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

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Running title: Effects of maternal age and parity in horses

Abstract:

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

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Introduction

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

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

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

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

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

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

Ovary and the estrus cycle (Table 1)

Follicular reserve

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

Estrus cycle and follicular waves

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

Hormonal profiles prior to ovulation

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

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

Ovulation

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

Induction of ovulation

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

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

Luteal phase

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

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

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

Follicular organization, oocytes and embryos (Table 2)

Follicular organization

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

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

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

Oocyte morphology

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

Oocyte quality

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

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

Fertilisation

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

Embryo development

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

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

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

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

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

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

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

Oviduct

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

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

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

Uterus – uterine cysts and vascularization

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

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

Uterus – endometrial degenerative changes

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

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

Endometritis

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

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

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

Effects of age and parity of recipient for embryo transfer

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

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

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

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

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

Gestational length (Table 4)

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

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

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

Gestation (Table 5)

Early feto-placental development

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

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

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

Blood flow and metabolism

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

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

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

Placenta (Table 6)

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

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

Placentas at term are lighter and less voluminous in primiparous compared to multiparous mares (Wilsher and Allen 2003; Elliott *et al.* 2009; Meirelles *et al.* 2017; Robles *et al.* 2018) except in one study (Robles *et al.* 2019). Moreover, placentas from primiparous mares were shown to present a reduced microcotyledonary surface compared to placentas from multiparous dams (Wilsher and Allen 2003; Elliott *et al.* 2009; Meirelles *et al.* 2017). Another study, however, showed that only the microcotyledonary conjunctive tissue is reduced in placentas from young primiparous mares (Robles *et al.* 2018) and another did not show difference in microcotyledonary area according to mares' parity (Robles *et al.* 2019). The only study performed on three different placental sites (pregnant horn, non-pregnant horn and placental body) showed that the haemotrophic trophoblast surface density is reduced in the placental body of primiparous compared to multiparous mares (Meirelles *et al.* 2017). The authors also observed a decreased microcotyledonary length in the three zones of primiparous compared to multiparous placentas (Meirelles *et al.* 2017). The reduced capacity of placentas from primiparous mares might be explained by a lack of elasticity in the uterus which need to be “primed” by a first pregnancy to reach its maximum productivity and development (Wilsher and Allen 2003). Effects of maternal parity on placental efficiency (as calculated as foal weight/m² of placenta) differ according to studies: efficiency is either reduced (Wilsher and Allen 2003; Robles 2018) or increased (Meirelles *et al.* 2017) in primiparous mares.

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

Fetal and foal development (Table 7)

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

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

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

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

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

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

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

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

Post-partum complications

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

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

Maternal behavior

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

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

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

Colostrum

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

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

Milk

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

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

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

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

Foal suckling

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

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

Conclusion

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

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

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

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

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

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.

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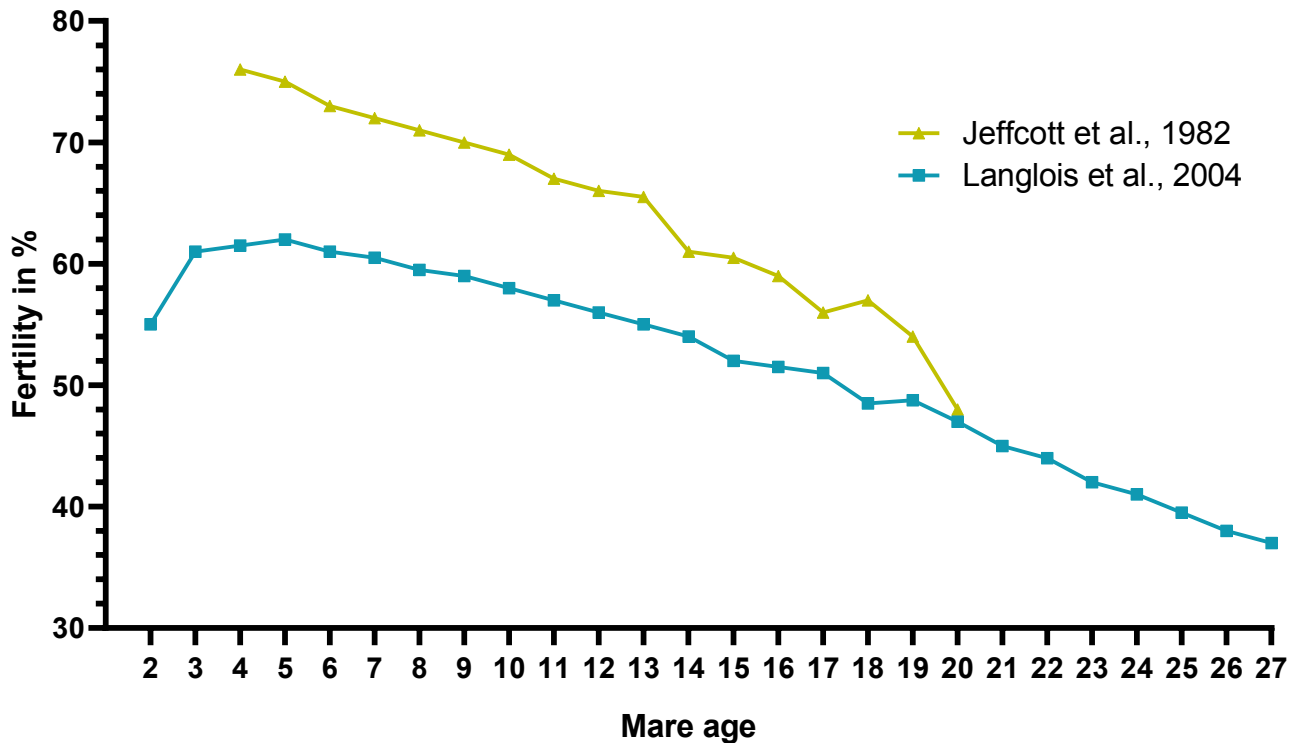
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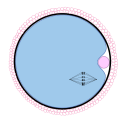






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		 PrimiparityVery high Parity (>10)Very young (<3-4)Aging (>10-12)Old (15-20)Very old (>20)				
Pre-implantation until blastocyst stage	Ovaries & folliculogenesis 	?			Delayed breeding season ↗ multiple ovulations	Hormonal dysregulation ↗ inter-ovulatory interval, ↘ pre-ovulatory follicle diameter
	Follicles & oocytes 	?			Altered gene expression in granulosa cells No difference in oocyte diameter ↘ Mt DNA copy number ↘ oocyte maturation potential ↗ aneuploidy, ↗ spindle instability ↘ follicular shape regularity ↗ IGF1, differences in miRNAs in follicular fluid Oocyte abnormalities	
	Embryo 	?			↘ diameter of embryonic vesicle by ultrasound ↘ embryo movement in the uterus ↘ Mt DNA copy number ↘ quality & development competence Delayed development*	
From blastocyst to post-natal period	Oviducts, uterus and cervix 	Aged maiden mares with signs of advanced chronic degenerative endometrial disease ↗ number of uterine cysts with parity ↗ frequency and severity of angiosis, ↗ vascular degenerations (elastosis in larger vessels of myometrium) with ↗ parity ↗ slope of uterine-cervical junction in multiparous, ↘ uterine clearance		↘ uterine vascular changes in luteal phase	↗ number of oviductal masses and uterine cysts with age ↗ frequency and severity of angiosis with mare age ↗ frequency of moderate to severe sclerotic vascular lesions Age = best predictor of endometrial grade ↗ size of uterine cysts ↘ uterine clearance and vascular changes in luteal phase and gestation Endometrial elastosis and fibrosis	
	Gestation 	↗ gestation length ? ↗ combined thickness of uterus and placenta ↘ metabolic adaptations to pregnancy	↘ fetal eCG and earlier eCG peak with ↗ parity ↗ diameter of uterine artery with ↗ parity		No clear difference in gestational length ↘ fetal size and placental development at implantation ↗ combined thickness of uterus and placenta ↗ diameter of both uterine arteries in >7 years old ↗ uterine artery pulsatility index in >7 years old	
	Placenta 	↘ placental weight and volume ↘ microcotyledonary surface and density	↗ placental weight with ↗ parity		No clear effect on weight and microcotyledonary structure	
	Post-partum complications Maternal behavior	↗ dystocia ↗ foal rejections			↗ placental retention ↗ dystocia	↗ placental retention
	Foal 	↗ dystocia ↘ birthweight and height Delayed metabolic and testicular maturation	↗ birthweight with ↗ parity		↗ foal mortality with maternal age before and after 15 days of age ↗ foal morbidity with maternal age before 15 days of age ↗ foal birthweight when excluded youngest and oldest mares	
	Lactation 	↘ fat and ↗ protein concentrations	↗ Milk production for parity ≥2 ↘ Ratio saturated/polyunsaturated fatty acid in parity <5		↗ colostrum calcium concentrations Modifications in fatty acid proportions ↘ colostrum IgG concentrations	

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

	Age categories	Sample size	Breed	Results	Source
Effect of age					
Ovarian activity	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)
Anti-Müllerian hormone concentration	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)
Breeding period and follicular activity	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)
	Exp 1: Young (5-7yo) Old (≥14yo) Exp 2: Young (3-7yo) Intermediate (17-19yo) Old (≥20yo)	12 16 16 10 9	Pony	Exp 1: ↗ Follicular activity in 5-7yo Exp 2: 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)
Hormonal control of follicular growth	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)
Ovulation	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
	Effect of age					
Follicular organization	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
Oocyte morphology	Young (2-7 yo) ^a Middle aged (8-14 yo) ^a Aged (≥ 15 yo) ^a	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
	Young (2-7 yo) ^a Middle aged (8-14 yo) ^a Aged (≥ 15 yo) ^a	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 ^a ≥ 12 yo ^a	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) ^α Old (20-26 yo) ^α	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 ^α Young (3.5-7 yo) Intermediate (15-19 yo) Old (≥20 yo) Exp 2 ^α 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 ovocytes 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) ^α Old (15-20 yo) ^α	7 6	One beginning of gestation/mare	Mixed breed	↘ embryo diameter measured by ultrasonography from Day 15 to 19 post ovulation High embryo mobility for both groups from Day 10 to 12 ↘ embryo movements earlier in old mares Similar day of fixation in the cornual-body junction for both groups	Camargo Ferreira et al., 2019
	Young (5-7 yo) ^α Old (≥ 15 yo) ^α	9 22	One beginning of gestation/mare	Pony mares	No difference in embryonic vesicle diameter measured by ultrasonography on day 12 and the day of fixation Similar embryo mobility between groups Fixation on 1.5 days later in old mares	Carnevale and Ginther 1992
	Young (6-10 yo) ^α Old (20-26 yo) ^α	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	Tendency to and ↘ oocyte-donor mean age for a positive pregnancy diagnosis by, respectively Day 17 and 50 post ovulation No difference in blastomere size, number of cells, presence of fragments of ooplasm nor extruded membrane through zona pellucida after ICSI No difference in the number of hours from ICSI until cleavage At transfer, ↗ number of cells for 20-23 yo compared to 3-13 and 14-19 yo groups ↘ time to transfer for 20-23 yo compared to 3-13 and 14-19 yo groups Earlier detection of embryonic vesicle in 14-19 yo compared to 20-23 yo group No difference in the diameter of vesicles at detection nor the embryonic vesicle diameter per day of detection	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	No difference in embryo diameter Alteration of gene expression in trophoblast and inner cell mass according to maternal age related to mitochondria, translation, transcription, chromatin modification In inner cell mass, disturbance on mitosis, signaling, adhesion and embryo development pathways In trophoblast, disturbance of ion movement related genes	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	↘ number of cells in embryos from old mares at Day 1 post ICSI No difference in the number of cells at Day 2 post ICSI	Altermatt et al., 2009
	Young (6-13 yo) Old (20-27 yo)	7 8	8 embryos 10 embryos	Light horse	↘ Basal oxygen consumption rate in Day 2 embryos from old mares No difference in basal extracellular acidification rate in Day 2 embryos No difference in cell numbers of the Day 2 embryos	Catandi et al., 2021
	4 - 11 yo 17-22 yo	A total of 10 mares	8 8	Slaughtered mares Breed not defined	↘ mtDNA copy number in old mares Day 8 old mare blastocysts No difference in the expression of 5 genes related to mitochondria	Hendriks et al., 2015

Yo: years old

^α: 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
Effect of age						
Oviducts	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 ^a (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
Uterine cysts and vascularization	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
Uterine endometrial degenerative changes	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) ^α Old (≥ 15 yo) ^α	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 gladular 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
Endometritis	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)	Adams et al., 1987
	Young (5-7 yo) ^α Old (≥ 15 yo) ^α	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) ^α Old (15-20 yo) ^α	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 Metallopeptidase 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 ₂ and 6-keto-PGF _{1α} in supernatants Without endometritis, ↘ leukotriene B4 and C4 concentration in supernatant with mare age ↗ concentration of PGE ₂ 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 _{1α} 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 ^α 10-18 yo ^α	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) ^α Old (≥ 15 yo) ^α	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
	Effect of parity					
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
Uterine endometrial degenerative changes	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
Endometritis	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
Recipients for embryo transfer	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
Effect of age and parity						
Uterine cysts and vascularization	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
Uterine endometrial degenerative changes	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
Endometritis	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
Effect of age					
Gestation longer in older mares	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)
No difference	3-7 years old 8-14 years old ≥15 years old	16 35 19	Criollo	No difference	Winter et al., (2007)
	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)
Gestation shorter in older mares	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	Kuhl et al., (2015)
	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				
Effect of parity					
Gestation longer in primiparous	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)
No difference	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)
Gestation shorter in primiparous	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
Effect of age					
Early fetoplacental development	4-8 years old >8 years old	11 5	Brandenburg	↘ conceptus size at 30-45 dpo in >8 years old ↗ eCG plasma concentration at 50-100 dpo in >8 years old	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)
Maternal metabolism	3.5-4 years old 8-19 years old	6 7	Croatian cold-blood	↗ parathyroid hormone ↘ osteocalcin serum concentration 50-70 days before foaling in 8-19 years old	Filipović et al., (2014)
Uterine vascularization	7-8 years old 16-21 years old	6 6	Thoroughbred	↘ uterine artery doppler indices (peak systolic velocity and total blood flow volume) with age ↘ total blood flow volume in 16-21 years old	Ousey et al., (2012)
	3-7 years old 8-11 years old 12-16 years old	16 17 18	Unspecified Warmblood	↗ diameter of both uterine arteries in >7 years old ↗ uterine artery pulsatility index in >7 years old	Klewitz et al., (2015)
Effect of parity					
Early fetoplacental development	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	↗ ventral combined thickness of uterus and placenta between 250 dpo and foaling in primiparous ↘ fetal eye diameter in primiparous until 200 dpo ↗ fetal eye diameter in primiparous after 200 dpo ↗ fetal biparietal diameter in primiparous after 200 dpo	Hendriks et al., (2009)
Maternal metabolism	Primiparous Multiparous 2-4	6 7	French Anglo-Arabian	↘ whole blood basal glucose concentration at 150 dpo in primiparous ↗ FSIVGTT** disposition index + ↗ glycaemia and insulinemia after meals in primiparous = ↘ ability to metabolically adapt to gestation ↗ amino acid maternal plasma concentration in primiparous at foaling	Robles <i>et al.</i> , (2019)
Uterine vascularization	Primiparous – Multiparous 3 Multiparous 4-5 Multiparous 6-9	7 23	Dutch Warmblood	↗ diameter of uterine artery as parity ↗ ↘ uterine artery total blood flow in primiparous-multiparous 3 compared to multiparous >3 ↗ pulsatility index as parity ↗ only in first trimester	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
Effect of age					
Term placental measurements	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)
Placental structure	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)
Effect of parity					
Term placental measurements	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)
Placental structure	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)
Placental gene expression	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)
Placental methylation	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 μm^{-1})
 Surface = surface density x placental volume (total microcotyledon surface, expressed in 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
Effect of age					
Foal birth weight	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)
Foal growth and measurements	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)
Risk of disease	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)
Offspring performance	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)
Effect of parity					
Foal birth weight	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)
Foal growth and measurements	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)
Foal metabolism and development	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)
Risk of disease	Primiparous Multiparous 2 – 6 as individual groups Multiparous ≥7	5 45 6	Thoroughbred Flat race	↘ risk of fracture in primiparous	Verheyen et al., (2007)
Offspring performance	Parity considered as continuous variable	Total = 875	Thoroughbred Flat race	Flat race performance increases until the 5 th parity and declines after the 10 th 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
Effect of age					
Retention of fetal membrane (RFM)	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)
Post-partum fever	<10yo ≥10yo	91 67	125 Crossbred 33 Percheron	No difference	(Aoki et al., 2014)
Dystocia	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)
Foal mortality	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)
Maternal behavior	3 yo 5yo	6 4	Jeju pony	↗ foal aggression by herd and ↗ maternal protection at 3yo	Rho et al. 2009
Effect of parity					
Retention of fetal membrane	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)

Post-partum fever	1 ≥2	23 112	125 Crossbred 33 Percheron	No difference	(Aoki et al., 2014)
Dystocia	1 2 ≥3	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 1	(Sabbagh et al., 2014)
Foal mortality	1 2 ≥3	Not defined	38,877 Breton 35,764 Comtois 13,274 Percheron 11,229 Ardenais	↗ at 1	(Sabbagh et al., 2014)
Maternal behavior	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
Effet of age					
Passive transfer of immunity	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)
Colostrum quality	≤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)
Milk quantity	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)
Milk quality	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)
Effect of parity					
Passive transfer of immunity	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)
Colostrum quality	≤5 >5	7 5	Konik breed	↘ Ratio saturated fatty acid/polyunsaturated fatty acid in parity <5	(Pikul et al., 2008)
Milk quantity	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)
Milk quality	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)
Foal suckling	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)