1  
221 (IGF-1) concentrations in follicular fluid are higher in old compared to young mares (Ginther *et al.*  
222 2008). The analysis of follicular fluid's exosomes and microvesicles reveals an effect of maternal age  
223 on miRNAs, suggesting an altered communication between granulosa cells and oocyte in old mares (da

224 Silveira *et al.* 2012, 2015). Interestingly, the addition of follicular fluid from old mares in *in vitro* oocyte  
225 maturation medium tended to improve horse blastocyst development compared to follicular fluid of  
226 young mares (Spacek and Carnevale 2018).

### 227 *Oocyte morphology*

228 The effect of maternal age on general morphology of immature and mature oocytes is controversial:  
229 some studies failed to observe any differences (Brinsko *et al.* 1995; Carnevale *et al.* 1999; Frank *et al.*  
230 2019) while others observed different characteristics (irregular shape, irregularities in organelle  
231 distribution in the cytoplasm) in oocytes from old mares associated with development failure  
232 (Carnevale *et al.* 2005; Altermatt *et al.* 2009). Moreover, using light microscopy, a larger incidence of  
233 oocyte abnormalities such as large cytoplasmic vesicles was described in old mares compared to young  
234 mares, which is also a sign of cell development defect (Carnevale *et al.* 1999).

### 235 *Oocyte quality*

236 If effects of age on oocyte morphology do not seem obvious, oocyte developmental potential is clearly  
237 affected by maternal age. Indeed, old mares' oocytes have been shown to have four times less chance  
238 to reach metaphase II after *in vitro* maturation compared to oocytes from young mares (Brinsko *et al.*  
239 1995), suggesting that maternal age affects oocyte quality. During *in vivo* development, the  
240 quantitative and temporal expression of several genes essential for oocyte maturation differs  
241 according to maternal age, suggesting an age-related desynchronization during oocyte maturation  
242 (Campos-Chillon *et al.* 2015). In cumulus-oocytes complexes, moreover, maternal age alters the  
243 expression of 48 genes identified in humans to be related to maternal aging and aneuploidy, which  
244 may be associated to the reduced developmental competence observed in old mares' oocytes (Cox *et al.*  
245 *et al.* 2015). After *in vitro* maturation (IVM), predisposition to chromosome misalignment on the  
246 metaphase II spindle have been observed in oocytes from old mares (Rizzo *et al.* 2019), possibly linked  
247 to a weakened centromeric cohesion of spindle (Rizzo *et al.* 2020), as confirmed by the reduced  
248 expression of *Mono Polar Spindle 1 kinase (MPS1)* in IVM oocytes from old mares leading to meiotic  
249 spindle instability, which could in return predispose to poorer chromosome segregation (Rizzo *et al.*  
250 2021). Loss of cohesion and poor spindle stability induce premature and poor separation of sister  
251 chromatids during the end of the first meiotic division, conducive to embryonic aneuploidy (Rizzo *et al.*  
252 *et al.* 2019, 2020, 2021). Surprisingly, the vitrification of oocytes is more deleterious for young mares'  
253 compared to old mares' oocytes (Ducheyne *et al.* 2019). Authors hypothesize that, in old mares,  
254 mechanisms related to spindle are already compromised before vitrification, limiting impacts of  
255 vitrification on oocyte quality in old mares (Ducheyne *et al.* 2019).

256 High numbers of mitochondria are present in the oocyte, and their number and activity have been  
257 described to decline with age in women (for review van der Reest *et al.* 2021). In equine, oocyte mtDNA  
258 copy numbers were shown to be decreased in old mare oocytes, while in young mares, they remains  
259 stable during *in vitro* (Rambags *et al.* 2014) or *in vivo* maturation (Campos-Chillon *et al.* 2015). One  
260 study, however, did not observe any difference according to maternal age but reduced metabolic  
261 function and capacity in oocytes and day-2 embryos originating from oocytes of old when compared  
262 to young mares. In mature oocytes, however, mtDNA was quantified by real-time PCR and was not  
263 different between the age groups (Catandi *et al.* 2021). Nevertheless, in this study, the abundance of  
264 free fatty acids was reduced in old mares' oocytes and developmental capacity was impaired,  
265 suggesting that mitochondrial copy numbers may not be indicative of mitochondrial function (Catandi  
266 *et al.* 2021). After *in vitro* maturation but not before, old mares' oocytes mitochondria display more  
267 swelling and less cristae compared to controls, suggesting a predisposition to mitochondrial  
268 degeneration with aging (Rambags *et al.* 2014). In contrast, others reported that maternal aging does  
269 not affect ATP content nor the expression of selected genes involved in mitochondria replication and  
270 function in oocytes (Hendriks *et al.* 2015).

#### 271 *Fertilisation*

272 Fertilization does not seem to be impaired by maternal age as the number of embryos collected via  
273 oviductal flushing at Day 2 post ovulation is similar between young, fertile mares and old, subfertile  
274 mares (Ball *et al.* 1986). Cytoskeleton differences at fertilization between oocytes from young and old  
275 mares, however, are observed. On one hand, *in vitro* sperm-injected oocytes from younger mares  
276 present more microtubule multiasters related to oocyte activation (Ruggeri *et al.* 2015). On the other  
277 hand, oocytes from older mares present more actine vesicles, indicating an age-related cytoskeletal  
278 remodeling (Ruggeri *et al.* 2015). How these differences affect embryo development remains  
279 unknown. Using either artificial insemination, oocyte or Day 4 embryo transfer into young recipient  
280 mares, less pregnancies are obtained when donor mares are old (Ball *et al.* 1986, 1989; Carnevale and  
281 Ginther 1995; Carnevale *et al.* 2005). Conversely, in most studies, maternal age is not shown to affect  
282 the number of *in vitro* blastocysts produced from oocytes (Altermatt *et al.* 2009; Cuervo-Arango *et al.*  
283 2019a; b) although some authors find different results (Catandi *et al.* 2021). It appears, therefore, that  
284 age of the mare leads to oocyte deficiency by altering its developmental competency but that oocyte  
285 environmental manipulation, such as *in vitro* maturation, may partly reduce the deleterious effects of  
286 mare age.

#### 287 *Embryo development*

288 Effects of maternal age on embryo morphology are not clear. At various developmental stages after *in*  
289 *vivo* or *in vitro* development, some studies showed that embryos from old mares are smaller (Woods  
290 *et al.* 1985; Carnevale, Griffin *et al.* 1993; Brinsko *et al.* 1994; Willmann *et al.* 2011; Panzani *et al.* 2014;  
291 Camargo Ferreira *et al.* 2019), while others do not observed any difference in size (Carnevale and  
292 Ginther 1992, 1995; Carnevale, Griffin *et al.* 1993; Morel *et al.* 2005; Frank *et al.* 2019; Derisoud *et al.*  
293 2021). At Day 1.5 post ovulation, *in vivo* oviductal embryos have the same number of cells regardless  
294 of the mare's age although, when embryos are recovered from old mares, cells are less uniform (size  
295 and texture) and present more imperfections and abnormalities (Carnevale, Griffin *et al.* 1993). At Day  
296 3 post ovulation, in addition to the poorer morphology, embryo cell number is reduced in old mares  
297 (Carnevale, Griffin *et al.* 1993). Conversely, at Day 2 and 4 post ovulation, other studies did not find  
298 any difference related to maternal age for diameter, number, size or texture of blastomeres in *in vivo*  
299 oviductal embryos (Ball *et al.* 1986, 1989). In *in vitro* produced embryos, the number of blastomeres  
300 was reduced on Day 1 post intra-cytoplasmic sperm injection (ICSI) but not any more on Day 2  
301 (Altermatt *et al.* 2009). On Day 7 to day 10 post ovulation, uterine *in vivo* embryos collected during  
302 commercial embryo transfer program are smaller (Woods *et al.* 1985; Panzani *et al.* 2014). Poorer  
303 embryo quality was also reported in one study (Woods *et al.* 1985) but not the other (Panzani *et al.*  
304 2014). *In vitro*, ICSI produced embryos from young and old mare oocytes had comparable blastomere  
305 size, ooplasm fragments and membrane extrudates at 24h post-ICSI (Frank *et al.* 2019). At the time of  
306 transfer, however, embryos from mares older than 20 years old had a higher cell count (Frank *et al.*  
307 2019). In another study, after 7 days of culture, blastocysts derived from Day 2 *in vivo* embryos  
308 collected in old mares were smaller with less cells and had a poorer quality score than the ones  
309 collected in young mares (Brinsko *et al.* 1994). As for oocytes, these data suggest that *in vitro*  
310 production of embryos may at least partially erase deleterious effects of maternal aging, maybe due  
311 to the low efficiency of the technique.

312 In terms of gene expression, differences observed in oocytes are also found in embryos. Indeed, in Day  
313 2 *in vitro* produced embryos derived from oocytes of old mares, metabolic function and capacity were  
314 also reduced, as observed directly in oocytes (Catandi *et al.* 2021). In Day 7 embryos recovered from  
315 old mares, a reduced quantity of mitochondrial DNA copy number, representing the number of  
316 mitochondria, is observed, while genomic DNA, representing the number of cells, remains stable,  
317 indicating that embryos from old mares have fewer mitochondria per cell (Hendriks *et al.* 2015), which  
318 can compromise preimplantation development. A recent study from our laboratory showed that in  
319 Day 8 blastocysts, maternal age affects the expression of genes related to mitosis, translation and  
320 transcription, cell signaling and adhesion, mitochondrial function and cell commitment, which may  
321 affect further embryo development (Derisoud *et al.* 2021).

322 Subsequently, maternal age appears to impact embryo mobility and day of fixation although studies  
323 are controversial. One recent study showed that embryo mobility is prematurely reduced in old mares  
324 without affecting the day of fixation (Camargo Ferreira *et al.* 2019). Conversely, an older study shows  
325 that embryo mobility is not affected by maternal age although fixation of embryos from old mares  
326 occurs one day later compared to embryos from young mares (Carnevale and Ginther 1992).

327 Finally, in a study looking exclusively at embryo recipients of different ages, the age of the recipient  
328 did not affect initial pregnancy rates up to 15-18 years of age but embryo loss between first pregnancy  
329 diagnosis and D50 was 20.5% in mares aged from 10 to 18 versus 13.3% in younger mares (Carnevale  
330 *et al.* 2000). This latter observation may be due to endometrial ageing, as discussed in the following  
331 section.

332

333 **In summary, despite some discrepancies in the literature, there are clear effects of mare age on**  
334 **follicle, oocyte and embryo quality. Disturbed gene expression was observed in old mares' granulosa**  
335 **cells, among which genes involved in immunity and inflammation. Both oocyte morphology and**  
336 **quality are disturbed in older mares, including alterations of oocyte spindle stability that could lead**  
337 **to embryo aneuploidy, altered gene expression, reduced mitochondrial DNA copy numbers, all**  
338 **leading to reduced oocyte developmental potential and altered cell numbers and metabolism in the**  
339 **embryo. Indeed, although fertilization rates appear to be globally similar between young and older**  
340 **mares, development to the blastocyst stage is in general reduced in mares older than 16, together**  
341 **with reduced embryo diameter and an increased incidence of morphological abnormalities.**

342 **The little data available for effects of parity do not indicate any effect.**

343

### 344 **The reproductive tract: oviducts, uterus and cervix (Table 3)**

#### 345 *Oviduct*

346 Fertilization and early embryo development take place in the oviducts and the oviductal environment  
347 may be affected by maternal age. Old mares' oviducts have a higher risk of developing fibrosis  
348 compared to young mares (Pinto-Bravo *et al.* 2018). *Collagen type 3* gene, related to endometrial  
349 fibrosis, is less expressed in oviducts of mares older than 10-year-old (Pinto-Bravo *et al.* 2018).  
350 Moreover, macroscopic structures now known as fibroblast masses have been reported in horse  
351 oviducts (Niekerk and Gerneke 1966; Onuma and Ohnami 1975; Liu *et al.* 1990). Cells from ovaries

352 initiate masses formation, most likely due to degenerated unfertilized oocytes and cumulus cells.  
353 These masses are not identified in oviducts from prepubertal mares and maternal age is positively  
354 correlated with the number of masses observed on oviduct (Aguilar *et al.* 1997). They could interfere  
355 with fertilization and/or migration of the ova, spermatozooids or the embryo and be a potential cause  
356 of infertility (Zent *et al.* 1993).

357 Using electrophoresis, a 25 year old study identified a relatively comparable protein composition in *in*  
358 *vitro* secretions from cultured oviductal epithelial cells collected from old and subfertile or young and  
359 fertile mares, although 9 non-identified proteins were less abundant in old mares' oviductal secretions,  
360 suggesting that oviductal environment may be partly responsible for failure to sustain embryo  
361 development in older animals (Brinsko *et al.* 1996). When embryos are co-cultured with oviductal  
362 epithelial cells from young fertile *versus* old and subfertile mares, however, no difference in blastocyst  
363 development rate was observed, although young mares' Day 7 blastocysts were bigger and with a  
364 higher quality score when co-cultured with oviductal cells from young and fertile mares (Brinsko *et al.*  
365 1994).

#### 366 *Uterus – uterine cysts and vascularization*

367 After 5-6 days in the oviduct, the embryo induces its own passage in the uterus at the late morula/early  
368 blastocyst stage (Weber *et al.* 1991, 1992, 1995). Maternal age and parity have been shown to induce  
369 degenerative changes in the uterus. Indeed, uterine cysts covering a large uterine surface are  
370 commonly observed in old and multiparous mares (Adams *et al.* 1987; Chevalier-Clément 1989;  
371 Bracher *et al.* 1992; Eilts *et al.* 1995; Tannus and Thun 1995; Ferreira *et al.* 2008). The effect of cysts  
372 on pregnancy rates are controversial before and after implantation (Adams *et al.* 1987; Eilts *et al.* 1995;  
373 Tannus and Thun 1995). It has been demonstrated that cysts could prevent embryo movements in the  
374 uterus while this movement is mandatory for maternal recognition of pregnancy in horses (McDowell  
375 *et al.* 1988). Cysts might also interfere with embryo fixation and implantation. Furthermore, a larger  
376 cystic area is positively correlated with reduced vascular perfusion in the uterus (Ferreira *et al.* 2008).  
377 In old and multiparous mares with uterine cysts, gravid uterine perfusion is reduced (Ousey *et al.*  
378 2012). When cysts are not present, however, the perfusion of old or young multiparous uteri is similar  
379 (Ferreira *et al.* 2008) although uterine artery resistance is increased (Bollwein 1998). Regardless of  
380 cysts, vascular alterations are observed in early gestation in old compared to young mares (Ferreira *et*  
381 *al.* 2015). Decreased uterine perfusion has been associated with elastosis, characterized as increased  
382 presence of elastic fibers (Esteller-Vico *et al.* 2015). Frequent angiosclerotic lesions, such as mild to  
383 moderate perivascular and intimal sclerosis, are observed in old multiparous mares (Grüninger *et al.*  
384 1998). Furthermore, both an hyperplasia of intimal elastic fibers, related to elastosis, and a thinning of

385 the medial smooth musculature, have been observed in uterine arteries as mares become older  
386 (Oikawa *et al.* 1993; Nambo *et al.* 1995) or parity increases (Esteller-Vico *et al.* 2012). One study  
387 showed that vascular degeneration is clearly related to the number of previous foaling and not to the  
388 mares' age (Esteller-Vico *et al.* 2012).

389

#### 390 *Uterus – endometrial degenerative changes*

391 Most studies showed that severe endometrial changes are more frequent in endometrial biopsies from  
392 old and/or multiparous mares in comparison to biopsies from young and/or nulliparous mares  
393 (Ricketts 1975; Doig *et al.* 1981; Waelchli 1990; Ricketts and Alonso 1991; Brinsko *et al.* 1994;  
394 Grüninger *et al.* 1998; Woodward *et al.* 2012; Esteller-Vico *et al.* 2012, 2015; Ferreira *et al.* 2015). Only  
395 one study did not find any differences according to age (Annandale *et al.* 2018). Observed changes  
396 were related to inflammatory and fibrotic modifications (Kenney 1978) with severity of uterine fibrosis  
397 being positively correlated with mares' age (Doig *et al.* 1981; Siemieniuch *et al.* 2017). Moreover, the  
398 height of the lining and glandular endometrium epithelium's stratum compactum is less variable  
399 between estrus and diestrus (Dubrovskaya *et al.* 2019) in old compared to younger mares. In addition,  
400 fewer and larger uterine glands are present in old mares' endometrium, both in estrus and diestrus  
401 (Carnevale and Ginther 1992; Dubrovskaya *et al.* 2019), with more extensive inflammatory cell  
402 infiltrations and fibrotic changes (Carnevale and Ginther 1992).

403 Parity in young mares does not seem to influence the ultrastructure of the endometrium (Tunón *et al.*  
404 1995). In contrast, old maiden mares also develop endometrial degenerative changes (Ricketts and  
405 Alonso 1991), suggesting that maternal aging is mostly responsible for these degenerative changes,  
406 over semen challenge, infection, pregnancy, parturition and post-partum involution (Ricketts and  
407 Alonso 1991; Esteller-Vico *et al.* 2012).

#### 408 *Endometritis*

409 For old mares, a higher incidence of endometritis has been demonstrated in old compared to young  
410 mares (Adams *et al.* 1987; Carnevale and Ginther 1992; Barbacini *et al.* 1999; Woodward *et al.* 2012)  
411 whereas mare primiparity does not affect the prevalence of endometritis (Barbacini *et al.* 1999).  
412 Delayed uterine clearance (LeBlanc *et al.* 1994) and endometrial degeneration (Troedsson *et al.* 1993)  
413 are identified factors in mares with endometritis. In old mares, uterine clearance is reduced (Evans *et*  
414 *al.* 1987), resulting in an increased incidence of intrauterine fluid retention (Woodward *et al.* 2012).  
415 Uterine clearance is facilitated by uterine contractility, which is also reduced and inconsistent in old  
416 mares (Carnevale and Ginther 1992; Camargo Ferreira *et al.* 2019). Moreover, uterine clearance is

417 facilitated by a good uterine position (LeBlanc *et al.* 1998). Increased parity contributes to a more  
418 ventral position of the uterus that could impair uterine clearance (LeBlanc *et al.* 1998). Cervical dilation  
419 is a key factor for a good uterine clearance (LeBlanc *et al.* 1994). Nulliparous mares have poorer cervical  
420 dilation, and it was suggested that old nulliparous are more likely to develop endometriosis as a result  
421 of the combination of altered cervical dilation and uterine conformation and as well as increased  
422 incidence of pneumovagina (LeBlanc *et al.* 1994). The only study comparing uterine fluids according to  
423 mare age and parity showed that although mare age does not affect uterine fluid total protein  
424 concentration in healthy mares, the concentration of one protein (not precisely identified but with a  
425 molecular weight ranging from 60 to 71kDa, possibly albumin) is higher in the uterine fluid of young  
426 compared to old mares (Tunón *et al.* 1998). Mare parity, however, did not affect the composition of  
427 uterine fluids (Tunón *et al.* 1998).

428 In addition to the impaired uterine clearance, old mares have also a longer vulva (Pascoe 1979), which  
429 represents a larger entrance for bacteria and could explain why bacteria counts are increased in old  
430 compared to younger mare uteri (Evans *et al.* 1987). A recent study showed an impairment of the  
431 expression of genes involved in local uterine immune response in old mares (Crociati *et al.* 2019 p.  
432 201). Studying endometrial tissue cultures from mares affected by subclinical endometritis, an  
433 increased production of prostaglandin E2 (PGE2), 6-oxoprostaglandin F1alpha (6-keto-PGF1a) and  
434 leukotriene C4 (LTC4) is observed in old mares in comparison to younger mares (Siemieniuch *et al.*  
435 2017). This dysregulation of selected arachidonic acid metabolites, known to be endocrine modulators  
436 of reproductive processes and immunological mediators, may be responsible for the reduced fertility  
437 of aged mares affected with endometritis (Siemieniuch *et al.* 2017).

#### 438 *Effects of age and parity of recipient for embryo transfer*

439 For embryo transfer recipient mares, neither mare age nor parity were shown to affect the likelihood  
440 of pregnancy after blastocyst transfer (Carnevale *et al.* 2000; Panzani *et al.* 2016; Claes *et al.* 2019).  
441 The number of cells and morphology grade of Day 2 post ovulation embryos transferred for 2 days in  
442 oviduct of recipients mares were not affected by recipient age (Carnevale, Griffin *et al.* 1993). However,  
443 embryo death was increased older mares (Carnevale *et al.* 2000) but no difference was observed  
444 according to mare parity (Panzani *et al.* 2016; Claes *et al.* 2019).

445

446 **In summary, fertility may be impaired in old mares due to the increased presence of oviductal**  
447 **masses that may disturb the migration of the fertilized embryo to the uterus. The old mare uterus**  
448 **as well as that from mares with high parity undergoes morphological changes including the presence**



449 of uterine cysts and fibrosis, regardless of parity. In addition, endometritis is more common in old  
450 mares, partly due to poorer conformation and delayed uterine clearance. Since chronic degenerative  
451 endometrial disease has been reported also in aged maiden mares, a direct effect of age is suspected.

452

## 453 **Gestation, placenta, fetal and foal development (Tables 4-7)**

454 Increasing age and/or parity appear to affect the reproductive tract with structural and functional  
455 degeneration. These alterations, along with a decreased embryo quality could affect placental, fetal  
456 intrauterine and offspring post-natal growth and long-term health, in the context of the  
457 Developmental Origins of Health and Diseases (DOHaD).

### 458 *Gestational length (Table 4)*

459 Gestational length is known to vary according to season of birth, foals' age and breed among other  
460 factors (Valera *et al.* 2006). Strict control of all these factors is difficult and may explain the variability  
461 of the reported data.

462 In terms of age, studies with large animal samples mostly observed that old mares have longer  
463 gestations (Satué *et al.* 2011; Langlois and Blouin 2012; Reilas *et al.* 2014; Ewert *et al.* 2018; Rodrigues  
464 *et al.* 2020). The studies that indicate a similar gestation length have usually smaller sample numbers  
465 (Winter *et al.* 2001, 2007; Davies Morel *et al.* 2002; Sevinga *et al.* 2004; Heidler *et al.* 2004; Aoki *et al.*  
466 2013; Bene *et al.* 2014; Reilas *et al.* 2014; Meirelles *et al.* 2017; Malinska *et al.* 2019; Popova 2020;  
467 Carluccio *et al.* 2020; Milojevic *et al.* 2020). Only three studies reported a shortening of gestation  
468 length with increasing maternal age (Bos and van der Mey 1980; Valera *et al.* 2006; Kuhl *et al.* 2015),  
469 but these study did not consider any very old mare nor had very high sample size of the same breed.

470 In terms of parity, on one hand, primiparous mares have been shown to have a shorter gestation in  
471 only one study (Robles 2018) while most studies showed that primiparous mares have a longer  
472 gestation, with increases varying from a few days up to 15 days (Pool-Anderson *et al.* 1994; Valera *et al.*  
473 2006; Satué *et al.* 2011; Reilas *et al.* 2014; Talluri *et al.* 2016). On the other hand, no differences  
474 according to maternal parity have been observed in some studies (Aoki *et al.* 2013; Meirelles *et al.*  
475 2017; Ewert *et al.* 2018).

476

### 477 *Gestation (Table 5)*

#### 478 Early feto-placental development

479 Although during the preimplantation period, effects of maternal age on embryo morphology differ  
480 between studies, at the time of implantation, fetal size is reduced in mares older than 8 compared to

481 younger dams (Willmann *et al.* 2011), probably due to prior delayed development that did not lead to  
482 pregnancy loss.

483 Effects of maternal age and parity on equine Chorionic Gonadotropin (eCG) production are  
484 controversial. A 75-year-old study on ponies did not report any correlation between maternal eCG  
485 serum concentration and mares' age or parity although it might be due to the lack of accuracy in age  
486 and parity estimation. Nevertheless, they highlighted decreased maternal eCG serum concentrations  
487 in successive pregnancies (Day and Rowlands 1946). In a more recent study, old mares have been  
488 shown to produce more eCG throughout the endometrial cups' lifespan compared to younger mares  
489 but no association with fetal development has been observed (Willmann *et al.* 2011). Finally, fetal eCG  
490 production was shown to start and stop earlier, beginning from the 3<sup>rd</sup> pregnancy, compared to the  
491 first two in young mares, leading to a non-significantly reduced eCG production over time (Wilsher and  
492 Allen 2011).

493

494 Blood flow and metabolism

495 Uterine blood flow during gestation has been demonstrated to be reduced during pregnancy in older  
496 compared to younger mares (Ousey *et al.* 2012). Another study showed that maternal parity had a  
497 more important effect on blood flow than age (for mares younger than 16) with larger uterine arteries'  
498 diameter correlated to an increased blood flow volume in multiparous compared to primiparous  
499 (Klewitz *et al.* 2015). From 100 to 250 days of gestation, in primiparous mares, the combined thickness  
500 of uterus and placenta increases, associated, with larger fetal eye cross-sectional and biparietal cross-  
501 sectional surface area after 220 days of gestation compared to mares with a higher parity (Hendriks *et*  
502 *al.* 2009). In contrast, maternal age negatively affects fetal aorta diameter when mares are 15 years  
503 old or older (Hendriks *et al.* 2009).

504 Studies performed on maternal age and parity related to modifications of mares' metabolism during  
505 pregnancy are scarce. Mares older than 8 have been shown to have increased plasma concentrations  
506 of parathyroid hormone, decreased osteocalcin and no change in IGF-1 during the last 2 months of  
507 gestation compared to mares aged 3-4, suggesting a different bone metabolism adaptation to  
508 pregnancy as mares age increases (Filipović *et al.* 2014). Furthermore, during gestation, basal glucose  
509 whole blood concentration is reduced in young primiparous mares compared to older multiparous  
510 mares, although parity does not alter urea plasmatic concentrations (Robles *et al.* 2019). In addition,  
511 at 280 days of gestation, late-gestation adaptations of the carbohydrate metabolism (i.e., increased  
512 insulin resistance) are less pronounced in primiparous mares compared to multiparous mares, which  
513 could possibly affect glucose transfer to the fetus (Robles *et al.* 2019). At foaling, moreover, increased  
514 plasma amino acid concentrations are observed in primiparous mares, in association with decreased

515 plasma amino acid concentrations in their newborns, suggesting impaired placental amino acid  
516 transfer to the fetus in primiparous pregnancies (Robles *et al.* 2019).

517

### 518 *Placenta (Table 6)*

519 Term placental weight has been studied by different authors, however, its relationship with mare age  
520 remains controversial (Wilsher and Allen 2003; Elliott *et al.* 2009; Ousey *et al.* 2012; Meirelles *et al.*  
521 2017; Robles *et al.* 2019). Young multiparous mares produce placentas with a larger microcotyledonary  
522 surface than older multiparous mares (Wilsher and Allen 2003; Ousey *et al.* 2012). Furthermore, old  
523 mares have an increased total volume of microcotyledons, allantoic vessels and height of  
524 microcotyledons in the placental body compared to mares younger than 8 (Meirelles *et al.* 2017).

525 Reduced placental microcotyledonary development is observed in older and subfertile compared to  
526 younger and fertile mares (Bracher *et al.* 1996). Furthermore, one study showed that endometrial  
527 degenerative changes induced by age affect placental development throughout gestation and lead to  
528 reduced microplacentome development and fetal growth (Abd-Elnaeim *et al.* 2006). In old mares,  
529 altered fetoplacental development could be explained by the presence of uterine cysts and  
530 degenerative changes in uterus (see section above). Moreover, age and parity are generally positively  
531 correlated and their respective impact is still to be determined.

532 Placentas at term are lighter and less voluminous in primiparous compared to multiparous mares  
533 (Wilsher and Allen 2003; Elliott *et al.* 2009; Meirelles *et al.* 2017; Robles *et al.* 2018) except in one study  
534 (Robles *et al.* 2019). Moreover, placentas from primiparous mares were shown to present a reduced  
535 microcotyledonary surface compared to placentas from multiparous dams (Wilsher and Allen 2003;  
536 Elliott *et al.* 2009; Meirelles *et al.* 2017). Another study, however, showed that only the  
537 microcotyledonary conjunctive tissue is reduced in placentas from young primiparous mares (Robles  
538 *et al.* 2018) and another did not show difference in microcotyledonary area according to mares' parity  
539 (Robles *et al.* 2019). The only study performed on three different placental sites (pregnant horn, non-  
540 pregnant horn and placental body) showed that the haemotrophic trophoblast surface density is  
541 reduced in the placental body of primiparous compared to multiparous mares (Meirelles *et al.* 2017).  
542 The authors also observed a decreased microcotyledonary length in the three zones of primiparous  
543 compared to multiparous placentas (Meirelles *et al.* 2017). The reduced capacity of placentas from  
544 primiparous mares might be explained by a lack of elasticity in the uterus which need to be "primed"  
545 by a first pregnancy to reach its maximum productivity and development (Wilsher and Allen 2003).  
546 Effects of maternal parity on placental efficiency (as calculated as foal weight/m<sup>2</sup> of placenta) differ  
547 according to studies: efficiency is either reduced (Wilsher and Allen 2003; Robles 2018) or increased  
548 (Meirelles *et al.* 2017) in primiparous mares.

549 At term, the expression of several genes is affected by maternal parity and age, suggesting placental  
550 nutrient transfer alterations (Robles *et al.* 2019). Moreover, the global DNA methylation in term  
551 placentas is decreased in young primiparous compared to older multiparous mares, suggesting  
552 different placental epigenetic regulations that may impact fetal intra-uterine growth (Robles *et al.*  
553 2019).

554

#### 555 *Fetal and foal development (Table 7)*

556 Maternal age has been demonstrated to affect foal morphology: both youngest and oldest dams  
557 produce lighter and smaller foals at birth and until 510 days of age (Hintz *et al.* 1979). Without  
558 considering extreme age ranges, the birth foal weight appears to increase with maternal age although  
559 this association is mainly due to mare parity (Elliott *et al.* 2009; Fernandes *et al.* 2014; Meirelles *et al.*  
560 2017; Malinska *et al.* 2019). In almost all previously cited studies, younger mares are primiparous while  
561 older are multiparous. Only one study compared young and old multiparous dams and showed that  
562 foal birthweight is higher when mares are between 10 and 15 years old (Wilsher and Allen 2003).  
563 Nevertheless, to the authors knowledge, no study compared birthweight of foals born to young and  
564 old primiparous, while in breeding farms it is common to see old primiparous broodmares.

565 Foals from primiparous mares remain smaller until at least one year of age and lighter until 4 months  
566 of age in most studies (Pool-Anderson *et al.* 1994; Cymbaluk and Laarveld 1996; Fernandes *et al.* 2014;  
567 Meirelles *et al.* 2017), except in one study (Robles *et al.* 2019).

568 Depending on breeds, increased maternal age reduces the size of foals until at least 6 months of age  
569 (Hintz *et al.* 1979; Klewitz *et al.* 2015; Meirelles *et al.* 2017; Robles 2018). One study on flat racehorses,  
570 however, showed that mare age and parity do not influence morphology of the competing adult  
571 (Paksoy and Ünal 2019). These differences in growth have been related to reduced plasma IGF-1  
572 concentration in foals from primiparous dams until 4 months of age (Cymbaluk and Laarveld 1996).  
573 Furthermore, foals from young and primiparous mares have a more immature regulation of glucose  
574 homeostasis and testicular development (Robles 2018). It has also been shown by epidemiological  
575 analysis that maternal age does not influence the risk of morbidity between 15 days and 1 year after  
576 birth although, with increasing mare age, foal mortality risk after 15 days of age is also increased  
577 (Morley and Townsend 1997).

578 In contrast to effects of age, the effects of parity are very clear with many studies showing that  
579 primiparous dams produce smaller and lighter foals at birth than multiparous mares, regardless of  
580 breeds or whether they are the result of embryo transfer (Doreau *et al.* 1991; Lawrence *et al.* 1992;  
581 Pool-Anderson *et al.* 1994; Cymbaluk and Laarveld 1996; Wilsher and Allen 2003; Elliott *et al.* 2009;  
582 Hendriks *et al.* 2009; Fernandes *et al.* 2014; Klewitz *et al.* 2015; Affonso *et al.* 2016; Talluri *et al.* 2016;

583 Meirelles *et al.* 2017; Robles *et al.* 2018, 2019). The comparison of the same mares over their first and  
584 second gestation gave the same result with the first foal being lighter (Wilsher and Allen 2003).  
585 Epidemiological studies give different conclusions regarding effects of maternal age and parity on foals'  
586 performance. Indeed, in flat race, the third and fourth foals of a mare perform better while the first,  
587 the ninth foal and over are less successful (Barron 1995). In a more recent study, however, neither  
588 maternal age nor parity significantly affected the racing performance of a total of 431 horses trained  
589 at 2 and 3 years old (Wilsher *et al.* 2006). In French show jumping horses, primiparous mares were  
590 shown to produce less performant horses, especially when mares were older than 10 years old (Palmer  
591 *et al.* 2018). Maternal age also significantly reduces (Barron 1995) or tends to reduce (Morley and  
592 Townsend 1997) performance of foals in flat races while in French show jumping, maternal age,  
593 without considering parity, does not affect foals' performances (Palmer *et al.* 2018). Two other studies,  
594 however, did not show any effect of mares' age and parity on foals' flat race performances (Wilsher *et*  
595 *al.* 2006). Maternal age does not seem to influence racing time but affects the earnings of the flat race  
596 foals (Köseman and Özbeyaz 2009). In flat races, the first foal is also less likely to suffer from a fracture  
597 than the following ones and this risk also decreases with maternal age (Verheyen *et al.* 2007).

598

599 **Altogether, the studies described above show that effects of age and parity on pregnancy, fetal**  
600 **development and post-natal growth and health are highly dependent on studies, suggesting effects**  
601 **of genetics and/or breeding conditions but maybe also due to different age criteria and statistical**  
602 **power in the different studies. Robles et al. even showed different results in two successive studies**  
603 **in the same experimental farm, demonstrating the difficulty to obtain repeatable results.**

604 **Nevertheless, analysis of the data indicate that primiparous mares tend to have increased pregnancy**  
605 **length by up to 15 days. Then, gestational length seems to generally increase with advancing mare's**  
606 **age, especially in older mares. Gestational changes in terms of endocrinology, maternal metabolism**  
607 **and uterine artery blood flow don't evidence major differences although blood flow to the uterus**  
608 **appears to be increased in older and multiparous mares. The placenta is in general smaller and**  
609 **appears less efficient in terms of microtyledonary structure in primiparous dams. As a consequence,**  
610 **primiparity has a clear negative effect on fetoplacental growth, with post-natal carry-over of the**  
611 **small size and weight, possibly leading to altered glucose metabolism in yearlings and poorer sports**  
612 **performance in older animals. Foal birthweight increases with maternal age and parity together with**  
613 **performance. Apart from one study where genetic value was taken into consideration as a cofactor**  
614 **(Palmer *et al.* 2018), data on post-natal offspring sport performance are hindered by the lack of**  
615 **information on parental genetics and post-natal environmental constraints.**

616

617 **Post-partum complications, maternal behavior, lactation quality and quantity**  
618 **(Tables 8 and 9)**

619 *Post-partum complications*

620 One of the most common problem encountered after foaling is the retention of fetal membrane (RFM)  
621 occurring in 2 to 10% of foalings (Frazer 2003). Few studies assessed the impact of age and/or parity  
622 on this subject. An increase of RFM in young (4 years old) and old mares (above 17 years old) was  
623 observed, compared to middle-aged mares (between 5 and 17 years old) (Sevinga *et al.* 2004). In a  
624 more recent study (Schürmann *et al.* 2019), however, no effect of neither age nor parity was  
625 highlighted. In terms of age, those results are in accordance with earlier results (Provencher *et al.*  
626 1988). Regarding post-partum fever (Aoki *et al.* 2014), another post-partum complication, no impact  
627 of age or parity was found.

628 Dystocia is the main cause of neonatal foal mortality and is generally the result of foal malpresentation  
629 (Ginther and Williams 1996). No interaction between dystocia and mare age was identified in Quarter  
630 horses (McCue and Ferris 2012; Rioja *et al.* 2012). In contrast, in draft horses, mares aged 3 were shown  
631 to have more difficult foalings and increased neonatal foal mortality than older mares (Sabbagh *et al.*  
632 2014). From 4 years of age to more than 10, no apparent effect was highlighted although a trend for  
633 more difficulties occurring in mares aged 4 than in mares ages 5 to 9 years of age was identified  
634 (Sabbagh *et al.* 2014). In the same study, authors also highlighted the effect of parity with more  
635 dystocia and stillbirth associated with the first foaling compared to the second and the third. Thus,  
636 they conclude that the most disadvantageous combination was a mare aged 3 having her first foal  
637 although a mare aged 10 years or more in her first parity encountered almost the same risks. In terms  
638 of foal mortality, an effect of age for mare under 3 years of age was also identified although it was  
639 correlated with poor maternal behavior toward the foal: when maternal behavior was taken into  
640 account, the effect of age was no more significant (Haas *et al.* 1996).

641

642 *Maternal behavior*

643 Pony foals born to young (3 years old) mares compared to older mares (5 years old) were shown to be  
644 more frequently harassed by other mares from the same herd due to their lower hierarchical position  
645 (Rho *et al.* 2009). Possibly as a result of these aggressions, these young mares are more protective of  
646 their foal.

647 Several studies have reported that primiparous mares are more likely to reject their foal than  
648 multiparous mares, demonstrating the importance of previous experience for maternal behavior  
649 (Chavatte 1991; Houpt and Lieb 1994; Juarbe-Díaz *et al.* 1998). After foals have been adopted,  
650 however, there is no difference in maternal behavior in terms of proximity index and reactivity to foal

651 handling (Søndergaard and Jago 2010). Nevertheless, increased maternal parity was associated with  
652 lower foal heart rates at 6 weeks of age when foals were introduced to a new object (Søndergaard and  
653 Jago 2010). In addition, maternal parity did not affect the onset of conflicts over suckling during the  
654 socialization period nor did it affect maternal protectiveness (Heitor and Vicente 2008). Foals born to  
655 higher parity mares were also more socially active in the herd (Heitor and Vicente 2008).

656

#### 657 *Colostrum*

658 Colostrum is produced by the mare during the first 24 hours after foaling and is essential for the passive  
659 transfer of immunity to the foal. Thus, efficiency of this transfer was assessed regarding both age  
660 and/or parity and, in most studies, no effect was identified in terms of immunoglobulin G (IgG)  
661 concentrations in foal plasma (Morris *et al.* 1985; LeBlanc *et al.* 1992; Raidal 1996; Erhard *et al.* 2001),  
662 mare plasma (Morris *et al.* 1985; Kenzig *et al.* 2009) or mare colostrum (Erhard *et al.* 2001; Kenzig *et al.*  
663 *et al.* 2009). A study, however, mentioned an effect of mare age on colostral IgG (LeBlanc *et al.* 1992)  
664 with mare younger than 10 showing higher concentrations. The increased foal morbidity and mortality  
665 risks observed in mares aged above 15 (Morley and Townsend 1997) may be related to the poorer  
666 passive transfer of immunity observed in these older mares (LeBlanc *et al.* 1992). Moreover, while not  
667 finding an effect of mare age, a study showed that foals from multiparous mares were less likely to  
668 present failure of passive transfer (Franco Ayala and Oliver-Espinosa 2016).

669 Factors influencing colostrum quality in the mare, however, are not well documented, especially when  
670 looking at age and parity. A study highlighted that colostrum calcium concentrations are higher in  
671 mares older than 7 compared to younger animals (Ramin *et al.* 2018). Age also affects colostrum fatty  
672 acid profiles with mares older than 10 producing a colostrum richer in palmitic acid (C16:0) and in  
673 polyunsaturated fatty acids compared to mares up to 10 years of age (Pikul *et al.* 2008). In the same  
674 study, an effect of parity was also identified with mares having foaled less than 5 times showing a  
675 decreased colostrum saturated fatty acid:polyunsaturated fatty acid ratio compared to mares with a  
676 parity over 5. More specifically, decreased colostrum fatty acid proportions of caprylic (C8:0), capric  
677 (C10:0), lauric (C12:0), myristic (C14:0) acids and increased oleic (C18:1*cis*9), palmitoleic (C16:1*cis*7)  
678 and arachidonic (C20:4) acids are observed (Pikul *et al.* 2008). The significance of those changes  
679 remains to be elucidated.

680

#### 681 *Milk*

682 Different factors have an impact on mares' lactation and, although lactation stage appears to be the  
683 strongest, age and parity also affect both milk quantity and quality. Concerning milk quantity, the effect  
684 of age appears non-linear during the mare's life and depends on the mare's breed, with greater milk  
685 production in multiparous mares in the first two thirds of lactation (Pool-Anderson *et al.* 1994). For

686 nursing mares, maximum milk production is reached around 7, while, for dairy mares, milk production  
687 reaches a peak between 11 and 15 years of age (Doreau and Martuzzi 2006).

688 A very limited number of studies assessed the question of how mares' age and parity affect milk  
689 quality. Two cited studies examined milk quality without highlighting any effect of parity on milk solid  
690 content (Gibbs *et al.* 1982 p. 198; Doreau *et al.* 1991). Mares' parity, however, did not affect fat  
691 contents while an impact on milk protein contents was observed with mares of parity 1 and 2  
692 producing milk richer in protein than mares at or above a parity of 3 (Gibbs *et al.* 1982). More recently,  
693 a study focused on macro-mineral, protein and urea contents without detecting any effect of age  
694 (Ramin *et al.* 2018). A decrease in milk lactose concentrations, however, was reported in multiparous  
695 mares, leading to the same trend for defatted dry extract (Barreto *et al.* 2020). This study also  
696 considered mares' age but no effect was detected. Nevertheless, authors mentioned the correlation  
697 between age and parity in their study and thus, effects of parity previously mentioned could be  
698 partially due to age. When focusing on milk fatty acid profiles, both age appears to have an impact.  
699 When mares up to 10 years of age were compared to older mares, the younger mares were shown to  
700 produce milk richer in myristoleic (C14:1), palmitic (C16:0) and palmitoleic (C16:1*cis*9) fatty acids and  
701 poorer in arachidonic acid (C20:4) (Pikul *et al.* 2008). The significance of those variations, in animals  
702 receiving a similar diet, remains to be understood.

703 In terms of parity, little is known as results between studies are contradictory. Two studies  
704 demonstrated an increase in milk production in multiparous mares compared to primiparous (Gibbs *et*  
705 *al.* 1982; Pool-Anderson *et al.* 1994). In other studies, however, no effect of parity was detected  
706 (Doreau *et al.* 1991; Trottier *et al.* 2006). In the last two above-mentioned studies, however, the  
707 number of mares studied (4 vs 4 in (Doreau *et al.* 1991) and 3 vs 3 in (Trottier *et al.* 2006)) might explain  
708 the lack of results.

709

#### 710 *Foal suckling*

711 As for milk quality and quantity few studies assessed the effect of age and parity on foal suckling bouts  
712 duration and frequency with only two studies assessing the effect of parity and none concerning the  
713 effect of mare age. Number and duration of suckling bouts per day does not seem to be influenced by  
714 mare parity (Trottier *et al.* 2006). Another study, however, indicates an increase of bout duration in  
715 foals from primiparous (Duncan *et al.* 1984).

716

717 **In summary, so far, little attention has been paid to effects of age or parity on lactation and so far,**  
718 **apart from a greater production after the first lactation, and a peak lactation yield for middle aged**  
719 **mares (between 7 and 15).**

720



721

## 722 **Conclusion**

723 In conclusion, both maternal age and parity affect horse reproduction, as summarized in Figure 2.

724 Mares' age affects cyclicity, folliculogenesis, oocyte and embryo quality as well as oviductal masses  
725 and uterine tract function. If effects of ovarian function and gamete/embryo quality and the  
726 accumulation of unfertilized eggs in the oviduct were to be expected, it was interesting to observe that  
727 age alone affects uterine quality in terms of fibrosis and the presence of endometrial cysts. The  
728 threshold for observable deleterious effects on these different factors needs to be better determined,  
729 as an epidemiological study demonstrates that ageing actually induces a reduction in fertility as soon  
730 as 8 years of age.

731 Maternal parity appears to have a non-linear effect. Primiparity has a major influence on placental and  
732 concomitant foal development, with smaller foals in first gestation that remain smaller postnatally.  
733 Moreover, dystocia is more frequent in primiparous mares, at least in draft mares and the prevalence  
734 of foal rejection is increased. Although colostrum quality does not seem affected, milk yield is reduced  
735 compared to subsequent lactations. After the first gestation, that one could consider as a "priming",  
736 endometrial quality and uterine clearance capacities progressively decline with increasing parity,  
737 whilst placental and foal birthweight and milk production increase.

738 In most studies, however, maternal age and parity are confounding factors as younger mares are  
739 usually nulliparous and older mares are multiparous. Interaction between parity and age is essentially  
740 observed in very young or very old primiparous mares.

741 Practically, since first pregnancies are compulsory, the choice of a pony as stallion should be  
742 considered so as to produce a small foal that would fit into the pony's morphological standards. In  
743 terms of recipient mares, recipients having already had a foal should be preferred. If this is not possible,  
744 large mares should be chosen, so as to reduce the effects of primiparity on foal size. In addition, for the  
745 management of aging mares with progressive fertility decline, caution should be increased at the time  
746 of foaling as placental retention was shown to be more frequent. Embryo transfer is recommended  
747 when the uterine tract is not able to sustain pregnancy any more, and ICSI if the quality of collected  
748 embryos is too low.

749

750

751 Figure 1: Decline in fertility in mares in relation to age (adapted from Langlois and Blouin, 2004 and  
752 Jeffcott et al., 1982)

753 Figure 2: Summary of effects of maternal age and parity on maternal factors affecting foal production.

754

755 **References**

- 756 Abd-Elnaeim M. M. M., Leiser R., Wilsher S., and Allen W. R. (2006). Structural and Haemovascular  
757 Aspects of Placental Growth Throughout Gestation in Young and Aged Mares. *Placenta* 27, 1103–1113.  
758 doi:10.1016/j.placenta.2005.11.005
- 759 Adams G. P., Kastelic J. P., Bergfelt D. R., and Ginther O. J. (1987). Effect of uterine inflammation and  
760 ultrasonically-detected uterine pathology on fertility in the mare. *J Reprod Fertil Suppl* 35, 445–454.
- 761 Affonso F. J., Meirelles M. G., Alonso M. A., Guimaraes C. F., Lemes K. M., Nichi M., and Fernandes C.  
762 B. (2016). Influence of mare parity on weight, height, thoracic circumference and vitality of neonatal  
763 foals. *Journal of Equine Veterinary Science* 41, 67. doi:10.1016/j.jevs.2016.04.053
- 764 Aguilar J. J., Woods G. L., Miragaya M. H., and Olsen L. M. (1997). Living fibroblast cells in the oviductal  
765 masses of mares. *Equine Veterinary Journal* S25, 103–108. doi:10.1111/j.2042-3306.1997.tb05112.x
- 766 Allen W. R., Brown L., Wright M., and Wilsher S. (2007). Reproductive efficiency of Flatrace and  
767 National Hunt Thoroughbred mares and stallions in England. *Equine Veterinary Journal* 39, 438–445.  
768 doi:10.2746/042516407X1737581
- 769 Altermatt J. L., Marolf A. J., Wrigley R. H., and Carnevale E. M. (2012). Effects of FSH and LH on ovarian  
770 and follicular blood flow, follicular growth and oocyte developmental competence in young and old  
771 mares. *Animal Reproduction Science* 133, 191–197. doi:10.1016/j.anireprosci.2012.06.021
- 772 Altermatt J. L., Suh T. K., Stokes J. E., and Carnevale E. M. (2009). Effects of age and equine follicle-  
773 stimulating hormone (eFSH) on collection and viability of equine oocytes assessed by morphology and  
774 developmental competency after intracytoplasmic sperm injection (ICSI). *Reprod. Fertil. Dev.* 21, 615.  
775 doi:10.1071/RD08210
- 776 Alves K. A., Alves B. G., Gastal G. D. A., Haag K. T., Gastal M. O., Figueiredo J. R., Gambarini M. L., and  
777 Gastal E. L. (2017). Preantral follicle density in ovarian biopsy fragments and effects of mare age.  
778 *Reprod. Fertil. Dev.* 29, 867. doi:10.1071/RD15402
- 779 Annandale A., Stroehle R. M., Schulman M. L., Sibeko-Matjila K. P., Fosgate G. T., Handler J., Vemming  
780 D. C., and Clift S. J. (2018). Influence of cycle stage, age and endometrial biopsy score on oxytocin  
781 receptor distribution and gene expression in the cervix and uterus of non-pregnant mares.  
782 *Theriogenology* 120, 1–9. doi:10.1016/j.theriogenology.2018.07.013
- 783 Aoki T., Yamakawa K., and Ishii M. (2013). Factors Affecting Gestation Length in Heavy Draft Mares.

784 Journal of Equine Veterinary Science 33, 437–440. doi:10.1016/j.jevs.2012.07.011

785 Aoki T., Yamakawa K., and Ishii M. (2014). Factors affecting the incidence of postpartum fever in heavy  
786 draft mares. *Journal of Equine Veterinary Science* 34, 719–721.

787 Avci M., Tirpan M. B., Tekin K., and Akçay E. (2016). The influence of insemination time, age and semen  
788 dose on fertility of mares inseminated with frozen semen. *Ankara Üniversitesi Veteriner Fakültesi*  
789 *Dergisi* 63, 359–363. doi:10.1501/Vetfak\_0000002753

790 Bain A. M. (1969). Foetal losses during pregnancy in the thoroughbred mare: A record of 2,562  
791 pregnancies. *New Zealand Veterinary Journal* 17, 155–158. doi:10.1080/00480169.1969.33811

792 Baker C. B., Little T. V., and McDOWELL K. J. (1993). The live foaling rate per cycle in mares. *Equine*  
793 *Veterinary Journal* 25, 28–30. doi:10.1111/j.2042-3306.1993.tb04819.x

794 Ball B. A., El-Sheikh Ali H., Scoggin K. E., Riddle W. T., Schnobrich M., Bradekamp E., Agnew M., Squires  
795 E. L., and Troedsson M. H. T. (2019). Relationship between anti-Müllerian hormone and fertility in the  
796 mare. *Theriogenology* 125, 335–341. doi:10.1016/j.theriogenology.2018.11.005

797 Ball B. A., Little T. V., Hillman R. B., and Woods G. L. (1986). Pregnancy rates at Days 2 and 14 and  
798 estimated embryonic loss rates prior to day 14 in normal and subfertile mares. *Theriogenology* 26,  
799 611–619. doi:10.1016/0093-691X(86)90168-8

800 Ball B. A., Little T. V., Weber J. A., and Woods G. L. (1989). Survival of Day-4 embryos from young,  
801 normal mares and aged, subfertile mares after transfer to normal recipient mares. *Reproduction* 85,  
802 187–194. doi:10.1530/jrf.0.0850187

803 Barbacini S., Marchi V., and Zavaglia G. (1999). Equine frozen semen: results obtained in Italy during  
804 the 1994-1997 period. *Equine Veterinary Education* 11, 109–112. doi:10.1111/j.2042-  
805 3292.1999.tb00930.x

806 Barbacini S., Zavaglia G., Gulden P., Marchi V., and Necchi D. (2000). Retrospective study on the efficacy  
807 of hCG in an equine artificial insemination programme using frozen semen. *Equine Veterinary*  
808 *Education* 12, 312–317. doi:10.1111/j.2042-3292.2000.tb00067.x

809 Barreto Í. M., Urbano S. A., Oliveira C. A., Macêdo C. S., Borba L. H., Chags B. M., and Rangel A. H.  
810 (2020). Chemical composition and lipid profile of mare colostrum and milk of the quarter horse breed.  
811 *PloS one* 15, e0238921.

812 Barron J. K. (1995). The effect of maternal age and parity on the racing performance of Thoroughbred

813 horses. *Equine Veterinary Journal* 27, 73–75. doi:10.1111/j.2042-3306.1995.tb03036.x

814 Bene S., Benedek Z., Szabó F., and Polgár P. (2014). SOME EFFECTS ON GESTATION LENGTH OF  
815 TRADITIONAL HORSE BREEDS IN HUNGARY. *j. cent. eur. agric.* 15, 1–10. doi:10.5513/JCEA01/15.1.1402

816 Benhajali H., Richard-Yris M.-A., Ezzaouia M., Charfi F., and Hausberger M. (2010). Factors influencing  
817 conception rates of Arab mares in Tunisia. *Animal Reproduction Science* 117, 106–110.  
818 doi:10.1016/j.anireprosci.2009.04.010

819 Bollwein H. (1998). Transrectal color Doppler sonography of the arteria uterina in cyclic mares.  
820 *THERIOGENOLOGY* 49, 1483–1488.

821 Bos H., and van der Mey G. J. W. (1980). Length of gestation periods of horses and ponies belonging to  
822 different breeds. *Livestock Production Science* 7, 181–187. doi:10.1016/0301-6226(80)90105-0

823 Bosh K. A., Powell D., Neibergs J. S., Shelton B., and Zent W. (2009). Impact of reproductive efficiency  
824 over time and mare financial value on economic returns among Thoroughbred mares in central  
825 Kentucky. *Equine Veterinary Journal* 41, 889–894. doi:10.2746/042516409X456059

826 Bracher V., Mathias S., and Allen W. R. (1992). Videoendoscopic evaluation of the mare's uterus: II.  
827 Findings in subfertile mares. *Equine Veterinary Journal* 24, 279–284. doi:10.1111/j.2042-  
828 3306.1992.tb02835.x

829 Bracher V., Mathias S., and Allen W. R. (1996). Influence of chronic degenerative endometritis  
830 (endometrosis) on placental development in the mare. *Equine Veterinary Journal* 28, 180–188.  
831 doi:10.1111/j.2042-3306.1996.tb03771.x

832 Brinsko S. P., Ball B. A., and Ellington J. E. (1995). In vitro maturation of equine oocytes obtained from  
833 different age groups of sexually mature mares. *Theriogenology* 44, 461–469. doi:10.1016/0093-  
834 691X(95)00218-W

835 Brinsko S. P., Ball B. A., Miller P. G., Thomas P. G. A., and Ellington J. E. (1994). In vitro development of  
836 day 2 embryos obtained from young, fertile mares and aged, subfertile mares. *Reproduction* 102, 371–  
837 378. doi:10.1530/jrf.0.1020371

838 Brinsko S. P., Ignatz G. G., Ball B. A., Thomas P. G. A., Currie W. B., and Ellington J. E. (1996).  
839 Characterization of polypeptides synthesized and secreted by oviductal epithelial cell explants  
840 obtained from young, fertile and aged, subfertile mares. *American Journal of Veterinary Research* 57,  
841 1346–1353.

842 Brück I., Anderson G., and Hyland J. (1993). Reproductive performance of Thoroughbred mares on six  
843 commercial stud farms. *Australian Vet J* 70, 299–303. doi:10.1111/j.1751-0813.1993.tb07979.x

844 Burkhardt J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *J. Agric.*  
845 *Sci.* 37, 64–68. doi:10.1017/S0021859600013083

846 Camargo Ferreira J., Linhares Boakari Y., Sousa Rocha N., Saules Ignácio F., Barbosa da Costa G., and  
847 de Meira C. (2019). Luteal vascularity and embryo dynamics in mares during early gestation: Effect of  
848 age and endometrial degeneration. *Reprod Dom Anim* 54, 571–579. doi:10.1111/rda.13396

849 Campos-Chillon F., Farmerie T. A., Bouma G. J., Clay C. M., and Carnevale E. M. (2015). Effects of aging  
850 on gene expression and mitochondrial DNA in the equine oocyte and follicle cells. *Reprod. Fertil. Dev.*  
851 27, 925. doi:10.1071/RD14472

852 Carluccio A., Bucci R., Fusi J., Robbe D., and Veronesi M. C. (2020). Effect of age and of reproductive  
853 status on reproductive indices in horse mares carrying mule pregnancies. *Heliyon* 6, e05175.  
854 doi:10.1016/j.heliyon.2020.e05175

855 Carnevale E. M. (2008). The mare model for follicular maturation and reproductive aging in the woman.  
856 *Theriogenology* 69, 23–30. doi:10.1016/j.theriogenology.2007.09.011

857 Carnevale E. M., Bergfelt D. R., and Ginther O. J. (1993). Aging effects on follicular activity and  
858 concentrations of FSH, LH, and progesterone in mares. *Animal Reproduction Science* 31, 287–299.  
859 doi:10.1016/0378-4320(93)90013-H

860 Carnevale E. M., Bergfelt D. R., and Ginther O. J. (1994). Follicular activity and concentrations of FSH  
861 and LH associated with senescence in mares. *Animal Reproduction Science* 35, 231–246.  
862 doi:10.1016/0378-4320(94)90039-6

863 Carnevale E. M., Coutinho da Silva M. A., Panzani D., Stokes J. E., and Squires E. L. (2005). Factors  
864 affecting the success of oocyte transfer in a clinical program for subfertile mares. *Theriogenology* 64,  
865 519–527. doi:10.1016/j.theriogenology.2005.05.008

866 Carnevale E. M., and Ginther O. J. (1992). Relationships of age to uterine function and reproductive  
867 efficiency in mares. *Theriogenology* 37, 1101–1115. doi:10.1016/0093-691X(92)90108-4

868 Carnevale E. M., and Ginther O. J. (1995). Defective Oocytes as a Cause of Subfertility in Old Mares.  
869 *Biology of Reproduction* 52, 209–214. doi:10.1093/biolreprod/52.monograph\_series1.209

870 Carnevale E. M., Griffin P. G., and Ginther O. J. (1993). Age-associated subfertility before entry of

871 embryos into the uterus in mares. *Equine Veterinary Journal* 25, 31–35. doi:10.1111/j.2042-  
872 3306.1993.tb04820.x

873 Carnevale E. M., Hermetet M. J., and Ginther O. J. (1997). Age and pasture effects on vernal transition  
874 in mares. *Theriogenology* 47, 1009–1018. doi:10.1016/S0093-691X(97)00058-7

875 Carnevale E. M., Ramirez R. J., Squires E. L., Alvarenga M. A., Vanderwall D. K., and McCue P. M. (2000).  
876 Factors affecting pregnancy rates and early embryonic death after equine embryo transfer.  
877 *Theriogenology* 54, 965–979. doi:10.1016/S0093-691X(00)00405-2

878 Carnevale E., Uson M., Bozzola J., King S., Schmitt S., and Gates H. (1999). Comparison of oocytes from  
879 young and old mares with light and electron microscopy. *Theriogenology* 51, 299. doi:10.1016/S0093-  
880 691X(99)91858-7

881 Catandi G. D., Obeidat Y. M., Broeckling C. D., Chen T. W., Chicco A. J., and Carnevale E. M. (2021).  
882 Equine maternal aging affects oocyte lipid content, metabolic function and developmental potential.  
883 *Reproduction* 161, 399–409. doi:10.1530/REP-20-0494

884 Chavatte P. (1991). Maternal behaviour in the horse; theory and practical applications to foal rejection  
885 and fostering. *Equine Veterinary Education* 3, 215–220. doi:10.1111/j.2042-3292.1991.tb01525.x

886 Chevalier-Clément F. (1989). Pregnancy loss in the mare. *Animal Reproduction Science* 20, 231–244.  
887 doi:10.1016/0378-4320(89)90088-2

888 Claes A., Ball B. A., Scoggin K. E., Esteller-Vico A., Kalmar J. J., Conley A. J., Squires E. L., and Troedsson  
889 M. H. T. (2015). The interrelationship between anti-Müllerian hormone, ovarian follicular populations  
890 and age in mares: Anti-Müllerian hormone and ovarian follicular populations. *Equine Vet J* 47, 537–  
891 541. doi:10.1111/evj.12328

892 Claes A., Ball B. A., Scoggin K. E., Roser J. F., Woodward E. M., Davolli G. M., Squires E. L., and Troedsson  
893 M. H. T. (2017). The influence of age, antral follicle count and diestrus ovulations on estrous cycle  
894 characteristics of mares. *Theriogenology* 97, 34–40. doi:10.1016/j.theriogenology.2017.04.019

895 Claes A., Cuervo-Arango J., Broek J., Galli C., Colleoni S., Lazzari G., Deelen C., Beitsma M., and Stout T.  
896 A. (2019). Factors affecting the likelihood of pregnancy and embryonic loss after transfer of  
897 cryopreserved in vitro produced equine embryos. *Equine Vet J* 51, 446–450. doi:10.1111/evj.13028

898 Cox L., Vanderwall D. K., Parkinson K. C., Sweat A., and Isom S. C. (2015). Expression profiles of select  
899 genes in cumulus–oocyte complexes from young and aged mares. *Reprod. Fertil. Dev.* 27, 914.  
900 doi:10.1071/RD14446

901 Crociati M., Capomaccio S., Mandara M. T., Stradaoli G., Sylla L., Monaci M., and Cappelli K. (2019).  
902 Different expression of Defensin-B gene in the endometrium of mares of different age during the  
903 breeding season. *BMC Veterinary Research* 15, 1–10. doi:<https://doi.org/10.1186/s12917-019-2215-z>

904 Cuervo-Arango J., Claes A. N., and Stout T. A. E. (2019a). Mare and stallion effects on blastocyst  
905 production in a commercial equine ovum pick-up–intracytoplasmic sperm injection program. *Reprod.*  
906 *Fertil. Dev.* 31, 1894. doi:10.1071/RD19201

907 Cuervo-Arango J., Claes A. N., and Stout T. A. (2019b). A retrospective comparison of the efficiency of  
908 different assisted reproductive techniques in the horse, emphasizing the impact of maternal age.  
909 *Theriogenology* 132, 36–44. doi:10.1016/j.theriogenology.2019.04.010

910 Cymbaluk N. F., and Laarveld B. (1996). The ontogeny of serum insulin-like growth factor-I  
911 concentration in foals: Effects of dam parity, diet, and age at weaning. *Domestic Animal Endocrinology*  
912 13, 197–209. doi:10.1016/0739-7240(96)00014-8

913 Davies Morel M. C. G., Newcombe J. R., and Hayward K. (2010). Factors affecting pre-ovulatory follicle  
914 diameter in the mare: the effect of mare age, season and presence of other ovulatory follicles (multiple  
915 ovulation). *Theriogenology* 74, 1241–1247. doi:10.1016/j.theriogenology.2010.05.027

916 Davies Morel M. C. G., Newcombe J. R., and Holland S. J. (2002). Factors affecting gestation length in  
917 the Thoroughbred mare. *Animal Reproduction Science* 74, 175–185. doi:10.1016/S0378-  
918 4320(02)00171-9

919 Davies Morel M. C. G., and O’Sullivan J. A. M. (2001). Ovulation rate and distribution in the  
920 thoroughbred mare, as determined by ultrasonic scanning: the effect of age. *Animal Reproduction*  
921 *Science* 66, 59–70. doi:10.1016/S0378-4320(01)00092-6

922 Davis S. R., Lambrinouadaki I., Lumsden M., Mishra G. D., Pal L., Rees M., Santoro N., and Simoncini T.  
923 (2015). Menopause. *Nat Rev Dis Primers* 1, 15004. doi:10.1038/nrdp.2015.4

924 Day F. T., and Rowlands I. W. (1946). SERUM GONADOTROPHIN IN WELSH AND SHETLAND PONIES.  
925 *Journal of Endocrinology* 5, 1–8. doi:10.1677/joe.0.0050001

926 Derisoud E., Jouneau L., Dubois C., Archilla C., Jaszczyszyn Y., Legendre R., Daniel N., Peynot N., Dahirel  
927 M., Auclair-Ronzaud J., Wimel L., Duranthon V., and Chavatte-Palmer P. (2021). Maternal age affects  
928 equine Day 8 embryo gene expression both in trophoblast and inner cell mass. *bioRxiv*  
929 2021.04.07.438786. doi:10.1101/2021.04.07.438786

930 Doig P. A., McKNIGHT J. D., and Miller R. B. (1981). The Use of Endometrial Biopsy in the Infertile Mare.



931 Can Vet J 22, 72–76.

932 Doreau M., Boulot S., and Martin-Rosset W. (1991). Effect of parity and physiological state on intake,  
933 milk production and blood parameters in lactating mares differing in body size. *Animal Science* 53,  
934 111–118.

935 Doreau M., and Martuzzi F. (2006). Milk yield of nursing and dairy mares. *PUBLICATION-EUROPEAN*  
936 *ASSOCIATION FOR ANIMAL PRODUCTION* 120, 57.

937 Driancourt M.-A., Prunier A., Palmer E., and Mariana J.-C. (1983). Seasonal effects on ovarian follicular  
938 development in pony mares. *Reprod. Nutr. Dévelop.* 23, 207–215. doi:10.1051/rnd:19830205

939 Dubrovskaya A. B., Lebedeva L. F., and Schukis K. A. (2019). Comparative histomorphological  
940 characteristics of the endometrium of young and aged mares in estrus and diestrus. *IOP Conf. Ser.:*  
941 *Earth Environ. Sci.* 341, 012067. doi:10.1088/1755-1315/341/1/012067

942 Ducheyne K. D., Rizzo M., Daels P. F., Stout T. A. E., and de Ruijter-Villani M. (2019). Vitriifying immature  
943 equine oocytes impairs their ability to correctly align the chromosomes on the MII spindle. *Reprod.*  
944 *Fertil. Dev.* 31, 1330. doi:10.1071/RD18276

945 Duncan P., Harvey P. H., and Wells S. M. (1984). On lactation and associated behaviour in a natural  
946 herd of horses. *Animal Behaviour* 32, 255–263. doi:10.1016/S0003-3472(84)80345-0

947 Duranthon V., and Chavatte-Palmer P. (2018). Long term effects of ART: What do animals tell us?  
948 *Molecular Reproduction and Development* 85, 348–368. doi:10.1002/mrd.22970

949 Eilts B. E., Scholl D. T., Paccamonti D. L., Causey R., Klimczak J. C., and Corley J. R. (1995). Prevalence of  
950 Endometrial Cysts and Their Effect on Fertility. *Biology of Reproduction* 52, 527–532.  
951 doi:10.1093/biolreprod/52.monograph\_series1.527

952 Elliott C., Morton J., and Chopin J. (2009). Factors affecting foal birth weight in Thoroughbred horses.  
953 *Theriogenology* 71, 683–689. doi:10.1016/j.theriogenology.2008.09.041

954 Erhard M. H., Luft C., Remler H.-P., and Stangassinger M. (2001). Assessment of colostral transfer and  
955 systemic availability of immunoglobulin G in new-born foals using a newly developed enzyme-linked  
956 immunosorbent assay (ELISA) system. *J Anim Physiol Anim Nutr* 85, 164–173. doi:10.1046/j.1439-  
957 0396.2001.00313.x

958 Esteller-Vico A., Liu I. K., and Couto S. (2012). Uterine vascular degeneration is present throughout the  
959 uterine wall of multiparous mares. Colinearity between elastosis, endometrial grade, age and parity.

960 Theriogenology 78, 1078–1084. doi:10.1016/j.theriogenology.2012.04.014

961 Esteller-Vico A., Liu I. K. M., Vaughan B., Steffey E. P., and Brosnan R. J. (2015). Effects of vascular  
962 elastosis on uterine blood flow and perfusion in anesthetized mares. Theriogenology 83, 988–994.  
963 doi:10.1016/j.theriogenology.2014.11.032

964 Evans M. J., Hamer J. M., Gason L. M., and Irvine C. H. G. (1987). Factors affecting uterine clearance of  
965 inoculated materials in mares. Journal of Reproduction and Fertility. Supplement 35, 327–334.

966 Ewert M., Lüders I., Böröcz J., Uphaus H., Distl O., and Sieme H. (2018). Determinants of gestation  
967 length in Thoroughbred mares on German stud farms. Animal Reproduction Science 191, 22–33.  
968 doi:10.1016/j.anireprosci.2018.02.001

969 Farquhar V. J., M. McCue P., Vanderwall D. K., and Squires E. L. (2000). Efficacy of the gnRH agonist  
970 deslorelin acetate for inducing ovulation in mares relative to age of mare and season. Journal of Equine  
971 Veterinary Science 20, 722–725. doi:10.1016/S0737-0806(00)80183-5

972 Fernandes C. B., Meirelles M. G., Guimaraes C. F., Nichi M., Affonso F. J., Fonte J. S., Onoe E., and Alonso  
973 M. A. (2014). Which paternal, maternal and placental parameters influence foal size and vitality?  
974 Journal of Equine Veterinary Science 34, 225–227. doi:10.1016/j.jevs.2013.10.161

975 Ferreira J. C., Canesin H. S., Ignácio F. S., Rocha N. S., Pinto C. R., and Meira C. (2015). Effect of age and  
976 endometrial degenerative changes on uterine blood flow during early gestation in mares.  
977 Theriogenology 84, 1123–1130. doi:10.1016/j.theriogenology.2015.06.013

978 Ferreira J. C., Gastal E. L., and Ginther O. J. (2008). Uterine blood flow and perfusion in mares with  
979 uterine cysts: effect of the size of the cystic area and age. Reproduction 135, 541–550.  
980 doi:10.1530/REP-07-0447

981 Filipović N., Stojević Z., Plevnik N., Mašek T., Prvanović N., and Tuček Z. (2014). The influence of age on  
982 bone metabolism in mares during late pregnancy and lactation. Research in Veterinary Science 97,  
983 194–198. doi:10.1016/j.rvsc.2014.06.005

984 Franco Ayala M. S., and Oliver-Espinosa O. J. (2016). Risk Factors Associated With Failure of Passive  
985 Transfer of Colostral Immunoglobulins in Neonatal Paso Fino Foals. Journal of Equine Veterinary  
986 Science 44, 100–104. doi:10.1016/j.jevs.2016.05.006

987 Frank B. L., Doddman C. D., Stokes J. E., and Carnevale E. M. (2019). Association of equine oocyte and  
988 cleavage stage embryo morphology with maternal age and pregnancy after intracytoplasmic sperm  
989 injection. Reprod. Fertil. Dev. 31, 1812. doi:10.1071/RD19250

- 990 Frazer G. S. (2003). Post partum complications in the mare. Part 2: Fetal membrane retention and  
991 conditions of the gastrointestinal tract, bladder and vagina. *Equine Veterinary Education* 15, 91–100.
- 992 Gibbs P. G., and Davison K. E. (1992). A field study on reproductive efficiency of mares maintained  
993 predominately on native pasture. *Journal of Equine Veterinary Science* 12, 219–222.  
994 doi:10.1016/S0737-0806(06)81449-8
- 995 Gibbs P., Potter G., Blake R., and McMullan W. (1982). Milk Production of Quarter Horse Mares during  
996 150 Days of Lactation. 54, 496–499. doi:10.2527/jas1982.543496x
- 997 Ginther O. J. (1992). 'Reproductive biology of the mare : basic and applied aspects.' (Equiservices)
- 998 Ginther O. J., Carnevale E. M., and Bergfelt D. R. (1993). DELAY IN EMERGENCE OF THE OVULATORY  
999 FOLLICULAR WAVE IN OLD MARES. *JOURNAL OF EQUINE VETERINARY SCIENCE* 13, 5.
- 1000 Ginther O. J., Douglas R. H., and Lawrence J. R. (1982). Twinning in mares: a survey of veterinarians and  
1001 analyses of theriogenology records. *Theriogenology* 18, 333–347. doi:https://doi.org/10.1016/0093-  
1002 691X(82)90011-5
- 1003 Ginther O. J., Gastal M. O., Gastal E. L., Jacob J. C., and Beg M. A. (2009). Age-related dynamics of  
1004 follicles and hormones during an induced ovulatory follicular wave in mares. *Theriogenology* 71, 780–  
1005 788. doi:10.1016/j.theriogenology.2008.09.051
- 1006 Ginther O. J., Gastal M. O., Gastal E. L., Jacob J. C., Siddiqui M. A. R., and Beg M. A. (2008). Effects of  
1007 age on follicle and hormone dynamics during the oestrous cycle in mares. *Reprod. Fertil. Dev.* 20, 955.  
1008 doi:10.1071/RD08121
- 1009 Ginther O. J., and Williams D. (1996). On-the-farm incidence and nature of equine dystocias. *Journal*  
1010 *of Equine Veterinary Science* 16, 159–164. doi:10.1016/S0737-0806(96)80131-6
- 1011 Goncalves G. R., Morotti F., Colombo A. H. B., Bonato D. V., Bizarro-Silva C., Rosa C. O., Cavalieri F. L.  
1012 B., and Seneda M. M. (2020). Influence of age and ovarian antral follicle count on the reproductive  
1013 characteristics of embryo donor mares. *Veterinary Record* vetrec–2019–105526.  
1014 doi:10.1136/vr.105526
- 1015 Grüniger B., Schoon H.-A., Schoon D., Menger S., and Klug E. (1998). Incidence and morphology of  
1016 endometrial angiopathies in mares in relationship to age and parity. *Journal of Comparative Pathology*  
1017 119, 293–309. doi:10.1016/S0021-9975(98)80051-0
- 1018 Haadem C. S., Nødtvedt A., Farstad W., and Thomassen R. (2015). A retrospective cohort study on

1019 fertility in the Norwegian Coldblooded trotter after artificial insemination with cooled, shipped versus  
1020 fresh extended semen. *Acta Vet Scand* 57, 77. doi:10.1186/s13028-015-0161-8

1021 Haas S. D., Bristol F., and Card C. E. (1996). Risk factors associated with the incidence of foal mortality  
1022 in an extensively managed mare herd. *The Canadian Veterinary Journal* 37, 91.

1023 Hanlon D., Stevenson M., Evans M., and Firth E. (2012). Reproductive performance of Thoroughbred  
1024 mares in the Waikato region of New Zealand: 1. Descriptive analyses. *New Zealand Veterinary Journal*  
1025 60, 329–334. doi:10.1080/00480169.2012.693039

1026 Heidler B., Aurich J. E., Pohl W., and Aurich Chr. (2004). Body weight of mares and foals, estrous cycles  
1027 and plasma glucose concentration in lactating and non-lactating Lipizzaner mares. *Theriogenology* 61,  
1028 883–893. doi:10.1016/S0093-691X(03)00279-6

1029 Heitor F., and Vicente L. (2008). Maternal care and foal social relationships in a herd of Sorraia horses:  
1030 Influence of maternal rank and experience. *Applied Animal Behaviour Science* 113, 189–205.  
1031 doi:10.1016/j.applanim.2007.11.005

1032 Hemberg E., Lundeheim N., and Einarsson S. (2004). Reproductive Performance of Thoroughbred  
1033 Mares in Sweden. *Reprod Domest Anim* 39, 81–85. doi:10.1111/j.1439-0531.2004.00482.x

1034 Hendriks W. K., Colenbrander B., van der Weijden G. C., and Stout T. A. E. (2009). Maternal age and  
1035 parity influence ultrasonographic measurements of fetal growth in Dutch Warmblood mares. *Animal*  
1036 *Reproduction Science* 115, 110–123. doi:10.1016/j.anireprosci.2008.12.014

1037 Hendriks W. K., Colleoni S., Galli C., Paris D. B. B. P., Colenbrander B., Roelen B. A. J., and Stout T. A. E.  
1038 (2015). Maternal age and in vitro culture affect mitochondrial number and function in equine oocytes  
1039 and embryos. *Reprod. Fertil. Dev.* 27, 957. doi:10.1071/RD14450

1040 Hevia M. L., Quiles A. J., Fuentes F., and Gonzalo C. (1994). Reproductive performance of thoroughbred  
1041 horses in Spain. *Journal of Equine Veterinary Science* 14, 89–92. doi:10.1016/S0737-0806(06)81883-6

1042 Hintz R. L., Hintz H. F., and Vleck L. D. V. (1979). Growth rate of Thoroughbreds. Effect of age of dam,  
1043 year and month of birth, and sex of foal. *Journal of Animal Science* 48, 480–487.

1044 Haupt K., and Lieb S. (1994). A survey of foal rejecting mares. *Applied Animal Behaviour Science* 39,  
1045 188. doi:10.1016/0168-1591(94)90143-0

1046 Hutton C. A., and Meacham T. N. (1968). Reproductive Efficiency on Fourteen Horse Farms. *Journal of*  
1047 *Animal Science* 27, 434. doi:10.2527/jas1968.272434x

1048 Jeffcott L. B., Rosedale P. D., Freestone J., Frank C. J., and Towers-Clark P. F. (1982). An assessment of  
1049 wastage in Thoroughbred racing from conception to 4 years of age. *Equine Veterinary Journal* 14, 185–  
1050 198. doi:10.1111/j.2042-3306.1982.tb02389.x

1051 Juarbe-Díaz S. V., Houpt K. A., and Kusunose R. (1998). Prevalence and characteristics of foal rejection  
1052 in Arabian mares. *Equine Veterinary Journal* 30, 424–428. doi:10.1111/j.2042-3306.1998.tb04513.x

1053 Kareskoski M., Venhoranta H., Virtala A.-M., and Katila T. (2019). Analysis of factors affecting the  
1054 pregnancy rate of mares after inseminations with cooled transported stallion semen. *Theriogenology*  
1055 127, 7–14. doi:10.1016/j.theriogenology.2018.12.036

1056 Katila T., Reilas T., Nivola K., Peltonen T., and Virtala A.-M. (2010). A 15-year survey of reproductive  
1057 efficiency of Standardbred and Finnhorse trotters in Finland - descriptive results. *Acta Veterinaria*  
1058 *Scandinavica* 11.

1059 Kenney R. M. (1978). Cyclic and pathologic changes of the mare endometrium as detected by biopsy,  
1060 with a note on early embryonic death. *Journal of the American Veterinary Medical Association* 172,  
1061 241–262.

1062 Kenzig A. R., O’Meara K. M., Kremer C. J., Jogan K. S., Jack N. E., and Cole K. (2009). Colostral, Milk and  
1063 Serum Immunoglobulin G Concentrations in Quarter Horse Mares and Their Foals. *Journal of Equine*  
1064 *Veterinary Science* 29, 486–487. doi:10.1016/j.jevs.2009.04.177

1065 Klewitz J., Struebing C., Rohn K., Goergens A., Martinsson G., Orgies F., Probst J., Hollinshead F.,  
1066 Bollwein H., and Sieme H. (2015). Effects of age, parity, and pregnancy abnormalities on foal birth  
1067 weight and uterine blood flow in the mare. *Theriogenology* 83, 721–729.  
1068 doi:10.1016/j.theriogenology.2014.11.007

1069 Korkmaz Ö., Emre B., Polat İ. M., Zonturlu A. K., PiR Yağci İ., Pekcan M., Gürler Ş., Akkuş T., and Çebi  
1070 Şen Ç. (2020). The Correlation Between Anti-Müllerian Hormone Concentrations and Reproductive  
1071 Parameters in Different Age Groups in Purebred Arabian Mares. *Kafkas Univ Vet Fak Derg* 26, 53–57.  
1072 doi:10.9775/kvfd.2019.22227

1073 Köseman A., and Özbeyaz C. (2009). Some phenotypic and genetic parameters of racing performance  
1074 in Arabian horses. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 56, 219–224.  
1075 doi:10.1501/Vetfak\_0000002221

1076 Kuhl J., Stock K. F., Wulf M., and Aurich C. (2015). Maternal Lineage of Warmblood Mares Contributes  
1077 to Variation of Gestation Length and Bias of Foal Sex Ratio (EZ Cameron, Ed.). *PLoS ONE* 10, e0139358.

1078 doi:10.1371/journal.pone.0139358

1079 Lane E., Bijnen M., Osborne M., More S., Henderson I., Duffy P., and Crowe M. (2016). Key Factors  
1080 Affecting Reproductive Success of Thoroughbred Mares and Stallions on a Commercial Stud Farm.  
1081 *Reprod Dom Anim* 51, 181–187. doi:10.1111/rda.12655

1082 Langlois B., and Blouin C. (2004). Statistical analysis of some factors affecting the number of horse  
1083 births in France. *Reprod. Nutr. Dev.* 44, 583–595. doi:10.1051/rnd:2004055

1084 Langlois B., and Blouin C. (2012). Genetic parameters for gestation length in French horse breeds.  
1085 *Livestock Science* 146, 133–139. doi:10.1016/j.livsci.2012.02.028

1086 Lawrence L. M., DiPietro J., Ewert K., Parrett D., Moser L., and Powell D. (1992). Changes in body weight  
1087 and condition of gestating mares. *Journal of Equine Veterinary Science* 12, 355–358.  
1088 doi:10.1016/S0737-0806(06)81361-4

1089 LeBlanc M. M., Neuwirth L., Asbury A. C., Tran T., Mauragis D., and Klapstein E. (1994). Scintigraphic  
1090 measurement of uterine clearance in normal mares and mares with recurrent endometritis. *Equine*  
1091 *Veterinary Journal* 26, 109–113. doi:10.1111/j.2042-3306.1994.tb04346.x

1092 LeBlanc M. M., Neuwirth L., Jones L., Cage C., and Mauragis D. (1998). Differences in uterine position  
1093 of reproductively normal mares and those with delayed uterine clearance detected by scintigraphy.  
1094 *Theriogenology* 50, 49–54. doi:10.1016/S0093-691X(98)00112-5

1095 LeBlanc M. M., Tran T., Baldwin J. L., and Pritchard E. L. (1992). Factors that influence passive transfer  
1096 of immunoglobulins in foals. *J Am Vet Med Assoc* 200, 179–183.

1097 Liu I. K. M., Lantz K. C., Schlafke S., Bowers J. M., and Enders A. C. (1990). Clinical observations of  
1098 oviductal masses in the mare. *Proceedings of the annual convention of the American Association of*  
1099 *Equine Practitioners. Proceedings of the annual convention of the American Association of Equine*  
1100 *Practitioners* 36, 41–45.

1101 Malheiros de Souza J. R., Dias Gonçalves P. B., Bertolin K., Ferreira R., Sardinha Ribeiro A. S., Ribeiro D.  
1102 B., Mara da Encarnação Fiala Rechsteiner S., Roldi de Araújo R., Pimentel C. A., and Hammes Pimentel  
1103 A. M. (2020). Age-dependent effect of foal heat breeding on pregnancy and embryo mortality rates in  
1104 Thoroughbred mares. *Journal of Equine Veterinary Science* 102982. doi:10.1016/j.jevs.2020.102982

1105 Malinska M., Havlicek Z., Koru E., and Rezac P. (2019). Animal and environmental factors associated  
1106 with gestation length in the endangered Old Kladruber horse. *Theriogenology* 129, 116–120.  
1107 doi:10.1016/j.theriogenology.2019.02.013

1108 Marinone A. I., Losinno L., Fumuso E., Rodríguez E. M., Redolatti C., Cantatore S., and Cuervo-Arango  
1109 J. (2015). The effect of mare's age on multiple ovulation rate, embryo recovery, post-transfer  
1110 pregnancy rate, and interovulatory interval in a commercial embryo transfer program in Argentina.  
1111 *Animal Reproduction Science* 158, 53–59. doi:10.1016/j.anireprosci.2015.04.007

1112 Mata F., Bourbon J., Twigg-Flesner A., and Greening L. (2013). Investigating follicle growth, uterine  
1113 oedema and other factors affecting reproductive success in the Lusitano. 14.

1114 McCue P. M., and Ferris R. A. (2012). Parturition, dystocia and foal survival: a retrospective study of  
1115 1047 births. *Equine Veterinary Journal* 44, 22–25.

1116 McCue P. M., Wall J. J., Brink Z. A., and Jr G. E. S. (2009). 177 Live foal rate: effects of embryo transfer  
1117 and donor mare age. *Reproduction, Fertility and Development* 22, 247–247.  
1118 doi:10.1071/RDv22n1Ab177

1119 McDowell K. J., Powell D. G., and Baker C. B. (1992). Effect of book size and age of mare and stallion on  
1120 foaling rates in thoroughbred horses. *Journal of Equine Veterinary Science* 12, 364–367.  
1121 doi:10.1016/S0737-0806(06)81363-8

1122 McDowell K. J., Sharp D. C., Grubaugh W., Thatcher W. W., and Wilcox C. J. (1988). Restricted conceptus  
1123 mobility results in failure of pregnancy maintenance in mares. *Biology of Reproduction* 39, 340–348.

1124 Meirelles M. G., Veras M. M., Alonso M. A., de Fátima Guimarães C., Nichi M., and Fernandes C. B.  
1125 (2017). Influence of Maternal Age and Parity on Placental Structure and Foal Characteristics From Birth  
1126 up to 2 Years of Age. *Journal of Equine Veterinary Science* 56, 68–79. doi:10.1016/j.jevs.2017.03.226

1127 de Mestre A. M., Rose B. V., Chang Y. M., Wathes D. C., and Verheyen K. L. P. (2019). Multivariable  
1128 analysis to determine risk factors associated with early pregnancy loss in thoroughbred broodmares.  
1129 *Theriogenology* 124, 18–23. doi:10.1016/j.theriogenology.2018.10.008

1130 Milojevic M., Streten M., Ayaşan T., Zdralic T., and Djermanovic V. (2020). Effects of Age and Sex of The  
1131 Foal on The Gestation Length of Thoroughbred Mares. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım  
1132 ve Doğa Dergisi* 23, 1386–1392. doi:10.18016/ksutarimdog.vi.692961

1133 Monniaux D., Clément F., Dalbiès-Tran R., Estienne A., Fabre S., Mansanet C., and Monget P. (2014).  
1134 The Ovarian Reserve of Primordial Follicles and the Dynamic Reserve of Antral Growing Follicles: What  
1135 Is the Link?1. *Biology of Reproduction* 90,. doi:10.1095/biolreprod.113.117077

1136 Morel M. C. G. D., Newcombe J. R., and Swindlehurst J. C. (2005). The effect of age on multiple  
1137 ovulation rates, multiple pregnancy rates and embryonic vesicle diameter in the mare. *Theriogenology*

- 1138 63, 2482–2493. doi:10.1016/j.theriogenology.2004.09.058
- 1139 Morley P. S., and Townsend G. G. (1997). A survey of reproductive performance in Thoroughbred  
1140 mares and morbidity, mortality and athletic potential of their foals. *Equine Veterinary Journal* 29, 290–  
1141 297.
- 1142 Morris L. H. A., and Allen W. R. (2002). Reproductive efficiency of intensively managed Thoroughbred  
1143 mares in Newmarket. *Equine Veterinary Journal* 34, 51–60. doi:10.2746/042516402776181222
- 1144 Morris D. D., Meirs D. A., and Merryman G. S. (1985). Passive transfer failure in horses: incidence and  
1145 causative factors on a breeding farm. *Am J Vet Res* 46, 2294–2299.
- 1146 Nambo Y., Oikawa M.-A., Yoshihara T., Kuwano A., and Katayama Y. (1995). Age-related  
1147 Morphometrical Changes of Arteries of Uterine Wall in Mares. *Journal of Veterinary Medicine Series A*  
1148 42, 383–387. doi:10.1111/j.1439-0442.1995.tb00390.x
- 1149 Nath L., Anderson G., and McKinnon A. (2010). Reproductive efficiency of Thoroughbred and  
1150 Standardbred horses in north-east Victoria. *Australian Veterinary Journal* 88, 169–175.  
1151 doi:10.1111/j.1751-0813.2010.00565.x
- 1152 Niekerk V., and Gerneke W. H. (1966). Persistence and parthenogenetic cleavage of tubal ova in the  
1153 mare. *Onversrepoort J. ver. Res.* 31, 195–232.
- 1154 Nishikawa Y. (1959). 'Studies on reproduction in horses. Singularity and artificial control in  
1155 reproductive phenomena.' (Tokyo: Japan Racing Association.)
- 1156 Oikawa M., Katayama Y., Yoshihara T., Kaneko M., and Yoshikawa T. (1993). Microscopical  
1157 characteristics of uterine wall arteries in barren aged mares. *Journal of Comparative Pathology* 108,  
1158 411–415. doi:10.1016/S0021-9975(08)80214-9
- 1159 Onuma H., and Ohnami Y. (1975). Retention of tubal eggs in mares. *Journal of reproduction and*  
1160 *fertility. Supplement* 23, 507–511.
- 1161 Ousey J. C., Kölling M., Newton R., Wright M., and Allen W. R. (2012). Uterine haemodynamics in young  
1162 and aged pregnant mares measured using Doppler ultrasonography: Uterine haemodynamics in  
1163 mares. *Equine Veterinary Journal* 44, 15–21. doi:10.1111/j.2042-3306.2011.00446.x
- 1164 Paksoy Y., and Ünal N. (2019). Multivariate analysis of morphometry effect on race performance in  
1165 Thoroughbred horses. *R. Bras. Zootec.* 48, e20180030. doi:10.1590/rbz4820180030
- 1166 Palmer E., and Chavatte-Palmer P. (2020). Contribution of reproduction management and technologies



1167 to genetic progress in horse breeding. *Journal of Equine Veterinary Science* 103016.  
1168 doi:10.1016/j.jevs.2020.103016

1169 Palmer E., and Driancourt M. A. (1983). Some interactions of season of foaling, photoperiod and  
1170 ovarian activity in the equine. *Livestock Production Science* 10, 197–210. doi:10.1016/0301-  
1171 6226(83)90035-0

1172 Palmer E., Robles M., Chavatte-Palmer P., and Ricard A. (2018). Maternal Effects on Offspring  
1173 Performance in Show Jumping. *Journal of Equine Veterinary Science* 66, 221.  
1174 doi:10.1016/j.jevs.2018.05.108

1175 Panzani D., Rota A., Marmorini P., Vannozzi I., and Camillo F. (2014). Retrospective study of factors  
1176 affecting multiple ovulations, embryo recovery, quality, and diameter in a commercial equine embryo  
1177 transfer program. *Theriogenology* 82, 807–814. doi:10.1016/j.theriogenology.2014.06.020

1178 Panzani D., Vannozzi I., Marmorini P., Rota A., and Camillo F. (2016). Factors Affecting Recipients’  
1179 Pregnancy, Pregnancy Loss, and Foaling Rates in a Commercial Equine Embryo Transfer Program.  
1180 *Journal of Equine Veterinary Science* 37, 17–23. doi:10.1016/j.jevs.2015.10.011

1181 Pascoe R. R. (1979). Observations on the length and angle of declination of the vulva and its relation  
1182 to fertility in the mare. *J Reprod Fertil Suppl* 299–305.

1183 Pascoe D. R., Liu I. K. M., Spensley M. S., and Hughes J. P. (1985). Effect of endometrial pathology on  
1184 the success of non-surgical embryo transfer. *Equine Veterinary Journal* 17, 108–110.  
1185 doi:10.1111/j.2042-3306.1985.tb04610.x

1186 Pérez-Marín C. C., Vizquete G., and Galisteo J. J. (2017). Embryo recovery results in Hispano-Arabe horse  
1187 and Spanish donkey breeds. *Livestock Science* 206, 76–81. doi:10.1016/j.livsci.2017.10.011

1188 Physick-Sheard P. W. (1995). Demographic analysis of the Canadian Standardbred broodmare herd.  
1189 *Preventive Veterinary Medicine* 24, 285–299. doi:10.1016/0167-5877(95)00492-F

1190 Pikul J., Wójtowski J., Danków R., Kuczynska B., and Lojek J. (2008). Fat content and fatty acids profile  
1191 of colostrum and milk of primitive Konik horses (*Equus caballus gmelini* Ant.) during six months of  
1192 lactation. *The Journal of dairy research* 75, 302.

1193 Pinto-Bravo P., Ferreira-Dias G., Rebordão M. R., Amaral A., Fernandes C., Cuello C., Parrilla I., Martínez  
1194 E., Roberto da Costa R. P., and Skarzynski D. J. (2018). Is mare endometrosis linked to oviduct fibrosis?:  
1195 *PHK* 34, 43–46. doi:10.21836/PEM20180107

- 1196 Platt H. (1973). Aetiological aspects of abortion in the thoroughbred mare. *J Comp Pathol* 83, 199–205.
- 1197 Pool-Anderson K., Raub R. H., and Warren J. A. (1994). Maternal influences on growth and  
1198 development of full-sibling foals<sup>2</sup>. *Journal of Animal Science* 72, 1661–1666.  
1199 doi:10.2527/1994.7271661x
- 1200 Popova M. (2020). BREEDING AGE OF MARES FROM THE HAFLINGER BREED, DURATION OF  
1201 GESTATIONAL LENGTH AND THE INFLUENCE OF SOME PARATYPE FACTORS ON IT. *Scientific Papers:*  
1202 *Series D, Animal Science-The International Session of Scientific Communications of the Faculty of*  
1203 *Animal Science* 63, 8.
- 1204 Provencher R., Threlfall W. R., Murdick P. W., and Wearly W. K. (1988). Retained fetal membranes in  
1205 the mare: a retrospective study. *The Canadian Veterinary Journal* 29, 903.
- 1206 Raidal S. L. (1996). The incidence and consequences of failure of passive transfer of immunity on a  
1207 Thoroughbred breeding farm. *Australian Vet J* 73, 201–206. doi:10.1111/j.1751-0813.1996.tb10035.x
- 1208 Rambags B. P. B., van Boxtel D. C. J., Tharasanit T., Lenstra J. A., Colenbrander B., and Stout T. A. E.  
1209 (2014). Advancing maternal age predisposes to mitochondrial damage and loss during maturation of  
1210 equine oocytes in vitro. *Theriogenology* 81, 959–965. doi:10.1016/j.theriogenology.2014.01.020
- 1211 Ramin A., Bayrami M., Ramin S., and Kalantry L. (2018). Relationships among urea, protein and macro-  
1212 mineral concentrations in colostrums and whole milk in lactating mares. *International Journal of*  
1213 *Research in applied and basic medical sciences* 4, 15–22.
- 1214 van der Reest J., Nardini Cecchino G., Haigis M. C., and Kordowitzki P. (2021). Mitochondria: Their  
1215 relevance during oocyte ageing. *Ageing Research Reviews* 70, 101378. doi:10.1016/j.arr.2021.101378
- 1216 Reilas T., Virtala A. M., and Katila (2014). Gestation lengths of Finnhorse and Standardbred mares in  
1217 Finland: effects of breeding season and reproductive status: *PHK* 30, 45–51.  
1218 doi:10.21836/PEM20140107
- 1219 Rho J. R., Srygley R. B., and Choe J. C. (2009). Age of the Jeju pony (*Equus caballus*) at first reproduction:  
1220 potential costs for foals and mothers that first reproduce early in life. *J Ethol* 27, 483–488.  
1221 doi:10.1007/s10164-008-0146-4
- 1222 Ricketts S. W. (1975). Endometrial biopsy as a guide to diagnosis of endometrial pathology in the mare.  
1223 *Journal of reproduction and fertility. Supplement* 23, 341–345.
- 1224 Ricketts S. W., and Alonso S. (1991). The effect of age and parity on the development of equine chronic

1225 endometrial disease. *Equine Veterinary Journal* 23, 189–192. doi:10.1111/j.2042-3306.1991.tb02752.x

1226 Rioja E., Cernicchiaro N., Costa M. C., and Valverde A. (2012). Perioperative risk factors for mortality  
1227 and length of hospitalization in mares with dystocia undergoing general anesthesia: a retrospective  
1228 study. *The Canadian Veterinary Journal* 53, 502.

1229 Rizzo M., Ducheyne K. D., Deelen C., Beitsma M., Cristarella S., Quartuccio M., Stout T. A. E., and Ruijter-  
1230 Villani M. (2019). Advanced mare age impairs the ability of in vitro-matured oocytes to correctly align  
1231 chromosomes on the metaphase plate. *Equine Vet J* 51, 252–257. doi:10.1111/evj.12995

1232 Rizzo M., du Preez N., Ducheyne K. D., Deelen C., Beitsma M. M., Stout T. A. E., and de Ruijter-Villani  
1233 M. (2020). The horse as a natural model to study reproductive aging-induced aneuploidy and  
1234 weakened centromeric cohesion in oocytes. *aging*. doi:10.18632/aging.104159

1235 Rizzo M., Stout T. A. E., Cristarella S., Quartuccio M., Kops G. J. P. L., and De Ruijter-Villani M. (2021).  
1236 Compromised MPS1 Activity Induces Multipolar Spindle Formation in Oocytes From Aged Mares:  
1237 Establishing the Horse as a Natural Animal Model to Study Age-Induced Oocyte Meiotic Spindle  
1238 Instability. *Front. Cell Dev. Biol.* 9, 657366. doi:10.3389/fcell.2021.657366

1239 Robles M. (2018). Maternal parity affects placental development, growth and metabolism of foals until  
1240 1 year and a half. *Theriogenology* 108, 321–330.  
1241 doi:<https://doi.org/10.1016/j.theriogenology.2017.12.019>

1242 Robles M., Couturier-Tarrade A., Derisoud E., Geeverding A., Dubois C., Dahirel M., Aioun J., Prezelin  
1243 A., Calvez J., Richard C., Wimel L., and Chavatte-Palmer P. (2019). Effects of dietary arginine  
1244 supplementation in pregnant mares on maternal metabolism, placental structure and function and  
1245 foal growth. *Sci Rep* 9, 6461. doi:10.1038/s41598-019-42941-0

1246 Robles M., Dubois C., Gautier C., Dahirel M., Guenon I., Bouraima-Lelong H., Viguié C., Wimel L.,  
1247 Couturier-Tarrade A., and Chavatte-Palmer P. (2018). Maternal parity affects placental development,  
1248 growth and metabolism of foals until 1 year and a half. *Theriogenology* 108, 321–330.  
1249 doi:10.1016/j.theriogenology.2017.12.019

1250 Rodrigues J. A., Gonçalves A. R., Antunes L., Bettencourt E. V., and Gama L. T. (2020). Genetic and  
1251 Environmental Factors Influencing Gestation Length in Lusitano Horses. *Journal of Equine Veterinary*  
1252 *Science* 84, 102850. doi:10.1016/j.jevs.2019.102850

1253 Ruggeri E., DeLuca K. F., Galli C., Lazzari G., DeLuca J. G., and Carnevale E. M. (2015). Cytoskeletal  
1254 alterations associated with donor age and culture interval for equine oocytes and potential zygotes

1255 that failed to cleave after intracytoplasmic sperm injection. *Reprod. Fertil. Dev.* 27, 944.  
1256 doi:10.1071/RD14468

1257 Sabbagh M., Danvy S., and Ricard A. (2014). Genetic and environmental analysis of dystocia and  
1258 stillbirths in draft horses. *animal* 8, 184–191.

1259 Samper J. C., Vidament M., Katila T., Newcombe J. R., Estrada A., and Sargeant J. (2002). Analysis of  
1260 some factors associated with pregnancy rates of frozen semen: a multi-center study. *Theriogenology*  
1261 58, 647–650. doi:10.1016/S0093-691X(02)00754-9

1262 Satué K., Felipe M., Mota J., and Muñoz A. (2011). Gestational length in Carthusian broodmares: effects  
1263 of breeding season, foal gender, age of mare, year of parturition, parity and sire. *Polish Journal of*  
1264 *Veterinary Sciences* 14, 173–180. doi:10.2478/v10181-011-0027-6

1265 Schürmann C., Loose M., Failing K., and Wehrend A. (2019). Retained placenta in mares-an analysis of  
1266 121 cases. *Tierärztliche Praxis. Ausgabe G, Grosstiere/Nutztiere* 47, 373–379.

1267 Sessions-Bresnahan D. R., and Carnevale E. M. (2015). Age-associated changes in granulosa cell  
1268 transcript abundance in equine preovulatory follicles. *Reprod. Fertil. Dev.* 27, 906.  
1269 doi:10.1071/RD14467

1270 Sevinga M., Barkema H. W., Stryhn H., and Hesselink J. W. (2004). Retained placenta in friesian mares:  
1271 incidence, and potential risk factors with special emphasis on gestational length. *Theriogenology* 61,  
1272 851–859. doi:https://doi.org/10.1016/S0093-691X(03)00260-7

1273 Sharma S., Dhaliwal G. S., and Dadarwal D. (2010). Reproductive efficiency of Thoroughbred mares  
1274 under Indian subtropical conditions: A retrospective survey over 7 years. *Animal Reproduction Science*  
1275 117, 241–248. doi:10.1016/j.anireprosci.2009.05.011

1276 Siemieniuch M. J., Gajos K., Kozdrowski R., and Nowak M. (2017). Advanced age in mares affects  
1277 endometrial secretion of arachidonic acid metabolites during equine subclinical endometritis.  
1278 *Theriogenology* 103, 191–196. doi:10.1016/j.theriogenology.2017.07.043

1279 da Silveira J. C., Veeramachaneni D. N. R., Winger Q. A., Carnevale E. M., and Bouma G. J. (2012). Cell-  
1280 Secreted Vesicles in Equine Ovarian Follicular Fluid Contain miRNAs and Proteins: A Possible New Form  
1281 of Cell Communication Within the Ovarian Follicle. *Biology of Reproduction* 86, 1–10.  
1282 doi:https://doi.org/10.1095/biolreprod.111.093252

1283 da Silveira J. C., Winger Q. A., Bouma G. J., and Carnevale E. M. (2015). Effects of age on follicular fluid  
1284 exosomal microRNAs and granulosa cell transforming growth factor- $\beta$  signalling during follicle

1285 development in the mare. *Reprod. Fertil. Dev.* 27, 897. doi:10.1071/RD14452

1286 Søndergaard E., and Jago J. (2010). The effect of early handling of foals on their reaction to handling,  
1287 humans and novelty, and the foal–mare relationship. *Applied Animal Behaviour Science* 123, 93–100.  
1288 doi:10.1016/j.applanim.2010.01.006

1289 Spacek S. G., and Carnevale E. M. (2018). Impact of Equine and Bovine Oocyte Maturation in Follicular  
1290 Fluid From Young and Old Mares on Embryo Production in Vitro. *Journal of Equine Veterinary Science*  
1291 68, 94–100. doi:10.1016/j.jevs.2018.04.009

1292 Squires E., Barbacini S., Matthews P., Byers W., Schwenzer K., Steiner J., and Loomis P. (2006).  
1293 Retrospective study of factors affecting fertility of fresh, cooled and frozen semen. *Equine Veterinary*  
1294 *Education* 18, 96–99. doi:10.1111/j.2042-3292.2006.tb00425.x

1295 Stabenfeldt G. H., Hughes J. P., and Evans J. W. (1972). Ovarian Activity During the Estrous Cycle of the  
1296 Mare. *Endocrinology* 90, 1379–1384. doi:10.1210/endo-90-5-1379

1297 Talluri T. R., Arangasamy A., Singh J., Ravi S. K., Pal Y., Legha R. A., Alpha Raj M., Chopra A., Singh R. K.,  
1298 and Tripathi B. N. (2016). Factors affecting length of gestation in artificially inseminated Marwari mares  
1299 of India. *Asian Pacific Journal of Reproduction* 5, 481–489. doi:10.1016/j.apjr.2016.10.004

1300 Tannus R. J., and Thun R. (1995). Influence of Endometrial Cysts on Conception Rate of Mares. *Journal*  
1301 *of Veterinary Medicine Series A* 42, 275–283. doi:10.1111/j.1439-0442.1995.tb00378.x

1302 Troedsson M. H. T., deMoraes M. J., and Liu I. K. (1993). Correlations between histologic endometrial  
1303 lesions in mares and clinical response to intrauterine exposure with *Streptococcus zooepidemicus*. *Am*  
1304 *J Vet Res* 54, 570–572.

1305 Trottier N. L., Stamper T., Moore J., Liesman J., and Nielsen B. D. (2006). A weigh-suckle-weigh  
1306 approach for estimating milk yield in the Arabian mare. *EAAP PUBLICATION* (120, 2006-06-01) 163–  
1307 165.

1308 Tunón A.-M., Rodriguez-Martinez H., Haglund A., Albiñ A., Magnusson U., and Einarsson S. (1995).  
1309 Ultrastructure of the secretory endometrium during oestrus in young maiden and foaled mares. *Equine*  
1310 *Veterinary Journal* 27, 382–388. doi:10.1111/j.2042-3306.1995.tb04074.x

1311 Tunón A.-M., Rodriguez-Martinez H., Hultén C., Nummijarvi A., and Magnusson U. (1998).  
1312 Concentrations of total protein, albumin and immunoglobulins in undiluted uterine fluid of  
1313 gynecologically healthy mares. *Theriogenology* 50, 821–831. doi:10.1016/S0093-691X(98)00187-3

1314 Uliani R. C., Conley A. J., Corbin C. J., Friso A. M., Maciel L. F. S., and Alvarenga M. A. (2019). Anti-  
1315 Müllerian hormone and ovarian aging in mares. *Journal of Endocrinology* 147–156. doi:10.1530/JOE-  
1316 18-0391

1317 Uliani R. C., Ramires Neto C., Dell’Aqua Jr., Pessoa M. A., Camargo A. L., Alvarenga R., and Alvarenga  
1318 M. A. (2010). Effect of mare breed and age on embryo transfer efficiency. *Animal Reproduction Science*  
1319 121S, 303–304. doi:10.1016/j.anireprosci.2010.04.140

1320 Valera M., Blesa F., Santos R. D., and Molina A. (2006). Genetic study of gestation length in andalusian  
1321 and arabian mares. *Animal Reproduction Science* 95, 75–96. doi:10.1016/j.anireprosci.2005.09.008

1322 Vanderwall D. K., Woods G. L., Freeman D. A., Weber J. A., Rock R. W., and Tester D. F. (1993). Ovarian  
1323 follicles, ovulations and progesterone concentrations in aged versus young mares. *Theriogenology* 40,  
1324 21–32. doi:10.1016/0093-691X(93)90338-6

1325 Verheyen K. L. P., Price J. S., and Wood J. L. N. (2007). Fracture rate in Thoroughbred racehorses is  
1326 affected by dam age and parity. *The Veterinary Journal* 174, 295–301. doi:10.1016/j.tvjl.2006.07.023

1327 Vidament M., Dupere A. M., Julienne P., Evain A., Noue P., and Palmer E. (1997). Equine frozen semen:  
1328 freezability and fertility field results. *THERIOGENOLOGY* 48, 907–917.  
1329 doi:https://doi.org/10.1016/S0093-691X(97)00319-1

1330 Visser J. A., and Themmen A. P. N. (2005). Anti-Müllerian hormone and folliculogenesis. *Molecular and*  
1331 *Cellular Endocrinology* 234, 81–86. doi:10.1016/j.mce.2004.09.008

1332 Vogelsang S. G., and Vogelsang M. M. (1989). Influence of donor parity and age on the success of  
1333 commercial equine embryo transfer. *Equine Veterinary Journal* 21, 71–72. doi:10.1111/j.2042-  
1334 3306.1989.tb04679.x

1335 Waelchli R. O. (1990). Endometrial biopsy in mares under nonuniform breeding management  
1336 conditions: Prognostic value and relationship with age. *Can Vet J* 31, 379–384.

1337 Watkins A., Lucas E. S., and Fleming T. (2010). Impact of the periconceptual environment on the  
1338 programming of adult disease. *Journal of Developmental Origins of Health and Disease*, 1, 1–9.

1339 Weber J. A., Freeman D. A., Vanderwall D. K., and Woods G. L. (1991). Prostaglandin E2 secretion by  
1340 oviductal transport-stage equine embryos. *Biology of Reproduction* 45, 540–543.

1341 Weber J. A., Woods G. L., Freeman D. A., and Vanderwall D. K. (1992). Prostaglandin E2-specific binding  
1342 to the equine oviduct. *Prostaglandins* 43, 61–65. doi:10.1016/0090-6980(92)90065-2

- 1343 Weber J. A., Woods G. L., and Lichtenwalner A. B. (1995). Relaxatory Effect of Prostaglandin E2 on  
1344 Circular Smooth Muscle Isolated from the Equine Oviductal Isthmus<sup>1</sup>. *Biology of Reproduction* 52,  
1345 125–130. doi:10.1093/biolreprod/52.monograph\_series1.125
- 1346 Wesson J. A., and Ginther O. J. (1981). Influence of Season and Age on Reproductive Activity in Pony  
1347 Mares on the Basis of a Slaughterhouse Survey. *Journal of Animal Science* 52, 119–129.  
1348 doi:10.2527/jas1981.521119x
- 1349 Willmann C., Schuler G., Hoffmann B., Parvizi N., and Aurich C. (2011). Effects of age and altrenogest  
1350 treatment on conceptus development and secretion of LH, progesterone and eCG in early-pregnant  
1351 mares. *Theriogenology* 75, 421–428. doi:10.1016/j.theriogenology.2010.05.009
- 1352 Wilsher S., and Allen W. R. (2003). The effects of maternal age and parity on placental and fetal  
1353 development in the mare. *Equine Veterinary Journal* 35, 476–483. doi:10.2746/042516403775600550
- 1354 Wilsher S., and Allen W. R. (2011). Factors influencing equine chorionic gonadotrophin production in  
1355 the mare: Equine chorionic gonadotrophin production. *Equine Veterinary Journal* 43, 430–438.  
1356 doi:10.1111/j.2042-3306.2010.00309.x
- 1357 Wilsher S., Allen W. R., and Wood J. L. N. (2006). Factors associated with failure of Thoroughbred  
1358 horses to train and race. *Equine Veterinary Journal* 38, 113–118. doi:10.2746/042516406776563305
- 1359 Winter G. H. Z., Rubin M. I. B., De La Corte F. D., and Silva C. A. M. (2007). Gestational Length and First  
1360 Postpartum Ovulation of Criollo Mares on a Stud Farm in Southern Brazil. *Journal of Equine Veterinary*  
1361 *Science* 27, 531–534. doi:10.1016/j.jevs.2007.10.015
- 1362 Winter C. G., Wang B., Ballew A., Royou A., Karess R., Axelrod J. D., and Luo L. (2001). Drosophila Rho-  
1363 Associated Kinase (Drok) Links Frizzled-Mediated Planar Cell Polarity Signaling to the Actin  
1364 Cytoskeleton. *Cell* 105, 81–91. doi:10.1016/S0092-8674(01)00298-7
- 1365 Woods G. L., Baker C. B., Baldwin J. L., Ball B. A., Bilinski J., Cooper W. L., Ley W. B., Mank E. C., and Erb  
1366 H. N. (1987). Early pregnancy loss in brood mares. *Journal of reproduction and fertility. Supplement*  
1367 35, 455–459.
- 1368 Woods G. L., Baker C. B., Hillman R. B., and Schlafer D. H. (1985). Recent studies relating to early  
1369 embryonic death in the mare. *Equine Veterinary Journal* 17, 104–107. doi:10.1111/j.2042-  
1370 3306.1985.tb04609.x
- 1371 Woodward E. M., Christoffersen M., Campos J., Squires E. L., and Troedsson M. H. T. (2012).  
1372 Susceptibility to persistent breeding-induced endometritis in the mare: Relationship to endometrial

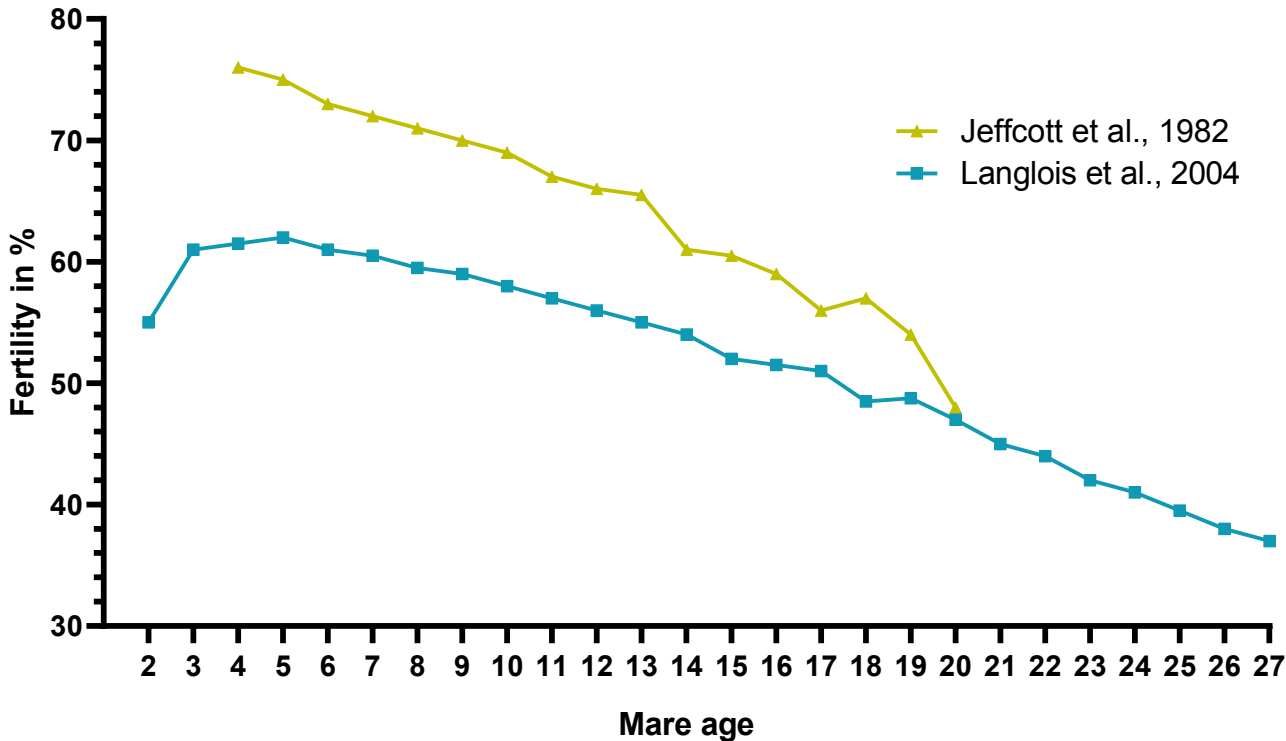
1373 biopsy score and age, and variations between seasons. *Theriogenology* 78, 495–501.  
1374 doi:10.1016/j.theriogenology.2012.02.028



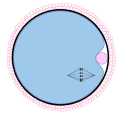


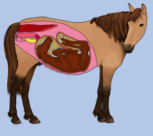



1375 Zent W. W., Liu I. K. M., and Spirito M. A. (1993). Oviduct flushing as a treatment for infertility in the  
1376 mare. *Equine Veterinary Journal* 15, 47–48. doi:10.1111/j.2042-3306.1993.tb04824.x

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		 Primiparity      Very high Parity (>10)      Very young (<3-4)      Aging (>10-12)      Old (15-20)      Very old (>20)		
Pre-implantation until blastocyst stage	Ovaries & folliculogenesis 	?	<ul style="list-style-type: none"> <li>Delayed breeding season</li> <li>↑ multiple ovulations</li> <li>Hormonal dysregulation               <ul style="list-style-type: none"> <li>↑ inter-ovulatory interval, ↓ pre-ovulatory follicle diameter</li> </ul> </li> </ul>	
	Follicles & oocytes 	?	<ul style="list-style-type: none"> <li>Altered gene expression in granulosa cells</li> <li>No difference in oocyte diameter               <ul style="list-style-type: none"> <li>↓ Mt DNA copy number</li> <li>↓ oocyte maturation potential                   <ul style="list-style-type: none"> <li>↑ aneuploidy, ↑ spindle instability</li> </ul> </li> <li>↓ follicular shape regularity                   <ul style="list-style-type: none"> <li>↑ IGF1, differences in miRNAs in follicular fluid</li> <li>Oocyte abnormalities</li> </ul> </li> </ul> </li> </ul>	
	Embryo 	?	<ul style="list-style-type: none"> <li>↓ diameter of embryonic vesicle by ultrasound</li> <li>↓ embryo movement in the uterus</li> <li>↓ Mt DNA copy number</li> <li>↓ quality &amp; development competence</li> <li>Delayed development*</li> </ul>	
From blastocyst to post-natal period	Oviducts, uterus and cervix 	<b>Aged maiden mares with signs of advanced chronic degenerative endometrial disease</b> <ul style="list-style-type: none"> <li>↑ number of uterine cysts with parity</li> <li>↑ frequency and severity of angiosis, ↑ vascular degenerations (elastosis in larger vessels of myometrium) with ↑ parity</li> <li>↑ slope of uterine-cervical junction in multiparous, ↓ uterine clearance</li> </ul>	<ul style="list-style-type: none"> <li>↑ number of oviductal masses and uterine cysts with age</li> <li>↑ frequency and severity of angiosis with mare age</li> <li>↑ frequency of moderate to severe sclerotic vascular lesions</li> <li>Age = best predictor of endometrial grade</li> <li>↑ size of uterine cysts</li> <li>↓ uterine clearance and vascular changes in luteal phase and gestation</li> <li>Endometrial elastosis and fibrosis</li> </ul>	
	Gestation 	<ul style="list-style-type: none"> <li>↑ gestation length?</li> <li>↑ combined thickness of uterus and placenta</li> <li>↓ metabolic adaptations to pregnancy</li> </ul>	<ul style="list-style-type: none"> <li>↓ fetal eCG and earlier eCG peak with ↑ parity</li> <li>↑ diameter of uterine artery with ↑ parity</li> </ul>	<ul style="list-style-type: none"> <li>No clear difference in gestational length</li> <li>↓ fetal size and placental development at implantation</li> <li>↑ combined thickness of uterus and placenta</li> <li>↑ diameter of both uterine arteries in &gt;7 years old</li> <li>↑ uterine artery pulsatility index in &gt;7 years old</li> </ul>
	Placenta 	<ul style="list-style-type: none"> <li>↓ placental weight and volume</li> <li>↓ microcotyledonary surface and density</li> </ul>	<ul style="list-style-type: none"> <li>↑ placental weight with ↑ parity</li> </ul>	<ul style="list-style-type: none"> <li>No clear effect on weight and microcotyledonary structure</li> </ul>
	Post-partum complications Maternal behavior	<ul style="list-style-type: none"> <li>↑ dystocia</li> <li>↑ foal rejections</li> </ul>		<ul style="list-style-type: none"> <li>↑ placental retention</li> <li>↑ dystocia</li> <li>↑ placental retention</li> </ul>
	Foal 	<ul style="list-style-type: none"> <li>↑ dystocia</li> <li>↓ birthweight and height</li> <li>Delayed metabolic and testicular maturation</li> </ul>	<ul style="list-style-type: none"> <li>↑ birthweight with ↑ parity</li> </ul>	<ul style="list-style-type: none"> <li>↑ foal mortality with maternal age before and after 15 days of age</li> <li>↑ foal morbidity with maternal age before 15 days of age</li> <li>↑ foal birthweight when excluded youngest and oldest mares</li> </ul>
Lactation 	<ul style="list-style-type: none"> <li>↓ fat and ↑ protein concentrations</li> </ul>	<ul style="list-style-type: none"> <li>↑ Milk production for parity ≥2</li> <li>↓ Ratio saturated/polyunsaturated fatty acid in parity &lt;5</li> </ul>	<ul style="list-style-type: none"> <li>↑ colostrum calcium concentrations</li> <li>Modifications in fatty acid proportions</li> <li>↓ colostrum IgG concentrations</li> </ul>	

rates. To our knowledge, no report on effects of parity on these parameters are available.

	Age categories	Sample size	Breed	Results	Source
<b>Effect of age</b>					
<b>Ovarian activity</b>	5-7yo 15-19yo ≥20yo	9 10 16	Pony	↘ Ovulation regularity ≥20yo	(Carnevale <i>et al.</i> , 1994)
	3-12yo 20-30yo	25 21	Arabian, Quarter Horse, Thoroughbred	Latest first ovulation for old mares ↘ Number of ovulatory cycles after 20yo	(Vanderwall <i>et al.</i> , 1993)
	<5yo >15yo	1,003	Pony	↘ Ovarian activity after 15yo	(Wesson and Ginther, 1981)
<b>Anti-Müllerian hormone concentration</b>	Young (3-8yo) Middle-aged (9-18yo) Old (19-27yo)	10 16 19	Light horse	↘ Number of antral follicles in 19-27yo (compared to 3-8yo and 9-18yo) ↘ AMH concentrations in 19-27yo (compared to 9-18yo) Weak AFC AMH correlation in 3-8yo	(Claes <i>et al.</i> , 2017)
	Young (4-8yo) Middle-aged (9-18yo) Old (19-25yo)	12 12 12	Arabian	↗ AMH concentration in 9-18yo	(Korkmaz <i>et al.</i> , 2020)
	4-8yo 9-18yo 19-25yo	1,101	Quarter Horse	↗ AMH concentration 5-10yo and 10-15yo ↘ AMH concentration ≥20yo	(Uliani <i>et al.</i> , 2019)
<b>Breeding period and follicular activity</b>	Young (4-9yo) Old (≥20yo)	22 15	Not defined	↘ Dominant follicle's growth ≥20yo	(Altermatt <i>et al.</i> , 2012)
	Group 1 (5-7yo) Group 2 (15-19yo) Group 3 (≥20yo)	9 11 11	Pony	Prolonged interovulatory interval for ≥20yo group ↗ Follicular activity in 15-19yo compared to ≥20yo	(Carnevale <i>et al.</i> , 1993)
	5-7yo 15-19yo ≥20yo	9 10 16	Pony	Delayed follicular activity in ≥20yo Prolonged follicular growth ≥20yo	(Carnevale <i>et al.</i> , 1994)
	<b>Exp 1:</b> Young (5-7yo) Old (≥14yo) <b>Exp 2:</b> Young (3-7yo) Intermediate (17-19yo) Old (≥20yo)	12 16 16 10 9	Pony	<b>Exp 1:</b> ↗ Follicular activity in 5-7yo <b>Exp 2:</b> Number of follicles highest in 3-7yo group and lowest in ≥20yo group	(Carnevale <i>et al.</i> , 1997)
	Young (3-8yo) Middle-aged (9-18yo) Old (19-27yo)	10 16 19	Light horse	↘ Number of follicles with age Prolonged interovulatory interval for 19-27yo group	(Claes <i>et al.</i> , 2017)
	5-7yo 15-19yo ≥20yo	9 10 11	Pony	Later primary wave for ≥20yo group	(Ginther <i>et al.</i> , 1993)

	Young (5-6yo) Intermediate (10-14yo) Old (≥18yo)	7 8 8	Pony (mixed breeds)	↗ Interovulatory interval in ≥18yo Number of follicles lower in ≥18yo group	(Ginther <i>et al.</i> , 2008)
	Young (5-6yo) Intermediate (10-14yo) Old (≥18yo)	14 waves 16 waves 15 waves	Pony (mixed breeds)	↗ Number and size of follicles in 5-6yo group	(Ginther <i>et al.</i> , 2009)
	Young (3-4yo) Middle-aged (5-10yo) Old (13-25yo)	42 95 49	Polo Argentino	Interovulatory interval = for 3-4 and 5-10yo groups ↗ Interovulatory interval in 13-25yo group	(Marinone <i>et al.</i> , 2015)
	3-12yo 20-30yo	25 21	Arabian, Quarter Horse, Thoroughbred	Delayed in 25-33yo Prolonged follicular growth ≥20yo	(Vanderwall <i>et al.</i> , 1993)
<b>Hormonal control of follicular growth</b>	Group 1 (5-7yo) Group 2 (15-19yo) Group 3 (≥20yo)	9 11 11	Pony	↗FSH concentrations in 15-19yo group ↘ LH concentration in ≥20yo group Progesterone concentration = in all groups	(Carnevale <i>et al.</i> , 1993)
	5-7yo 15-19yo ≥20yo	9 10 16	Pony	Elongated reproductive cycle ↗ FSH and LH concentrations Elongated reproductive cycle ↘ progesterone concentration	(Carnevale <i>et al.</i> , 1994)
	3-8yo 9-18yo 19-27yo	10 16 19	Light horse	Progesterone concentration ↗ with age ↗ FSH concentration in 19-27yo group	(Claes <i>et al.</i> , 2017)
	Young (5-6yo) Intermediate (10-14yo) Old (≥18yo)	7 8 8	Not defined	↗ FSH concentration in ≥18yo group ↗ Maximum LH concentration in 5-6yo group ↗ Estradiol concentration in 5-6yo group ↗Progesterone concentration in 10-14yo group	(Ginther <i>et al.</i> , 2008)
	Young (5-6yo) Intermediate (10-14yo) Old (≥18yo)	14 waves 16 waves 15 waves	Pony (mixed breeds)	↗ LH concentration in 5-6yo group Estradiol concentrations = in all groups	(Ginther <i>et al.</i> , 2009)
	3-12yo 20-30yo	25 21	Arabian, Quarter Horse, Thoroughbred	↗ Progesterone concentration in 25-33yo group	(Vanderwall <i>et al.</i> , 1993)
<b>Ovulation</b>	3-9yo 10-16yo >16yo	220 251 145	Warmblood	Ovulation induction using hCG have more chance to fail in >16yo mares	(Barbacini <i>et al.</i> , 2000)
	Group 1 (5-7yo) Group 2 (15-19yo) Group 3 (≥20yo)	9 11 11	Pony	Larger follicle in 15-19yo group Smaller follicles for >20yo group compared to 15-19yo group	(Carnevale <i>et al.</i> , 1993)
	Young (3-7yo) Intermediate (17-19yo) Old (≥20yo)	16 10 9	Pony	↘ Follicle size in >20yo group	(Carnevale <i>et al.</i> , 1997)
	Young (3-8yo) Middle-aged (9-18yo) Old (19-27yo)	10 16 19	Light horse	Number of ovulations = in all groups	(Claes <i>et al.</i> , 2017)

	3-5yo 6-8yo 9-11yo 12-14yo 15-18yo 18-22yo	1,136 mares 2,181 oestrous cycles	Thoroughbred	↗ Number of multiple ovulation with age	(Davies Morel and O'Sullivan, 2001)
	2-4yo 5-9yo 10-14yo 15-19yo ≥20yo	46 142 66 51 31	Light breed	Ovulation rate in response to doserelin is the highest in 10-14yo group ↘Ovulation rate in >20yo group	(Farquhar <i>et al.</i> , 2000)
	Young (5-6yo) Intermediate (10-14yo) Old (≥18yo)	14 waves 16 waves 15 waves	Pony (mixed breeds)	Ovulatory follicle size = in all groups	(Ginther <i>et al.</i> , 2009)
	Young (3-4yo) Middle-aged (5-10yo) Old (13-25yo)	36 98 37	Polo Argentino	↗ Multiple ovulation rate with age	(Marinone <i>et al.</i> , 2015)
	2-22yo, studied as a continuous variable	159 mares (309 cycles)	Lusitano	↘ Ovulatory follicle size with age	(Mata <i>et al.</i> , 2013)
	2-4yo 5-7yo 8-10yo 11-13yo 14-16yo 17-19yo 20-22yo ≥23yo	1581 mares (3,075 ovulations)	Thoroughbred	Multiple ovulation rate ↗ from 2-4yo to 17-19yo then ↘ until ≥23yo	(Morel <i>et al.</i> , 2005)
	2-10yo 11-15yo 16-20yo 21-24yo	198 embryo-donors (937 ovulations)	Arabian, Show Jumping, Haflinger, Quarter Horse, Standardbred	↗ Multiple ovulation rate in 11-15yo group (compared to 2-10yo group) ↗ Multiple ovulation rate in 16-20yo group (compared to all other groups)	(Panzani <i>et al.</i> , 2014)

Table 2: Summary of literature reports showing effects of mare's age on follicular organization, oocytes and embryos

	Categories	Number of animals	Sample size	Breed	Results	Source
<b>Effect of age</b>						
<b>Follicular organization</b>	5-6 yo 7-10 yo 11-16 yo	3 4 3	141 176 19 (Preantral follicles)	Light horse	5-6 yo with more normal follicles (in shape and number of granulosa cell layers around the oocyte) than 11-16 yo	Alves et al., 2017
	Young (4-14 yo) Mid-aged (15-19 yo) Old (20-27 yo)	12 9 14	Granulosa cells from one preovulatory follicle/mare	?	Analyze of granulosa cell gene expression: tendency to or ↗ for receptors of insulin, adiponectin and peroxisome proliferating factor-γ in both older groups than young mares ↗ <i>interleukin-6 (IL-6)</i> in Old versus Young mare ↗ <i>interleukin-6 signal transducer (IL6ST)</i> in old than in younger groups ↗ <i>tumor necrosis factor receptor superfamily member 1A (TNFRSF1A)</i> in mid-aged than young mares Tended to ↘ <i>tumor necrosis factor- inducible gene 6 (TNFAIP6)</i> in mid-aged vs old and young mares tended to ↗ <i>LH receptor (LHR)</i> and <i>steroidogenic acute regulatory protein (STAR)</i> in old vs Mid-aged and young, respectively ↗ <i>tissue-type plasminogen activator (PLAU)</i> in young and old compared to mid-aged ↗ <i>Thioredoxin-2 (TXN2)</i> in old than younger groups	Sessions-Bresnahan & Carnevale 2015
	Young (3-7 yo) Intermediate (8-17 yo) Old (≥ 18 yo)	11 23 10	One follicle/mare	Large ponies and pony-horse crosses	After 30h of <i>in vivo</i> maturation: No difference in follicle diameter, meiotic stage, spindle quality and orientation, thickness of the F-actin band and the percentage of the oocyte image with foci of microfilaments No difference in concentrations of estradiol, progesterone and testosterone in the follicular fluid ↗ Insulin like growth factor 1 concentrations in follicular fluid of old mares	Ginther et al., 2008
	Young (3-13 yo) Old (≥ 20 yo)	3 3	One dominant follicular fluid/mare	Light horse	Variation in quantity and in composition of miRNAs identified in exosomes from follicular fluid with the mare age	Da Silveira et al., 2012
	Young (3-12 yo) Old (20-26 yo)	6 6	One follicular fluid and granulosa cells/mare at deviation, mid-estrus and preovulation	Not defined	Differences in <i>interleukin-6 (IL6)</i> and <i>collagen, type I, alpha 2 (COL1A2)</i> during follicular development according to maternal age Alteration of the gene expression of <i>inhibitor of DNA binding 2 (ID2)</i> , <i>signal transducer and activator of transcription 1 (STAT1)</i> and <i>cell division cycle 25A (CDC25A)</i> in old mare granulosa cells	Da Silveira et al., 2015
	Young (4-13 yo) Old (21-26 yo)	15 18	One follicular fluid/mare	Not defined	Tendency to ↗ blastocyst development using follicular fluid in <i>in vitro</i> medium from old mares	Spacek and Carnevale 2018
<b>Oocyte morphology</b>	Young (2-7 yo) <sup>a</sup> Middle aged (8-14 yo) <sup>a</sup> Aged (≥ 15 yo) <sup>a</sup>	Not defined	38 ovaries/432 follicles/288 COCs 17 ovaries/209 follicles/125 COCs 26 ovaries/270 follicles/176 COCs	Slaughtered mares Breed not defined	No difference in the proportion of normal oocytes	Brinsko et al., 1995
	Young (3-10 yo) Old (≥ 19 yo)	10 10	One oocyte/mare	Not defined	No difference in oocyte diameter, quantification of organelles Tendency to ↘ area per section of oocyte occupied by cortical granules ↗ number of oocytes with large vesicles and number of large vesicles/oocyte from old mares ↗ morphological abnormalities (i.e. irregular shapes, areas of ooplasm without organelles)	Carnevale et al., 1999
	3-13 yo 14-19 yo 20- 23 yo 24-27 yo	A total of 93 mares	49 141 99 52 (oocytes)	Light horse	No difference in oocyte and inner zona pellucida volume ↗ oocyte and ooplasm diameter in 14-19 yo compared to 3-13 yo ↗ inner zona pellucida diameter in 14-19 yo compared to 3-13 yo In 14-19 and 20-23 yo groups, ↗ ooplasm volume compared to 3-13 and 24-27 yo groups.	Frank et al., 2019
	< 20 yo ≥ 20 yo	70 mare-year 67 mare -year	180 162	Light horse	↗ oocyte grade with mares' age	Carnevale et al., 2005
	Young (4-9 yo) Old (≥ 20 yo)	22 15	40 37	Light horse	No difference in the oocyte nor the ooplasm volume and diameter ↘ thickness of the zona pellucida and zona pellucida + matrix in old mare oocytes but not matrix thickness alone ↗ inner zona pellucida and perivitelline space volume in old mare oocytes	Altermatt et al., 2009
<b>Oocyte quality</b>	Young (2-7 yo) <sup>a</sup> Middle aged (8-14 yo) <sup>a</sup> Aged (≥ 15 yo) <sup>a</sup>	Not defined	38 ovaries/432 follicles/288 COCs 17 ovaries/209 follicles/125 COCs 26 ovaries/270 follicles/176 COCs	Slaughtered mares Breed not defined	x 3 and x 4 ↘ chances to reach MI and MII, respectively, for oocytes from aged mares	Brinsko et al., 1995

	Young (3-12 yo) Old (≥ 20 yo)	15 15	One oocyte, granulosa and cumulus cells/mare at 0, 6, 9 and 12 hours after LH administration	Light horse	Different gene expression profile of luteinizing hormone receptor (LHR) → asynchrony of the initiation of oocyte and follicle maturation Different gene expression profile of <i>amphiregulin (AREG)</i> and <i>epiregulin (EREG)</i> in granulosa cells, <i>phosphodiesterase 4D (PDE4D)</i> in cumulus cells and <i>phosphodiesterase 3A (PDE3A)</i> and <i>G-protein-coupled receptor 3 (GPR3)</i> in oocytes ↘ total gene expression levels of <i>growth differentiation factor 9 (GDF9)</i> and <i>bone morphogenetic protein 15 (BMP15)</i> in old follicles Temporal ↘ mtDNA copy numbers	Campos-Chillon et al., 2015
	3-12 yo ≥ 18 yo	10 5	14 COCs 10 COCs	Quarter horse-type	↘ gene expression of <i>mitochondrial translational initiation factor 3 (IF3)</i> , <i>heat shock transcription factor 5 (HSF5)</i> and <i>Y box binding protein 2 (YBX2)</i> in old oocytes ↗ <i>ADP-ribosylation factor-like 6 interacting protein 6 (ARL6IP6)</i> , <i>BCL2-associated X protein (BAX)</i> and <i>hypoxia upregulated 1 (HYOU1)</i> in old mare cumulus cells	Cox et al., 2015
	Young (2-14 Yo) Old (16-27 yo)	27 24	169 COCs/75 MII oocytes 134 COCs/ 54 MII oocytes (25 MII oocytes/analysis)	Slaughtered mares Breed not defined	↗ chromosome misalignment in old mare oocytes ↗ thickness of metaphase plate in old mare oocytes No difference in spindle morphometry	Rizzo et al., 2019
	Young (2-14 Yo) Old (16-27 yo)	106 115	641 COCs/319 MII oocytes 530 COCs/ 241 MII oocytes (4 pool of 10 oocytes/group → gene expression analysis + 394 → chromosome spreads)	Slaughtered mares Breed not defined	↘ gene expression of the centromere cohesion stabilizing protein, <i>Shugoshin 1 (SGOL1)</i> in old mare oocytes ↗ aneuploidy, premature sister chromatid separation and weakness of centromeric cohesion in old mare oocytes	Rizzo et al., 2020
	Young (2-14 Yo) Old (16-28 yo)	41 77	A total of 640 COCs (4 pool of 10 oocytes/group → gene expression analysis + 320 → analysis of inhibitors and immunofluorescence)	Slaughtered mares Breed not defined	↘ gene expression of <i>mono polar spindle 1 (MPS1)</i> , <i>SPC25</i> and <i>Aurora kinase C (AURKC)</i> (meiosis regulators) in old mare oocytes ↗ instability of spindle assembly when MPS1 is inhibited in old mare oocytes → Compromised MPS1 activity = predisposition to meiotic spindle instability in old mare oocyte	Rizzo et al., 2021
	Young (2-14 Yo)  Old (16-31 yo)	35  61	311 COCs 169 control → 75 MII oocytes 142 vitrified oocytes → 38 MII oocytes 294 COCs 150 control → 64 MII oocytes 144 vitrified oocytes → 48 MII oocytes (19 to 34/group for each analysis)	Slaughtered mares Breed not defined	↘ of severity of vitrification-related degenerations in spindle morphology and capacity to reach MII → Reflection of pre-existing compromise spindle assembly checkpoints	Ducheyne et al., 2019
	< 12 yo <sup>a</sup> ≥ 12 yo <sup>a</sup>	268	114 IVM COCs/127 not IVM oocytes 154 IVM COCs/106 not IVM oocytes (mtDNA quantity analysis → all + mitochondrial morphology → 5/group)	Slaughtered mares Breed not defined	↘ mtDNA copy number in old mare oocyte after IVM only ↗ swelling and ↘ number of cristae in mitochondria from old mare oocytes after IVM only	Rambags et al., 2014
	Exp 1 Young (7-11 yo) Old (20-26 yo)  Exp 2 Young (6-13 yo) Old (20-27 yo)	8 10  7 8	One follicle/mare → cumulus cells and oocytes from the same mare  9/10 oocytes 14/13 oocytes	Light horse	No difference in relative abundance of glucose, pyruvate, lactate and triglycerides in oocytes and cumulus cells ↗ and ↘ free fatty acids relative abundance in cumulus cells and oocytes, respectively, from old mares ↘ Basal and maximum oxygen consumption rate and extracellular acidification rate in oocytes from old mares No difference in mtDNA copy number	Catandi et al., 2021
	< 12 yo ≥ 12 yo	101	165 oocytes (77 not IVM/88 IVM) 170 oocytes (92 not IVM/78 IVM)	Slaughtered mares Breed not defined	No difference in gene expression nor ATP content in oocytes	Hendriks et al., 2015
Fertilisation	Normal (average of 4.9 yo + nulliparous) Subfertile (average of 17.4 yo + history of reproductive failure)	15 15	One embryo collection at day 2 post ovulation and one pregnancy diagnosis at 14 days post ovulation/mare	Standardbred  Standardbred + Thoroughbred	No difference in Day 2 embryo recovery rates ↘ number of positive diagnosis in subfertile mares	Ball et al., 1986
	Young (9-13 yo) Old (20-25 yo)	Not defined	11 32	Not defined	↘ tubulin multiasters in sperm-injected old mare oocytes ↗ actin vesicles in sperm-injected old mare oocytes = different cytoskeletal remodeling in sperm-injected oocytes according to maternal age	Ruggeri et al., 2015

	Normal (average of 5.7 yo + primiparous or multiparous) Subfertile (average of 19.4 yo + history of reproductive failure)	38  22	One embryo collection at day 4 post ovulation and transfer + pregnancy check at 16 days post ovulation/donor mare	Standardbred  Standardbred + Thoroughbred	No difference in Day 4 embryo recovery rates After transfer, ↘ embryo survival rates for subfertile donors	Ball et al., 1989
	< 20 yo ≥ 20 yo	70 mare-year 67 mare-year	Pregnancy diagnosis for each of the 548 oocyte transfer	Light horse	No difference in Day 16 and 50 positive pregnancy diagnosis rates	Carnevale et al., 2005
	Young (6-10 yo) <sup>α</sup> Old (20-26 yo) <sup>α</sup>	Not defined	12 transferred oocytes 26 transferred oocytes	Pony mares	After transfer, ↘ embryo survival rates for old donors	Carnevale and Ginther 1995
	Young (4-9 yo) Old (≥ 20 yo)	22 15	40 37	Light horse	No difference in cleavage rate, the incidence of delayed development, morula and blastocyst rates nor the rate of embryonic vesicles producing a heartbeat	Altermatt et al., 2009
	1-4 5-6 7-8 9-10 11-12 13-14 15-16 17-18 19-20 21-22 23-29	A total of 323 mares from February 2017 to the end of January 2019	49 OPU-ICSI 47 OPU-ICSI 44 OPU-ICSI 41 OPU-ICSI 43 OPU-ICSI 55 OPU-ICSI 73 OPU-ICSI 68 OPU-ICSI 50 OPU-ICSI 45 OPU-ICSI 37 OPU-ICSI	Warmblood + Arabian	No difference in the number of embryos produced by OPU-ICSI session, the blastocyst/injected oocyte nor the likelihood of success	Cuervo-Arango et al., 2019a
	Age considered as a continuous variable (from 1 to 29 years old)	A total of 473 mares			In median, mares selected for OPU-ICSI were older No difference in the post-ICSI cleavage rate, the blastocyst/injected or recovered oocyte nor the likelihood of pregnancy at Day 12 and 45 and the embryonic loss incidence	Cuervo-Arango et al., 2019b
	Young (6-14 yo) Old (20-28 yo)	13 11	29 24	Light horse	No difference in the number of cleaved embryos ↘ embryonic development to blastocyst stage per old mare sperm-injected oocytes or cleaved embryos	Catandi et al., 2021
Embryo development	Normal (average of 4 yo + maiden)  Subfertile (average of 18 yo + history of reproductive failure)	14  14	3 embryo collection/mare	Standardbred, Quarter horse and Morgan Standardbred and Thoroughbred	↘ number of flushes with one or more normal embryos ↗ number of flushes with only abnormal embryos ↘ diameter of embryos at day 7 and 8 post ovulation collected from subfertile mares	Woods et al., 1985
	Exp 1 <sup>α</sup> Young (3.5-7 yo) Intermediate (15-19 yo) Old (≥20 yo) Exp 2 <sup>α</sup> Young (2-9 yo) Old (≥20 yo)	18 14 20  18 18	One embryo collection at day 1.5 post ovulation/mare  Half of the mares in embryo collection at day 3 post ovulation and the other half in ultrasound follow-up	Pony mares	↘ number of cleaved oocytes at the collection time in old compared to young mares No difference in the mean number of cells per embryo ↗ mean embryo morphology grade in old compared to young and intermediate mares  ↗ mean embryo morphology grade in old compared to young mares At day 11, tendency to and at day 12 and 13 post ovulation, ↘ mean diameter of embryonic vesicle	Carnevale et al., 1993
	Young & Normal (2-7 yo)  Old & Subfertile (17-24 yo + multiparous, no foaling in the 2 previous years)	19  16	26 oviductal flush/16 <i>in vitro</i> cultured embryos  28 oviductal flush/16 <i>in vitro</i> cultured embryos	Light horse	No difference in embryonic development to blastocyst stage rates ↘ development for embryos from aged and subfertile mares ↘ quality score of embryos from old mares at the day of blastulation and after 7 days of culture Tendency to ↘ blastocyst diameter on day 7 of culture for embryos from old and subfertile mares Tendency to ↘ number of cells in blastocyst from old and subfertile mares	Brinsko et al., 1994
	4-8 yo > 8 yo	19 13	2 monitored beginning of gestation/mare	Brandenburg	↘ mean diameter of embryonic vesicle from day 12 to 22 in > 8 yo Negative correlation between the Day 20 post ovulation vesicle size and the age of the mare	Willmann et al., 2011
	2-10 yo 11-15 yo 16-20 yo	131 mares during 10	47 embryos 58 embryos 70 embryos	Different breeds (Arabian, Show Jumping, Haflinger,	↘ embryo diameter collected from Day 7-10 post ovulation in 16-20 and 21-24 yo compared to 2-10 and 11-15 yo groups No difference in embryo quality	Panzani et al., 2014



21-24 yo	breeding seasons	40 embryos	Quarter Horse and Standardbred)		
Young (5-6 yo) <sup>α</sup> Old (15-20 yo) <sup>α</sup>	7 6	One beginning of gestation/mare	Mixed breed	<ul style="list-style-type: none"> <li>↘ embryo diameter measured by ultrasonography from Day 15 to 19 post ovulation</li> <li>High embryo mobility for both groups from Day 10 to 12</li> <li>↘ embryo movements earlier in old mares</li> <li>Similar day of fixation in the cornual-body junction for both groups</li> </ul>	Camargo Ferreira et al., 2019
Young (5-7 yo) <sup>α</sup> Old (≥ 15 yo) <sup>α</sup>	9 22	One beginning of gestation/mare	Pony mares	<ul style="list-style-type: none"> <li>No difference in embryonic vesicle diameter measured by ultrasonography on day 12 and the day of fixation</li> <li>Similar embryo mobility between groups</li> <li>Fixation on 1.5 days later in old mares</li> </ul>	Carnevale and Ginther 1992
Young (6-10 yo) <sup>α</sup> Old (20-26 yo) <sup>α</sup>	Not defined	12 transferred oocytes 26 transferred oocytes	Pony mares	No difference in embryonic vesicle diameter measured by ultrasonography on days 11 to 14	Carnevale and Ginther 1995
2-5 yo 6-8 yo 9-11 yo 12-15 yo ≥16	A total of 1213 mares	90 embryos at day 13/ 74 at day 14 201 embryos at day 13/ 124 at day 14 195 embryos at day 13/ 144 at day 14 217 embryos at day 13/ 160 at day 14 128 embryos at day 13/ 104 at day 14	Thoroughbred and Thoroughbred-Warmblood	No difference in embryonic vesicle diameter measured by ultrasonography on days 13 to 14	Morel et al., 2005
3-13 yo 14-19 yo 20- 23 yo 24-27 yo	A total of 86 mares	178 ICSI-produced embryos	Light horse	<ul style="list-style-type: none"> <li>Tendency to and ↘ oocyte-donor mean age for a positive pregnancy diagnosis by, respectively Day 17 and 50 post ovulation</li> <li>No difference in blastomere size, number of cells, presence of fragments of ooplasm nor extruded membrane through zona pellucida after ICSI</li> <li>No difference in the number of hours from ICSI until cleavage</li> <li>At transfer, ↗ number of cells for 20-23 yo compared to 3-13 and 14-19 yo groups</li> <li>↘ time to transfer for 20-23 yo compared to 3-13 and 14-19 yo groups</li> <li>Earlier detection of embryonic vesicle in 14-19 yo compared to 20-23 yo group</li> <li>No difference in the diameter of vesicles at detection nor the embryonic vesicle diameter per day of detection</li> </ul>	Frank et al., 2019
Young (6 yo) Old (10-16 yo)	11 20	5 <i>in vivo</i> produced embryos 6 <i>in vivo</i> produced embryos	Saddlebred	<ul style="list-style-type: none"> <li>No difference in embryo diameter</li> <li>Alteration of gene expression in trophoblast and inner cell mass according to maternal age related to mitochondria, translation, transcription, chromatin modification</li> <li>In inner cell mass, disturbance on mitosis, signaling, adhesion and embryo development pathways</li> <li>In trophoblast, disturbance of ion movement related genes</li> </ul>	Derisoud et al., 2021
Normal (average of 4.9 yo + nulliparous) Subfertile (average of 17.4 yo + history of reproductive failure)	15 15	One embryo collection at day 2 post ovulation and one pregnancy diagnosis at 14 days post ovulation/mare	Standardbred Standardbred + Thoroughbred	No difference in diameter nor number of blastomeres in Day 2 post ovulation embryos	Ball et al., 1986
Normal (average of 5.7 yo + primiparous or multiparous) Subfertile (average of 19.4 yo + history of reproductive failure)	38 22	One embryo collection at day 4 post ovulation and transfer + pregnancy check at 16 days post ovulation/donor mare	Standardbred Standardbred + Thoroughbred	No difference in the morphology nor in diameter of embryos at Day 4 post ovulation	Ball et al., 1989
Young (4-9 yo) Old (≥ 20 yo)	22 15	40 37	Light horse	<ul style="list-style-type: none"> <li>↘ number of cells in embryos from old mares at Day 1 post ICSI</li> <li>No difference in the number of cells at Day 2 post ICSI</li> </ul>	Altermatt et al., 2009
Young (6-13 yo) Old (20-27 yo)	7 8	8 embryos 10 embryos	Light horse	<ul style="list-style-type: none"> <li>↘ Basal oxygen consumption rate in Day 2 embryos from old mares</li> <li>No difference in basal extracellular acidification rate in Day 2 embryos</li> <li>No difference in cell numbers of the Day 2 embryos</li> </ul>	Catandi et al., 2021
4 - 11 yo 17-22 yo	A total of 10 mares	8 8	Slaughtered mares Breed not defined	<ul style="list-style-type: none"> <li>↘ mtDNA copy number in old mares Day 8 old mare blastocysts</li> <li>No difference in the expression of 5 genes related to mitochondria</li> </ul>	Hendriks et al., 2015

Yo: years old

<sup>α</sup>: age determined based on dental characteristics

mtDNA: mitochondrial DNA

COCs: cumulus oocyte complexes

MII : Metaphase II

IVM : *in vitro* maturation

OPU : Ovum Pick-up

ICSI : Intra-Cytoplasmic Sperm Injection

Exp: experience

Table 3: Summary of literature reports showing effects of mare's age on the reproductive tract

	Categories	Number of animals	Sample size	Breed	Results	Source
<b>Effect of age</b>						
<b>Oviducts</b>	4-8 yo 10-12 yo ≥17 yo	Not defined	Not defined	Not defined	↘ expression of <i>collagen type III (COL3)</i> gene in the entire oviduct from mares older than 10 yo	Pinto-Bravo et al., 2018
	Age considered as a continuous variable <sup>a</sup> (from 2 to 20 yo)	A total of 250 mares	Oviducts and ovaries of each mare	Slaughtered mares Quarter Horse type	↗ number of masses with mare age	Aguilar et al., 1997
	Young, normal (2-7 yo) Old, subfertile (17-24 yo, multiparous, no foaling in the 2 previous years)	5 5	One oviductal cell explant/mare	Standardbred and Thoroughbred	Age-related differences in the synthesis and secretion of proteins from oviductal cell explants 9 and 7 non identified proteins, respectively, less and more abundant in old mare oviductal cell secretions	Brinsko et al., 1996
	Young & Normal (2-7 yo)  Old & Subfertile (17-24 yo + multiparous, no foaling in the 2 previous years)	19  16	16 co-cultures of oviductal cells with 8 <i>in vitro</i> embryos from young donors 8 <i>in vitro</i> embryos from old donors 16 co-cultures of oviductal cells with 8 <i>in vitro</i> embryos from young donors 8 <i>in vitro</i> embryos from old donors	Light horse	No difference in embryonic development to blastocyst stage rates related to the age of the oviductal cells donor No difference in embryo quality from young and old mares when co-cultured with oviductal cells from old mares while using oviductal cells from young mares, embryos from young donors with ↗ quality Tendency to ↘ the number of cells in embryos from young donors when co-cultured with old mare oviductal cells while no difference observed in embryos from old mares	Brinsko et al., 1994
<b>Uterine cysts and vascularization</b>	Age considered as a continuous variable (from 1.5 to ≥ 20 yo)	A total of 93 mares	One ultrasound examination/mare	Mixed breeds	Mares with uterine cysts older (mean of 13.4 yo) than mares without uterine cysts (mean of 6.9 yo) No difference in pregnancy rates at Day 11/12 post ovulation according to the presence, number and size of uterine cysts	Adams et al., 1987
	Age considered as a continuous variable (from 2 to 26 yo)	A total of 3740 mares on 5 breeding seasons	One ultrasound examination around Day 22 of pregnancy/mare	Thoroughbred Standardbred Saddlebred Pony Draught	Mares with uterine cysts older (mean of 14.7 yo) than mares without uterine cysts (mean of 9.3 yo)	Chevalier-Clément 1989
	Age considered as a continuous variable (from 5 to 23 years old)	A total of 87 mares	One videoendoscopic measurement/mare	Thoroughbred	Only 4 of the 48 mares with cysts < 10 yo Relation between mare age and cysts formation	Bracher et al., 1992
	< 11 yo ≥11 yo	A total of 215 mares	Several ultrasound examinations/mare	Thoroughbred	X 4.2 times more risk to present endometrial cysts in ≥ 11 yo	Eilts et al., 1995
	<7 yo 7-14 yo > 14 yo	116 117 26	One ultrasound examination/mare	Thoroughbred	↗ number of uterine cysts in ≥ 7yo mares ↗ uterine cyst size in >14 yo mares	Tannus and Thun 1995
	Exp 1: <7 yo 7-14 yo 15-25 yo Exp2: young (3-10 yo) Old (15-21 yo)	A total of 76 mares  11 11	One ultrasound examination/mare	Large ponies and pony-horse crosses	Exp 1: Mares with uterine cysts older (mean of 14.0 yo) than mares without uterine cysts (mean of 10.0 yo) ↗ frequency of mares with uterine cysts with age while the frequency of mares without uterine cyst ↗ for 7-14 yo group ↗ number and size of uterine cysts with age Exp 2: ↗ mesometrial blood flow velocities in old mares without cysts No age-related difference in uterine vascular perfusion in mares without cysts	Ferreira et al., 2008
	Age considered as a continuous variable	20	Blood-flow measurements and postmortem uterine biopsy/mare	Thoroughbred	Mares with elastosis (in average 18.6 and 20.0 yo) older than mares without elastosis (in average 9.0 and 10.0 yo) No difference in the mean arterial pressure and blood flow in uterus ↘ uterine blood perfusion (total volume of blood/min/100 g of uterus) with mare age and elastosis	Esteller-Vico et al., 2015
Young (4-6 yo) Adult (8-12 yo) Old (15-18 yo)	7 7 7	One ultrasound examination/day/mare from Day 12 to Day 20 of gestation	Mixed breed	More important uterine vascular changes throughout the study period in young and old compared to adult group	Ferreira et al., 2015	

					Similar perfusion of both uterine horns in old mares while in young and adult groups, more often ↘ perfusion in the uterine horn without compared to the one with the embryo	
	Young (7-8 yo) Aged (16-21 yo)	6 6	Ultrasound examination throughout gestation	Thoroughbred	↘ uterine artery blood flow throughout the gestation in old mares	Ousey et al., 2012
	2-7 8-12 13-17 18-31	32 33 28 19	One endometrial biopsy/mare	European warmblood, Thoroughbred, Standardbred, Ponies and Draught horses	↗ frequency and severity of angiosis with mare age ↗ frequency of moderate to severe sclerotic vascular lesions	Grüninger et al., 1998
	2-31 yo	A total of 13 mares	Uterine examination after death	Thoroughbred	↗ degree of internal elastic lamina thickening with mare age Atrophy and reduction in the number of medial smooth muscle cells in old mares	Nambo et al., 1995
	Age considered as a continuous variable	A total of 71 mares	Uterine examination after death	Thoroughbred, Quarter Horse, and Arabian horses	↗ vascular grade with age No difference in vascular degenerations with ↗ mare age	Esteller-Vico et al., 2012
<b>Uterine endometrial degenerative changes</b>	Not defined	A total of 700 mares	One endometrial biopsy/mare	Standardbred, Thoroughbreds and Pleasure breeds	↗ endometrial fibrosis with age	Doig et al., 1981
	4-11 yo 12-24 yo	104 88	One endometrial biopsy/mare	European warmblood, Thoroughbred, light draft, Standardbred and Arabian	↗ fibrotic nests, fibrosis of individual gland branches, cystic glandular degeneration and mononuclear cell infiltration of stratum compactum in older than 12 yo	Waelchli 1990
	Young (5-7 yo) <sup>α</sup> Old (≥ 15 yo) <sup>α</sup>	9 22	One endometrial biopsy/mare	Pony mares	↗ endometrial biopsy fibrotic changes for old mares ↘ score for gland density for old mares	Carnevale and Ginther 1992
	Young & Normal (2-7 yo)  Old & Subfertile (17-24 yo + multiparous, no foaling in the 2 previous years)	19  16	One endometrial biopsy/mare	Light horse	↗ severity of endometrial histologic changes in old and subfertile mares	Brinsko et al., 1994
	Age group 1 (2-8 yo) Age group 2 (9-16 yo) Age group 3 (17-27 yo)	9 22 26	One diestrus endometrial biopsy/mare	Mixed breeds	↗ Age related to a poorer biopsy score	Woodward et al., 2012
	Age considered as a continuous variable	A total of 71 mares	Uterine examination after death	Thoroughbred, Quarter Horse, and Arabian horses	↗ endometrial grade with age Age = best predictor of endometrial grade	Esteller-Vico et al., 2012
	Young (4-6 yo) Adult (8-12 yo) Old (15-18 yo)	7 7 7	One ultrasound examination/day/mare from Day 12 to Day 20 of gestation	Mixed breed	More important uterine vascular changes throughout the study period in young and old compared to adult group Similar perfusion of both uterine horns in old mares while in young and adult groups, more often ↘ perfusion in the uterine horn without compared to the one with the embryo	Ferreira et al., 2015
	Young (2-9 yo) included 6 mares of 2 yo Old (10-20 yo)	17 10	One endometrial biopsy/mare	Light horse	No difference in biopsy score, distribution and gene expression of oxytocin receptors	Annandale et al., 2018
	3-8 yo 12-19 yo	4 7	One endometrial biopsy/mare in estrus and diestrus	Draft and riding breeds	No difference in the height of the lining nor the glandular epithelium ↗ diameter of endometrial glands in the stratum spongiosum in estrus and diestrus in old mares ↘ density of endometrial glands in the stratum compactum in estrus and diestrus in old mares ↘ density of endometrial glands in the stratum spongiosum in diestrus only in old mares	Dubrovskaya et al., 2019
	<b>Endometritis</b>	Age considered as a continuous variable (from 1.5 to ≥ 20 yo)	A total of 93 mares	One ultrasound examination/mare	Mixed breeds	Mares with uterine fluid collection older (mean of 12.2 yo) than mares without (mean of 7.5 yo)
Young (5-7 yo) <sup>α</sup> Old (≥ 15 yo) <sup>α</sup>		9 22	One endometrial biopsy and ultrasound estrus monitoring/mare	Pony mares	↗ endometrial biopsy score for cellular infiltration for old mares ↗ ultrasonically detected intrauterine fluid collections for old mares	Carnevale and Ginther 1992

					↘ uterine contractility at 13 days post ovulation in old mares	
	3-9 yo 10-16 yo >16 yo	111 126 56	Monitoring of mares in a commercial artificial insemination center	Warmblood	↗ endometritis with mare age	Barbacini et al., 1999
	Age group 1 (2-8 yo) Age group 2 (9-16 yo) Age group 3 (17-27 yo)	9 22 26	One diestrus endometrial biopsy/mare	Mixed breeds	↗ incidence of uterine fluid at 48 and 96h post insemination in mares older than 9 yo	Woodward et al., 2012
	2-5 yo 11-16 yo 18-29 yo	A total of 12 mares	One uterine infusion and collection/mare	Not defined	↘ speed of uterine clearance in older mares Positive correlation between mare age and bacterial concentration or total number of bacteria Positive correlation between mare age and white blood cell concentration and total number ↘ physical clearance in older mares	Evans et al., 1987
	Young (5-6 yo) <sup>α</sup> Old (15-20 yo) <sup>α</sup>	7 6	One beginning of gestation/mare	Mixed breed	More often ↘ uterine contractility in old group during the first 20 days of gestation	Camargo Ferreira et al., 2019
	Age considered as a continuous variable (from 3 to 21 yo)	14 30	Scintigraphy during estrous prior ovulation/mare	Thoroughbred, Quarter Horse, Standardbred, Arabian and Saddlebred	↗ incidence of delayed uterine clearance of intrauterine fluids after mating in mares older than 14 yo	Leblanc et al., 1998
	Young (5-8 yo) Old (9-14 yo)	9 11	One uterine fluid collection on Day 2, 3 or 4 during estrus/mare	Warmblood and Standardbred	No difference in the concentration of total protein concentrations and albumin ↘ concentration of not identified proteins (molecular weight from 60-71 kDa) in old mare uterine fluid	Tunón et al., 1998
	Age considered as a continuous variable (from 3 to >20 yo)	A total of 9020 mares	One vulva measurement during estrus/mare	Thoroughbred, Standardbred and Arabian	↗ length of the vulva with age	Pascoe 1979
	<10 yo >10 yo	15 10	One endometrial biopsy/mare	Standardbred	↘ gene expression of <i>Defensin Beta 4B (DEFB4B)</i> , <i>Matrix Metalloproteinase 9 (MMP9)</i> and <i>Tumor Necrosis Factor (TNFA)</i> in old mare uterine biopsy ↗ Interleukin-6 (IL6) and Collagen Type III Alpha 1 Chain (COL3A1) in old mare uterine biopsy No difference in fibrosis and inflammation distribution in uterine biopsy	Crociati et al., 2019
	2-5yo 6-10 yo 11-15 yo 16-23 yo	17 23 22 20	One <i>in vitro</i> culture of endometrial biopsy/mare	Warmblood	Without endometritis no difference of PGE <sub>2</sub> and 6-keto-PGF <sub>1α</sub> in supernatants Without endometritis, ↘ leukotriene B4 and C4 concentration in supernatant with mare age ↗ concentration of PGE <sub>2</sub> in supernatants when biopsy from group 16-23 yo with endometritis compared to 2-5 and 11-15 yo groups with endometritis ↗ 6-keto-PGF <sub>1α</sub> in supernatants when biopsy from group 2-5 yo with endometritis compared to groups with endometritis compared to 6-10 yo group with endometritis ↘ leukotriene B4 in supernatants when biopsy from group 6-10 yo with endometritis compared to groups with endometritis compared to 16-23 yo group with endometritis ↗ leukotriene C4 in supernatants when biopsy from group 16-23 yo with endometritis compared to groups with endometritis compared to 6-10 and 16-23 yo groups with endometritis	Siemieniuch et al., 2017
Recipients for embryo transfer	2-9 yo <sup>α</sup> 10-18 yo <sup>α</sup>	412 120	A total of 638 embryos transferred into recipients	Not defined	No difference in pregnancy rates at 12- and 50-days post ovulation ↗ Embryo death in older recipients	Carnevale et al., 2000
	Not defined	A total of 261 mares	Not defined	Not defined	No difference in the likelihood of pregnancy nor embryonic loss	Claes et al., 2019
	Young (2-9 yo) <sup>α</sup> Old (≥ 15 yo) <sup>α</sup>	10 10	10 embryos transferred 10 embryos transferred	Pony	No difference in the mean morphology grade and no. of cells for embryos	Carnevale et al., 1993
<b>Effect of parity</b>						
Uterine cysts and vascularization	Mares continuously bred from 4 yo (from 5 to 23 years old)	A total of 87 mares	One videoendoscopic measurement/mare	Thoroughbred	↗ number of cysts with parity	Bracher et al., 1992
	Maiden 1-2 3-5 >5	32 33 28 19	One endometrial biopsy/mare	Thoroughbred Standardbred Ponies Draught	↗ frequency and severity of angiosis with mare parity Unaltered vessels only and no severe vascular changes in maiden mares ↘ number of clearly defined membrana elastica interna with parity	Grüninger et al., 1998

	Nulliparous Multiparous	29 49	Uterine examination after death	Thoroughbred, Quarter Horse, and Arabian horses	↗ vascular grade with parity Parity = best predictor of vascular grade ↗ vascular degenerations (mostly elastosis in larger vessels of myometrium) with ↗ number of foals	Esteller-Vico et al., 2012
<b>Uterine endometrial degenerative changes</b>	Maiden Multiparous (1-2)	7 7	Over a 3-year period, videoendoscopy and endometrial biopsy/mare	Swedish Warmblood and Standardbred	No endometritis in studied mares No difference in the morphology (i.e. epithelial status, presence of foreign cells, secretory status of the glands, fibrosis of cystic glands) of the endometrium No difference in the ultrastructure of the endometrium	Tunón et al., 1995
	Nulliparous Multiparous	29 49	Uterine examination after death	Thoroughbred, Quarter Horse, and Arabian horses	↗ endometrial grade with parity	Esteller-Vico et al., 2012
<b>Endometritis</b>	Maiden Multiparous	167 409	Monitoring of mares in a commercial artificial insemination center	Warmblood	No difference in endometritis prevalence	Barbacini et al., 1999
	Nulliparous Multiparous	14 30	Scintigraphy during estrous prior ovulation/mare	Thoroughbred, Quarter Horse, Standardbred, Arabian and Saddlebred	↗ incidence of delayed uterine clearance of intrauterine fluids after mating in mares older than 14 yo ↗ slope of uterine-cervical junction in multiparous	Leblanc et al., 1998
	Maiden Multiparous	10 10	One uterine fluid collection on Day 2, 3 or 4 during estrus/mare	Warmblood and Standardbred	No difference in the concentration of total protein concentrations and albumin No difference of any of the uterine proteins recorded	Tunón et al., 1998
<b>Recipients for embryo transfer</b>	Nulliparous Multiparous	A total of 164 mares	175 embryo transfers 176 embryo transfers	Standardbred and Haflinger	No difference in pregnancy on 14 and 40 days, foal born, the overall pregnancy loss, before 40 days after or 40 days rates	Panzani et al., 2016
	Maiden Multiparous	A total of 261 mares	62 embryo transfers 199 embryo transfers	Not defined	No difference in the likelihood of pregnancy nor embryonic loss	Claes et al., 2019
<b>Effect of age and parity</b>						
<b>Uterine cysts and vascularization</b>	Young (6-10 yo) nulliparous Old (12-13 yo) multiparous	4 2	One ultrasound examination/mare	Standardbred	↗ vascular resistance in the 2 old multiparous mares	Bollwein et al., 1998
	Young (2-12 yo) maiden Old (13-19 yo) maiden	29 6	One endometrial biopsy/mare	Thoroughbred Standardbred Ponies Draught	↗ frequency of mild degeneration of the membrana elastica interna, perifibrosis or perifibroelastosis in older maiden mares	Grüniger et al., 1998
	26-31 yo multiparous	3		Thoroughbred	Markedly thickened walls of arteries in the uterine wall Marked elastosis in the uterine wall arteries due to concentric multiplication of the layers of the internal elastic lamina Elastic fibers completely digested by elastase Atrophy and reduction in the number of medial smooth muscle cells	Oikawa et al., 1993
<b>Uterine endometrial degenerative changes</b>	Age considered as a continuous variable (from 2 to 25 years old) And parity not defined	A total of 3,804 mares	One endometrial biopsy/mare	Thoroughbred, part bred, Arabian, pony, heavy horse and Standardbred	↗ Chronic infiltrative endometritis and degenerative endometrial diseases with mare age and parity Histopathologic degree of chronic degenerative endometrial disease more correlated with age than mare parity Aged maiden mares with signs of advanced chronic degenerative endometrial diseases	Ricketts and Alonso, 1991
<b>Endometritis</b>	Group 1, Nulliparous 3-8 yo Group 2, Multiparous 15-20 yo	4 4	Scintigraphy during several time point in further estrus cycle/mare	Not defined	↗ speed of clearance in multiparous Poorer cervical dilation in nulliparous	Leblanc et al., 1994

Yo: years old

α: age determined based on dental characteristics

Exp: experience

Nulliparous: mares that did not previously foal, also known as maiden mares

Multiparous: mares that already foaled at least once (included lactating mares at the time of the study) \* number of foalings indicated when knew

Table 4: Summary of literature reports showing effects of mare's age and parity on gestational length. The age and parity are calculated at foaling (parity 1 = first foal for the mare)

	Categories	Number of animals	Breed	Results	Source
<b>Gestation longer in older mares</b>	4-7 years old 8-12 years old 13-17 years old	36 198 105	Carthusian Spanish bred	13-17 years old longer than 8-12 years old	Satué et al., (2011)
	Age considered as a continuous variable (from 2 to >20 years old)	Total = 355 150	Thoroughbred, Arabian, Anglo-Arabian, French Saddle, French Trotter, French draft horses	↗ with age	Langlois and Blouin, (2012)
	2-9 years old 10-13 years old 14-16 years old ≥17 years old	Total = 13 531 Detail not specified for groups	American Standardbred, Finnhorse	≥ 17 years old longer than 2-9 years old	Reilas et al., (2014)
	2-years groups from 2 to >19 years of age	Total = 10 294	Hanoverian	↗ with age	Christmann et al., (2017)
	≤6 years old 7-9 years old 10-12 years old 13-15 years old ≥16 years old	4606 4843 3455 1992 1330	Thoroughbred	↗ with age	Ewert et al., (2018)
	Age considered as a continuous variable (from 2 to >20 years old)	Total = 1 027	Lusitano	↗ with age	Rodrigues et al., (2020)
	<b>No difference</b>	3-7 years old 8-14 years old ≥15 years old	16 35 19	Criollo	No difference
Age considered as a continuous variable (from 3 to >24 years old)		Total = 433	Thoroughbred	No difference	Davies Morel et al., (2002)
4 years old 5-17 years old >17 years old		Total = 502	Freisian	No difference	Sevinga et al., (2004)
6-9 years old 10-13 years old 14-23 years old		15 15 16	Lipizzaner	No difference	Heidler et al., (2004)
Age considered as a continuous variable (from 3 to 19 years old)		Total = 209	Draft horses (crossbreds)	No difference	Aoki et al., (2013)
Each age is considered as a group (from 3 to 17 years old)		Total = 680	Furioso - north star, Hungarian cold-blood and sport horse, Kisberi, Lipizzaner, Nonius, Shagya	No difference	Bene et al., (2014)
4-8 years old 9-12 years old ≥ 13 years old		15 15 10	Brazilian sport horse, Mangalarga paulista	No difference	Meirelles et al., (2017)
4-6 years old 7-12 years old 13-23 years old		Total = 649	Old kladruber	No difference	Malinska et al., (2019)
2-years groups from 4 to 23 years of age		Total = 49	Haflinger	No difference	Popova (2020)
<10 years old 10-16 years old >16 years old		68 18 10	Draft mares inseminated with Martina Franca donkey	No difference	Caluccio et al., (2020)
Age considered as a continuous variable		Total = 138	Thoroughbred	No difference	Milojevic et al., (2020)
7-8 years old 16-21 years old		6 6	Thoroughbred	No difference	Ousey et al., (2012)
<b>Gestation shorter in older mares</b>		3 years old 4-8 years old 9-12 years old >12 years old	116 337 124 63	Brandenburg	4-8 years old shorter than 3 years old
	3 years old (Shetland) ≥4 years old (all breeds)	286 2041	Fjord, Haflinger, Draft horse, Shetland, Freisian	>3 years old shorter	Bos and Van der Mey, (1980)
	<4 years old 4-14 years old (each as a group)	Total = 8439	Andalousian, Arabian	↘ with age	Valera et al., (2006)



	>14 years old				
<b>Effect of parity</b>					
<b>Gestation longer in primiparous</b>	Primiparous Multiparous (?)	6 6	Quarter horse (Full siblings)	↗ in primiparous	Pool-Anderson et al., (1994)
	Parity considered as a continuous variable	Total = 8439	Andalousian, Arabian	↘ until 4/5 birth and ↗ thereafter	Valera et al., (2006)
	Primiparous Multiparous (?)	164 173	Carthusian Spanish bred	↗ in primiparous	Satué et al., (2011)
	Primiparous Multiparous 2-5 Multiparous 6-13	Total = 13 531 Detail not specified for groups	American Standardbred, Finnhorse	↘ with parity increase	Reilas et al., (2014)
	Primiparous Multiparous (?)	Total =126 Detail not specified for groups	Marwari	↗ in primiparous	Talluri et al., (2016)
<b>No difference</b>	Parity considered as a continuous variable	Total = 209	Draft horses (crossbreds)	No difference	Aoki et al., (2013)
	Primiparous Multiparous 2-4 Multiparous ≥5	12 18 10	Brazilian sport horse, Mangalarga Paulista	No difference	Meirelles et al., (2017)
	Primiparous – Multiparous 2 Multiparous 3-5 Multiparous ≥6	5996 6024 4206	Thoroughbred	No difference	Ewert et al., (2018)
<b>Gestation shorter in primiparous</b>	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ in primiparous	Robles et al., (2018)

Table 5: Summary of literature reports showing effects of mare's age and parity on gestation parameters

	Categories	Sample size	Breed	Results	Source
<b>Effect of age</b>					
<b>Early fetoplacental development</b>	4-8 years old >8 years old	11 5	Brandenburg	<ul style="list-style-type: none"> <li>↘ conceptus size at 30-45 dpo in &gt;8 years old</li> <li>↗ eCG plasma concentration at 50-100 dpo in &gt;8 years old</li> </ul>	Willmann et al. (2011)
	Between 2 and >10 years old (age estimated with teeth growth)	Total = 62	Welsh, Shetland	No difference in eCG serum concentration	Day and Rowlands, (1946)
	<15 years old ≥15 years old	26 4	Dutch Warmblood	↘ fetal aorta diameter in ≥15 years old from 100 dpo* to foaling	Hendriks et al., (2009)
	3-7 years old 8-11 years old 12-16 years old	16 17 18	Unspecified Warmblood	↗ combined thickness of uterus and placenta in >7 years old	Klewitz et al., (2015)
<b>Maternal metabolism</b>	3.5-4 years old 8-19 years old	6 7	Croatian cold-blood	<ul style="list-style-type: none"> <li>↗ parathyroid hormone</li> <li>↘ osteocalcin serum concentration 50-70 days before foaling in 8-19 years old</li> </ul>	Filipović et al., (2014)
<b>Uterine vascularization</b>	7-8 years old 16-21 years old	6 6	Thoroughbred	<ul style="list-style-type: none"> <li>↘ uterine artery doppler indices (peak systolic velocity and total blood flow volume) with age</li> <li>↘ total blood flow volume in 16-21 years old</li> </ul>	Ousey et al., (2012)
	3-7 years old 8-11 years old 12-16 years old	16 17 18	Unspecified Warmblood	<ul style="list-style-type: none"> <li>↗ diameter of both uterine arteries in &gt;7 years old</li> <li>↗ uterine artery pulsatility index in &gt;7 years old</li> </ul>	Klewitz et al., (2015)
	<b>Effect of parity</b>				
<b>Early fetoplacental development</b>	Unknown at the start of the study	Total = 62	Welsh, Shetland	↘ eCG serum concentration with succeeding pregnancies	Day and Rowlands, (1946)
	Primiparous Multiparous 2 Multiparous 3	9 9 9	Thoroughbred	No difference in eCG serum concentration Earlier eCG peak as parity ↗	Wilsher and Allen, (2011)
	Primiparous Multiparous (?)	7 23	Dutch Warmblood	<ul style="list-style-type: none"> <li>↗ ventral combined thickness of uterus and placenta between 250 dpo and foaling in primiparous</li> <li>↘ fetal eye diameter in primiparous until 200 dpo</li> <li>↗ fetal eye diameter in primiparous after 200 dpo</li> <li>↗ fetal biparietal diameter in primiparous after 200 dpo</li> </ul>	Hendriks et al., (2009)
<b>Maternal metabolism</b>	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	<ul style="list-style-type: none"> <li>↘ whole blood basal glucose concentration at 150 dpo in primiparous</li> <li>↗ FSIVGTT** disposition index + ↗ glycaemia and insulinemia after meals in primiparous = ↘ ability to metabolically adapt to gestation</li> <li>↗ amino acid maternal plasma concentration in primiparous at foaling</li> </ul>	Robles <i>et al.</i> , (2019)
<b>Uterine vascularization</b>	Primiparous – Multiparous 3 Multiparous 4-5 Multiparous 6-9	7 23	Dutch Warmblood	<ul style="list-style-type: none"> <li>↗ diameter of uterine artery as parity ↗</li> <li>↘ uterine artery total blood flow in primiparous-multiparous 3 compared to multiparous &gt;3</li> <li>↗ pulsatility index as parity ↗ only in first trimester</li> </ul>	Klewitz <i>et al.</i> , (2015)

\*Dpo = day post ovulation, \*\*FSIVGTT= frequently sampled intravenous glucose tolerance test

Table 6: Summary of literature reports showing effects of mare's age and parity on the placenta.

	Categories	Sample size	Breed	Results	Source
<b>Effect of age</b>					
<b>Term placental measurements</b>	5-9 years old 10-15 years old ≥16 years old	41 10 9	Thoroughbred	No difference in placental weight, surface and volume ↗ placental efficiency in ≥16 compared to 5-9 years old	Wilsher and Allen, (2003)
	4-6 years old 7-9 years old 10-12 years old >12 years old	79 110 84 75	Thoroughbred	Placental weight ↗ with age Effect was lost when parity was considered in the model	Elliott et al., (2009)
	7-8 years old 16-21 years old	6 6	Thoroughbred	No difference in placental weight, surface and volume No difference in placental efficiency	Ousey et al., (2012)
	4-8 years old 9-12 years old ≥ 13 years old	15 15 10	Brazilian sport horse, Mangalarga paulista	No difference in placental weight and volume No difference in placental efficiency	Meirelles et al., (2017)
<b>Placental structure</b>	5-9 years old 10-15 years old ≥16 years old	41 10 9	Thoroughbred	Microcotyledon surface density ↘ with age No difference in microcotyledon surface	Wilsher and Allen, (2003)
	7-8 years old 16-21 years old	6 6	Thoroughbred	↘ microcotyledon surface density in 16-21 years old No difference in microcotyledon surface	Ousey et al., (2012)
	4-8 years old 9-12 years old ≥ 13 years old	15 15 10	Brazilian sport horse, Mangalarga paulista	↗ microcotyledon surface density in pregnant horn in ≥ 13 years old ↗ microcotyledon volume in placental body in ≥ 9 years old ↗ allantoic vessel volume and microcotyledon height in placental body in ≥ 13 years old	Meirelles et al., (2017)
	4-12 years fertile 10-22 years subfertile	4 6	Thoroughbred, unspecified pony	↘ density of microcotyledons	Bracher et al., (1996)
<b>Effect of parity</b>					
<b>Term placental measurements</b>	Primiparous Multiparous (?)	24 60	Thoroughbred	↘ placental weight, surface and volume in primiparous ↗ placental efficiency in primiparous	Wilsher and Allen, (2003)
	Primiparous Multiparous 2 Subsequent pregnancies	11 11	Thoroughbred	No difference in placental weight, surface and volume ↗ placental efficiency in primiparous	Wilsher and Allen, (2003)
	Primiparous Multiparous 2-4 Multiparous 5-7 Multiparous >7	74 143 82 49	Thoroughbred	Placental weight ↗ with parity Effect remained significant when age was considered in the model	Elliott et al., (2009)
	Primiparous Multiparous 2-4 Multiparous ≥5	12 18 10	Brazilian sport horse, Mangalarga paulista	↘ placental weight in primiparous compared to multiparous ≥5 ↘ placental efficiency in primiparous	Meirelles et al., (2017)
	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ placental weight and volume in primiparous ↘ placental efficiency in primiparous	Robles et al., (2018)
	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	No difference in placental weight, surface and volume	Robles et al., (2019)
<b>Placental structure</b>	Primiparous Multiparous (?)	24 60	Thoroughbred	↘ microcotyledon surface density in primiparous compared to multiparous aged 5-9 years old ↘ microcotyledon surface and density in primiparous compared to multiparous aged 5-15 years old	Wilsher and Allen, (2003)
	Primiparous Multiparous 2 Subsequent pregnancies	11 11	Thoroughbred	↘ microcotyledon surface density and surface in primiparous	Wilsher and Allen, (2003)
	Primiparous Multiparous 2-4 Multiparous ≥5	12 18 10	Brazilian sport horse, Mangalarga paulista	↘ microcotyledon surface density and volume in placental body in primiparous ↘ haemotrophic trophoblast volume in non-pregnant horn in primiparous ↘ connective tissue volume in placental body in primiparous ↘ microcotyledon width in placental pregnant, non-pregnant horns and body in primiparous	Meirelles et al., (2017)
	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ volume of both allantoic and microcotyledonary connective tissue s in primiparous ↘ surface of allantoic connective tissue in primiparous ↘ surface of histotrophic trophoblast and microcotyledonary vessels in primiparous	Robles et al., (2018)

	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	↘ surface of allantoic connective tissue in primiparous	Robles et al., (2019)
<b>Placental gene expression</b>	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	↘ gene expression of <i>VEGF</i> in primiparous ↗ gene expression of <i>ODC1</i> , <i>SLC7A1</i> , <i>ARG2</i> in primiparous	Robles et al., (2019)
<b>Placental methylation</b>	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	↘ total DNA methylation in primiparous	Robles et al., (2019)

Surface density = surface of microcotyledon calculated within a unit reference volume (expressed in  $\mu\text{m}^{-1}$ )

Surface = surface density x placental volume (total microcotyledon surface, expressed in  $\text{cm}^2$ )

Placental efficiency = foal birthweight/placental surface

*VEGF* = Vascular endothelial growth factor (role = vascularization)

*ODC1* = Ornithine decarboxylase (role = catalyses L-ornithine into putrescine)

*SLC7A1* = Solute Carrier Family 7 Member 1 (role = cell transport of lysine and arginine)

*ARG2* = Arginase 2 (role = catalyses L-arginine into L-ornithine)

Table 7: Summary of literature reports showing effects of mare's age and parity on the foal

	Categories	Sample size	Breed	Results	Source
<b>Effect of age</b>					
<b>Foal birth weight</b>	Age considered as a quantitative variable	Total = 1 992	Thoroughbred	↗ with age	Hintz et al., (1979)
	4-6 years old 7-9 years old 10-12 years old >12 years old	79 110 84 75	Thoroughbred	↗ with age Effect was lost when parity was considered in the model	Elliott et al., (2009)
	Age considered as continuous variable	Total = 38	Brazilian sport horse, Mangalarga paulista	↗ with age	Fernandes et al., (2014)
	4-8 years old 9-12 years old ≥ 13 years old	15 15 10	Brazilian sport horse, Mangalarga paulista	↗ in ≥ 9 years old compared to younger mares	Meirelles et al., (2017)
	4-6 years old 7-12 years old 13-23 years old	Total = 649	Old kladruber	↗ in ≥ 9 years old compared to younger mares	Malinska et al., (2019)
	5-9 years old 10-15 years old ≥16 years old	41 10 9	Thoroughbred	↗ in 10-15 compared to 5-9 years old	Wilsher and Allen, (2003)
	3-7 years old 8-11 years old 12-16 years old	16 17 18	Unspecified Warmblood	↗ in 8-11 compared to 3-7 and 12-16 years old	Klewitz et al., (2015)
<b>Foal growth and measurements</b>	Age considered as a quantitative variable	Total = 1 992	Thoroughbred	↗ weight, size, cannon width and thoracic perimeter in 7-11 years old compared to younger mares	Hintz et al., (1979)
	4-8 years old 9-12 years old ≥13 years old	15 15 10	Brazilian sport horse, Mangalarga paulista	↗ height until 6 months of age in ≥ 13 compared to 4-8 years old	Meirelles et al., (2017)
	2-5 years old 6 to 12 years old as individual groups 13-15 years old 16-19 years old	230 2 073 291 294	Thoroughbred	No effect of age on adult horses' measurements	Paksoy and Ünal, (2019)
	Age considered as a quantitative variable	Total = 990	Thoroughbred	↗ risk of foal mortality with maternal age before and after 15 days of age ↗ risk of foal morbidity with maternal age before 15 days of age but not after	Morley and Townsend, (1997)
<b>Risk of disease</b>	5-6 years old 7 years old 8-9 years old 10-11 years old ≥12 years old	9 13 13 10 11	Thoroughbred Flat race	Risk of fracture ↘ with ↗ age	Verheyen et al., (2007)
	Age considered as a quantitative variable	Total = 562	Thoroughbred Flat race	No effect on flat race performance	Wilsher et al., (2006)
<b>Offspring performance</b>	3-4 years old 5-10 years old ≥11 years old	10 230 87 191 110 563	Show jumping horses competing in France (various breeds)	↗ show jumping performance in 3-4 years old group (within primiparous) ↗ performance in ≥11 compared to 5-10 years old (within multiparous)	Palmer et al., (2018)
	2-5 years old 6 to 12 years old as individual groups 13-15 years old 16-19 years old	230 2073 291 294	Thoroughbred Flat race	No effect of age on flat race performance, earnings and health problems	Paksoy and Ünal, (2019)

	9-12 years old 13-16 years old 17-20 years old ≥21 years old	1 814 2 045 1 266 1 078	Arabian Flatrace	No effect on racing time ↗ earnings in 9-12 and ≥21 years old compared to other groups	Köseman and Özbeyaz, (2009)
	Age considered as continuous variable	Total = 875	Thoroughbred Flat race	Flat race performance starts to decline when mares are aged 11 years old and more	Barron, (1995)
<b>Effect of parity</b>					
<b>Foal birth weight</b>	Primiparous Multiparous (?)	7 8	French Anglo-Arabian	↘ in primiparous	Doreau et al., (1991)
	Primiparous Multiparous (?)	Total = 12	Quarter horse	↘ in primiparous	Lawrence et al., (1992)
	Primiparous Multiparous (?)	6 6	Quarter horse (Full siblings)	↘ in primiparous	Pool-Anderson et al., (1994)
	Primiparous Multiparous (min 3)	16 20	Belgian-cross	↘ in primiparous	Cymbaluk and Laarveld, (1996)
	Primiparous Multiparous (?)	24 60	Thoroughbred	↘ in primiparous	Wilsher and Allen, (2003)
	Primiparous Multiparous 2 Subsequent pregnancies	11 11	Thoroughbred	↘ in primiparous	Wilsher and Allen, (2003)
	Primiparous Multiparous 2-4 Multiparous 5-7 Multiparous >7	74 143 82 49	Thoroughbred	↗ with parity Effect remained significant when age was considered in the model	Elliott et al., (2009)
	Primiparous Multiparous (?)	7 23	Dutch Warmblood	↘ in primiparous	Hendriks et al., (2009)
	Primiparous Multiparous	Total = 38	Brazilian sport horse, Mangalarga paulista	↘ in primiparous	Fernandes et al., (2014)
	Primiparous – Multiparous 3 Multiparous 4-5 Multiparous 6-9	7 23	Dutch Warmblood	↘ in primiparous	Klewitz et al., (2015)
	Primiparous Multiparous 2-4 Multiparous ≥5	14 15 9	Brazilian sport horse, Mangalarga paulista	↘ in primiparous	Affonso et al., (2016)
	Primiparous Multiparous (?)	Total =126 Detail not specified for groups	Marwari	↘ in primiparous	Talluri et al., (2016)
	Primiparous Multiparous 2-4 Multiparous ≥5	12 18 10	Brazilian sport horse, Mangalarga paulista	↘ in primiparous	Meirelles et al., (2017)
	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ in primiparous	Robles et al. (2018)
	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	↘ in primiparous	Robles et al., (2019)
<b>Foal growth and measurements</b>	Primiparous Multiparous (?)	6 6	Quarter horse (Full siblings)	↘ weight of foals until 29 days of age ↗ average daily gain from 90 days of age	Pool-Anderson et al., (1994)
	Primiparous Multiparous (min 3)	16 20	Belgian-cross	↘ weight and average daily gain until 17 days of age in primiparous	Cymbaluk and Laarveld, (1996)
	Age considered as continuous variable	Total = 38	Brazilian sport horse, Mangalarga paulista	↘ weight, height, cannon width and thoracic perimeter between 7 and 30 days of age in primiparous	Fernandes et al., (2014)

	Primiparous Multiparous 2-4 Multiparous ≥5	12 18 10	Brazilian sport horse, Mangalarga paulista	↘ height in primiparous until 360 days of age	Meirelles et al., (2017)
	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ foal weight until 360 days of age in primiparous ↘ foal height until 540 days of age ↘ thoracic perimeter until 540 days of age	Robles et al., (2018)
	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	No effect until 360 days of age	Robles et al., (2019)
<b>Foal metabolism and development</b>	Primiparous Multiparous (min 3)	16 20	Belgian-cross	↘ IGF1 serum concentration until 17 days of age in primiparous	Cymbaluk and Laarveld, (1996)
	Primiparous Multiparous 2-5	12 20	French Anglo-Arabian	↘ glucose area under curve after an IVGTT at 120 days of age in primiparous (=↗ insulin sensitivity-metabolic immaturity) No difference in thyroid hormones ↘ testicular maturity at 360 days of age in primiparous	Robles et al., (2018)
<b>Risk of disease</b>	Primiparous Multiparous 2 – 6 as individual groups Multiparous ≥7	5 45 6	Thoroughbred Flat race	↘ risk of fracture in primiparous	Verheyen et al., (2007)
<b>Offspring performance</b>	Parity considered as continuous variable	Total = 875	Thoroughbred Flat race	Flat race performance increases until the 5 <sup>th</sup> parity and declines after the 10 <sup>th</sup> parity	Barron, (1995)
	Parity considered as continuous variable	Total = 562	Thoroughbred Flat race	No effect on flat race performance	Wilsher et al., (2006)
	Primiparous Multiparous 2-5 Multiparous ≥6	5 5627 110 981 41 376	Show jumping horses competing in France (various breeds)	↘ show jumping performances in primiparous	Palmer et al., (2018)

IGF1=Insulin-like growth factor 1

Table 8: Summary of literature reports showing effects of mare's age and parity on post-partum complications

	Categories	Sample size	Breed	Results	Source
<b>Effect of age</b>					
<b>Retention of fetal membrane (RFM)</b>	4yo 5-17yo ≥18yo	46 56 40	Friesian	↗ in 4yo and ≥18yo	(Sevinga et al., 2004)
	3-23yo, continuous variable	121	69 Warmblood 21 Thoroughbred 12 Coldblood 10 Quarter Horse 11 Pony	No difference	(Schürmann et al., 2019)
	<6yo 6-10yo 11-15yo ≥16yo	175 687 366 128	Standardbred	No difference	(Provencher et al., 1988)
<b>Post-partum fever</b>	<10yo ≥10yo	91 67	125 Crossbred 33 Percheron	No difference	(Aoki et al., 2014)
<b>Dystocia</b>	3-29yo, continuous variable	1,047	35% Thoroughbred 41% Quarter Horse 24% other/unknown	No difference in stage II labor duration → no difference in terms of dystocia	(McCue and Ferris, 2012)
	4-7yo 8-11yo ≥12yo	Not defined	49 Hot-blood 4 Warmblood 8 Draft horse 4 Miniature	No difference	(Rioja et al., 2012)
	3yo 4yo 5-9yo ≥10yo	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 3yo Trend of ↗ at 4yo compared to 5-9yo No difference between 5-9yo and ≥10yo	(Sabbagh et al., 2014)
<b>Foal mortality</b>	3yo 4yo 5-9yo ≥10yo	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 3yo Trend of ↗ at 4yo compared to 5-9yo No difference between 5-9yo and ≥10yo	(Sabbagh et al., 2014)
	3yo 4yo ≥5yo	85 77 253	Belgian-cross	↗ at 3yo No difference if taking in account mare level of interest toward her foal	(Haas et al., 1996)
<b>Maternal behavior</b>	3 yo 5yo	6 4	Jeju pony	↗ foal aggression by herd and ↗ maternal protection at 3yo	Rho et al. 2009
<b>Effect of parity</b>					
<b>Retention of fetal membrane</b>	1 2 3 4 5 6 7 8 11 12 unkown	35 26 10 11 7 8 2 3 1 2 16	69 Warmblood 21 Thoroughbred 12 Coldblood 10 Quarter Horse 11 Pony	No difference	(Schürmann et al., 2019)



<b>Post-partum fever</b>	1 ≥2	23 112	125 Crossbred 33 Percheron	No difference	(Aoki et al., 2014)
<b>Dystocia</b>	1 2 ≥3	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 1	(Sabbagh et al., 2014)
<b>Foal mortality</b>	1 2 ≥3	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 1	(Sabbagh et al., 2014)
<b>Maternal behavior</b>	1 ≥2	101 34	Various breeds	↗ foal rejections at 1 (75% of rejectors)	(Haupt and Lieb, 1994)
		720 657	Arabian Paint	↗ foal rejections at 1 (5.13% in arabians and 1.93% in paint)	(Juarbe-Diaz et al. 1998)
		Not defined	Thoroughbred	↗ foal rejections at 1	(Chavatte, 1991)
	1 to 10	44	Standardbred	No effect of parity on maternal reaction to foal handling No effect of parity on mare-foal relationship ↘ heart rate in 6 week old foals in new object test with ↗ parity	(Søndergaard et Jago 2010)
	1 to 6 (only one primiparous)	30 mare-year	Sorraia horse	No effects on protectiveness nor conflicts over suckling ↗ foal sociability with ↗ parity	(Heitor and Vicente 2008)

Table 9: Summary of literature reports showing effects of mare's age and parity on lactation

	Categories	Sample size	Breed	Results	Source	
<b>Effect of age</b>						
<b>Passive transfer of immunity</b>	3-24yo, continuous variable	126	Standardbred	No difference	(Morris et al., 1985)	
	<10yo 10-15yo >15yo	110 83 50	140 Standardbred 95 Thoroughbred 59 Arabian	No difference on foal plasma IgG concentrations ↗ of colostrum IgG concentrations in <10yo	(LeBlanc et al., 1992)	
	3-5yo 6-8yo 9-12yo >13yo	16 21 11 12	Throughbred, Standardbred, Kaltbult, Haflinger	No difference	(Erhard et al., 2001)	
	?	8	Quarter Horse	No difference	(Kenzig et al., 2009)	
	≤6yo 6-10yo 10-15yo >15yo	?	192 Paso Fino	No difference	(Franco Ayala and Oliver-Espinosa, 2016)	
	<b>Colostrum quality</b>	≤10yo >10yo	5 7	Konik breed	↗ Palmitic acid proportion in >10yo ↗ Polyunsaturated fatty acid proportion in >10yo	(Pikul et al., 2008)
		5-10yo, studied as a continuous variable	17 colostrum samples	?	↗ Calcium concentration in >7yo Macro-mineral, protein, urea contents = for all ages	(Ramin et al., 2018)
<b>Milk quantity</b>	5yo 6-7yo	7 8	Anglo-Arabian, Selle Français	No difference	(Doreau et al., 1991)	
	5-18yo	14	Quarter Horse	Effect of age not studied	(Gibbs et al., 1982)	
<b>Milk quality</b>	3-5yo 6-10yo 11-19yo	34	Quarter Horse	Milk quality = for all age groups	(Barreto et al., 2020)	
	5yo 6-7yo	7 8	Anglo-Arabian, Selle Français	No difference	(Doreau et al., 1991)	
	5-18yo	15	Quarter Horse	Effect of age not studied	(Gibbs et al., 1982)	
	≤10yo >10yo	5 7	Konik breed	↗ Proportion of C14:1, C16:0, C16:1c9 for ≤10yo group ↘ Proportion of C20:4 for ≤10yo group	(Pikul et al., 2008)	
	5-10yo, studied as a continuous variable	39 milk samples	?	Macro-mineral, protein, urea contents = for all ages	(Ramin et al., 2018)	
<b>Effect of parity</b>						
<b>Passive transfer of immunity</b>	1-?, continuous variable	126	Standardbred	No difference	(Morris et al., 1985)	
	1 ≥2	26 317	Thoroughbred	No difference	(Raidal, 1996)	

	1 2-3 4-5 ≥6	13 18 17 12	Throughbred, Standardbred, Kaltbult, Haflinger	No difference	(Erhard et al., 2001)
	?	8	Quarter Horse	No difference	(Kenzig et al., 2009)
<b>Colostrum quality</b>	≤5 >5	7 5	Konik breed	↘ Ratio saturated fatty acid/polyunsaturated fatty acid in parity <5	(Pikul et al., 2008)
<b>Milk quantity</b>	1 ≥2	7 8	Anglo-Arabian, Selle Français	↘ Trend for milk yield for parity 1	(Doreau et al., 1991)
	1 2 ≥3	4 4 6	Quarter Horse	↗ Milk production for parity ≥3 Milk production = between parity 1 and 2	(Gibbs et al., 1982)
	1 ≥2	6 6	Quarter Horse	↗ Milk production for parity ≥2	(Pool-Anderson et al., 1994)
	1 ≥2	3 3	Arabian	No difference	(Trottier et al., 2006)
<b>Milk quality</b>	1-6, studied as continuous variable	34	Quarter Horse	↘ Lactose content with parity	(Barreto et al., 2020)
	1 ≥2	7 8	Anglo-Arabian, Selle Français	Milk solid content = for all parity groups ↘ Fat concentration for parity 1	(Doreau et al., 1991)
	1 2 ≥3	4 4 6	Quarter Horse	Milk solid content = for all parity groups Milk fat content = for all parity groups ↗ Milk protein content in parity 1 and 2	(Gibbs et al., 1982)
	<5 ≥5	7 5	Konik breed	↘ Content of middle-chain saturated fatty acids for <5 parity group ↗ Content of unsaturated fatty acids for <5 parity group	(Pikul et al., 2008)
<b>Foal suckling</b>	1 ≥2	2 5	Camargue	↗ Duration of suckling bout for parity 1	(Duncan et al., 1984)
	1 ≥2	3 3	Arabian	No difference	(Trottier et al., 2006)