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BEYOND FOALING: EXPLORING THE CRUCIAL POSTPARTUM PERIOD IN THE MARE

Presentata da: Francesca Perina

Coordinatore Dottorato

Carolina Castagnetti

Supervisore

Jole Mariella

Co-supervisore

Aliai Lanci

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Introduction

The mare is a seasonally polyestrous long-day species, with estrous occurring naturally between April and September of the Northern Hemisphere. The postpartum period in the mare represents a highly delicate phase, during which a thorough and timely evaluation is essential to safeguard both the animal's health and her future reproductive efficiency, two aspects that are inextricably linked. From a physiological perspective, this stage is characterized by a series rapid endocrine, anatomical, structural, and functional adaptations, reflecting the complexity of this period, including uterine involution, the resumption of ovarian cyclic activity, the reduction of uterine blood flow, and the endometrial regeneration. Nevertheless, in addition to these normal events, the postpartum period may also be complicated by a range of conditions associated with pregnancy and parturition, such as retention of the fetal membranes, postpartum metritis, uterine prolapse, and lacerations of the reproductive tract, as well as systemic complications and gastrointestinal disorders. Postpartum complications can delay the timely return to fertility and potentially compromise reproductive performance within the same breeding season or even cause permanent infertility. Therefore, the use of assisted reproductive technologies represents a strategic approach to preserve fertility, especially in mares of high genetic or economic value. Consequently, meticulous clinical assessment and appropriate management of the mare during this period are essential to ensure her well-being and to optimize long-term reproductive performance. In light of the complexities and critical nature of the postpartum period in mares, which profoundly affects reproductive performance, overall health, and foal well-being, this thesis aims to investigate the physiological and pathological dynamics occurring during this period. The goal is to improve understanding of the mechanisms underlying reproductive recovery and to emphasize the importance of proper management and monitoring during this delicate stage.

Chapter 1. The postpartum period in the mare

1.1. The resumption of ovarian activity

Compared to other species, mares have an early resumption of ovarian activity after parturition and the first ovulation typically occurs, despite lactation, within 9-20 days after foaling during the so-called "foal heat" (Nagy et al., 1998; Ginther 1992; Sharma et al., 2010; Melchert et al., 2024). It has been observed that mares are at different stages of follicular development at the time of foaling, and those that ovulate early postpartum tend to show more advanced follicular growth soon after parturition (Lemes et al., 2017). Melchert et al., (2024) discovered that follicular growth resumes prior to foaling, with follicles larger than 10 mm increasing in number and that the dominant follicle nearly doubling in size during the last two weeks of gestation. Moreover, before foaling, follicular growth was more pronounced in mares that ovulated within 15 days postpartum compared to mares with delayed ovulation. Most mares resume regular ovarian activity after the first postpartum ovulation, with incidences of 52%, 58%, and 79% reported by Palmer and Driancourt (1983), Gastal et al. (2021), and Pastorello et al. (2022), respectively. Nevertheless, alternative reproductive patterns in the postpartum period have also been described, including continuous ovarian inactivity (anestrous phase) and ovulation at foal heat followed by ovarian inactivity. Palmer and Driancourt (1983), Gastal et al., (2021) and Pastorello et al. (2022) reported ovarian inactivity after the first ovulation in 35%, 8%, 8% of mares, respectively, and anestrus in 13%, 12%, 12.5%, respectively. The first estrus after parturition has traditionally been associated with lower average fertility and higher rates of pregnancy loss (Nagy et al., 1998; Meyers et al., 199; Ginther 1992;). However, several large retrospective field studies have reported no reduction in fertility during foal heat (Loy 1980; Camillo et al., 1997; Blanchard et al., 2004;). The reduction in reproductive efficiency observed during the early postpartum period has been attributed to an inadequate uterine environment in mares bred at foal heat (Ginther, 1992). Lower pregnancy rates in these mares may result from incomplete endometrial involution, particularly in those with greater follicular growth and earlier ovulation (<10 days postpartum) (Loy, 1980). Furthermore, reduced fertility outcomes have been reported in mares bred before 22 days postpartum, as well as higher pregnancy loss rates in those bred before 13 days postpartum (Blanchard et al., 2012). Overall, evidence suggests that breeding at foal heat can be a viable strategy in broodmare management, provided that sufficient uterine involution has taken place; however, when the first ovulation occurs too early, incomplete uterine recovery may negatively affect fertility. Exceptions may include mares with a history of foaling complications, evidence of reproductive tract pathology, or situations in which semen quality or availability is limited (Camillo et al., 1997).

Several factors have been investigated for their influence on the resumption of ovarian activity postpartum. Mares foaling early in the year are subject to opposing influences: the endocrine stimulus of parturition, which promotes ovulation, and the inhibitory effect of the anovulatory season. A delayed onset of post-partum estrus has been reported in early-foaling mares (Heidler et al., 2004) and is attributed to the photoperiod-dependent reduction in luteinizing hormone (LH) secretion during winter (Palmer and Driancourt, 1983). Most mares that foaled before the spring equinox exhibited a longer postpartum interval (>22 days) (Gastal et al., 2021). This finding is consistent with other reports indicating that foaling-first ovulation length is affected by the month of parturition, with longer intervals typically occurring between January and March and shorter ones between April and May in the Northern Hemisphere (Loy, 1980; Palmer and Driancourt 1983; Nagy et al., 1998; Sharma et al., 2010; Gastal et al., 2022). Irregular estrus cycles have also been described in non-postpartum cycling mares during the spring season (Ginther, 1990; Donadeu and Ginther 2002). Parity seems to negatively affect cyclicity, particularly in primiparous mares foaling early in the season, likely due to the metabolic demands and physiological stress associated with first lactation (Nagy et al., 1998). The influence of body condition score (BCS), lactational status, and breed on the resumption of postpartum cyclicity remains unclear. While some studies report no significant effect of these factors (Nagy et al., 1998; Sharma et al., 2010), other findings suggest a possible role, particularly for BCS and lactation (Henneke et al., 1984; Hines et al., 1987; Ginther et al., 1994; Gastal et al., 2021). Follicular dynamics appear to be similar between mares ovulating early and those ovulating later after foaling, with development characteristics comparable to those described in normal estrous cycles of cycling mares (Lemes et al., 2017). Likewise, the first diestrous period after parturition appears to be similar to the normal diestrus in non-lactating mares (Lemes et al., 2017; Gastal et al., 2021). However, a shorter interval to ovulation has been observed in postpartum lactating mares that ovulate during foal heat (≤22 days) compared with those at the second postpartum ovulation or with non-postpartum mares. Longer foal heats were linked with foaling during spring, lower body condition scores, and body weight loss, which were also associated with smaller dominant follicle diameters (Gastal et al., 2022). Lower LH and FSH concentrations during foal heat compared with subsequent ovulations indicate a strong partum effect, although not sufficient to impair the rapid resumption of ovarian activity (Gastal et al., 2022; Pastorello et al., 2022).).

1.2. Hormone profile

During pregnancy in the mare, endocrine maintenance and the onset of parturition result from coordinated hormonal interactions between the mare, placenta, and developing fetus. In early gestation, maternal hormones such as progesterone, estrogens, and gonadotropins support pregnancy recognition and mantainance. As the placenta develops, it assumes important endocrine functions, producing eCG, relaxin, estrogens, and progestagens. From mid-gestation onward, the fetoplacental unit becomes the main source of steroid hormones, maintaining optimal uterine conditions for fetal growth. Toward the periparturient period, increased maternal and fetal endocrine activity prepares for foaling and postnatal adaptation (Figure 1-2).

Figure 1. Endocrinolgy during equine prgnancy. From: Allen, W. R. (2001)

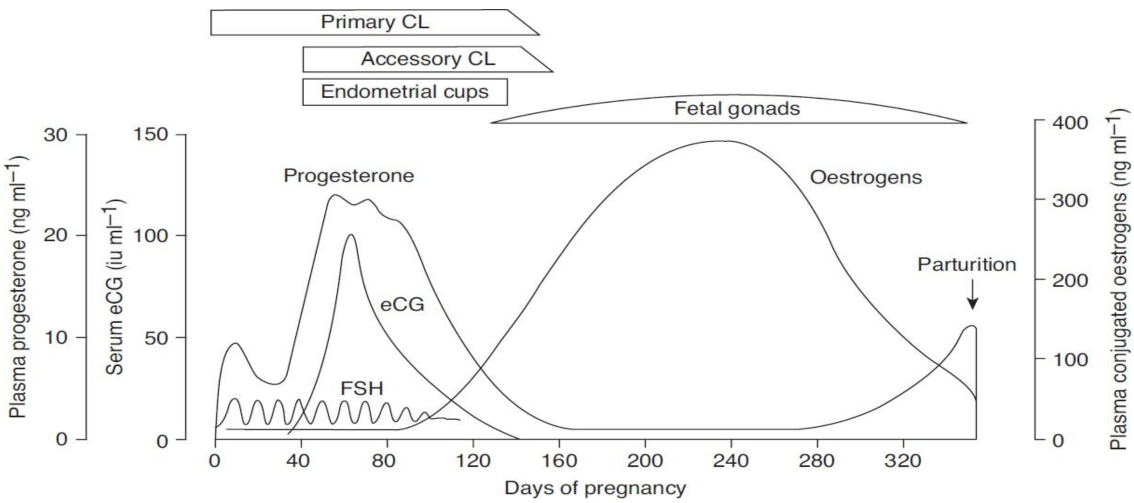
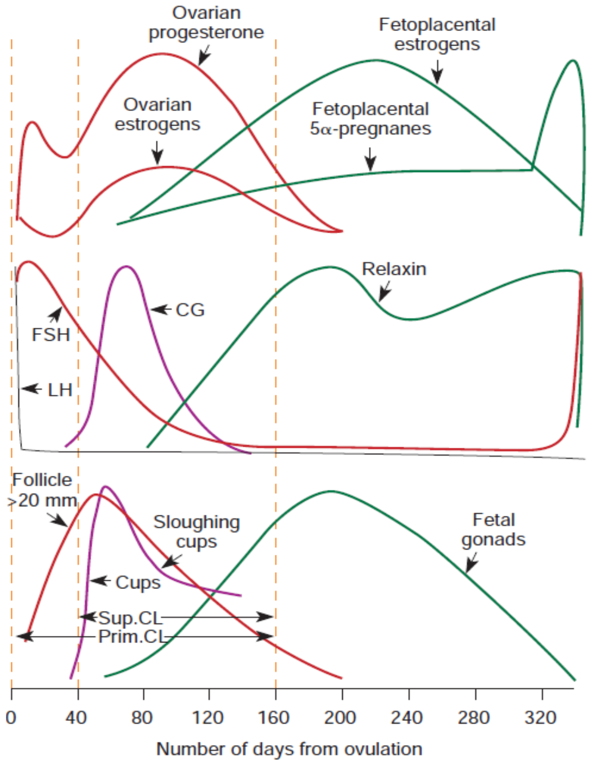


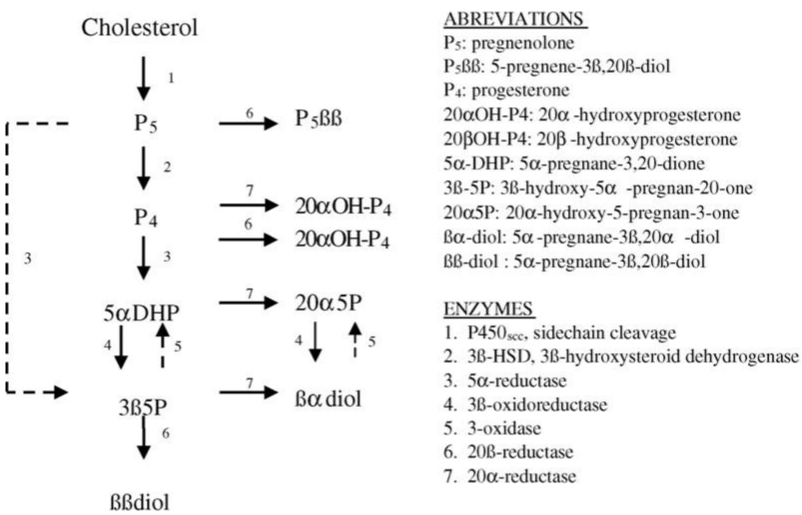
Figure 2. Endocrinology during equine pregnancy. In Brinsko. (2011), modified from Ginther, (1992).



During the postpartum period, mares undergo important hormonal adjustments aimed at supporting the high energy demands of lactation while gradually restoring ovarian activity. Changes in the hypothalamo-pituitary-adrenal (HPA) axis occur around parturition. Corticotropin-releasing hormone (CRH) stimulates adrenocorticotrophic hormone (ACTH) release and consequently cortisol secretion, a key mediator of stress responses (Özçelik et al., 2010; Holubová et al., 2016). Cortisol concentrations gradually increased during the last two weeks before foaling, with a sharp rise four days prior and a peak just before parturition (Nagel et al., 2012). ACTH levels resulted higher before foaling compared to the postpartum period, while cortisol levels were also elevated before foaling and shortly after, but declined later in the postpartum phase. A positive correlation was found between ACTH and cortisol throughout the peripartum period, indicating that the two hormones tended to rise and fall together. When compared with cycling mares, lactating mares showed lower ACTH concentrations after foaling, but higher cortisol levels before and immediately after parturition. The overall decrease in ACTH and cortisol observed during the early postpartum period suggests a reduced activity of the HPA axis in response to physical or psychological stress. This attenuation of the stress response may help the mare conserve energy for lactation, prevent stress-related inhibition of milk production, alleviate psychological stress, and support immune function. (Piccione et al., 2017; Arfuso et al., 2021).

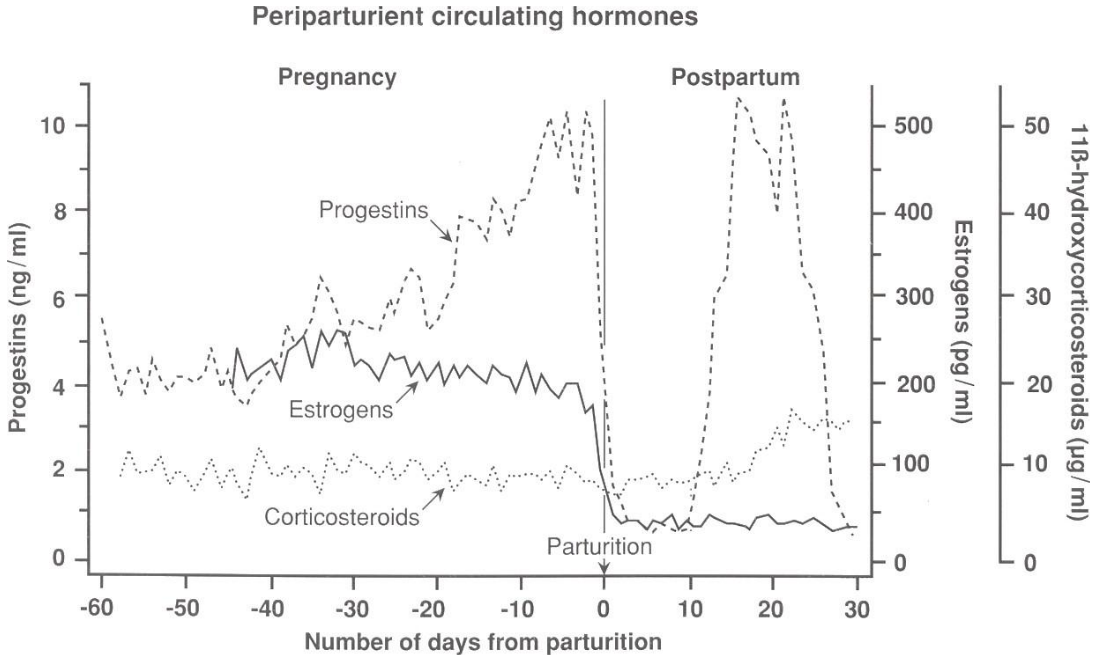
During the early stages of pregnancy, maintenance of myometrial quiescence is initially supported by progesterone secreted from the secondary corpora lutea. As these corpora lutea regress, maternal progesterone concentrations decline markedly during the final third of gestation. Indeed, during the second half of pregnancy, little progesterone is present, and the maintenance of uterine quiescence is sustained by progesterone and pregnenolone metabolites, known as progestogens (Figure 3), which are synthesized by the uteroplacental tissues (Ousey, 2004; Fowden et al., 2008). Among all progestogen, 5α-dihydroprogesterone (DHP) has been recognized as, perhaps, the only endogenous progestin with significant bioactivity other than progesterone itself (Scholtz et al., 2014). In the last month of gestation, total maternal progestagen concentrations progressively increase and reach a peak approximately 48 hours before parturition, followed by a steep decline within the final 24-48 hours preceding delivery (Conley, 2016). These endocrine changes are primarily regulated by the fetal HPA axis. As the fetal adrenal cortex grows and differentiates, it secretes pregnenolone, which serves as a precursor for uteroplacental progestagens synthesis. With further maturation, activation of the fetal adrenal cortex by a prenatal rise in fetal adrenocorticotropic hormone (ACTH) stimulates a functional shift from pregnenolone to cortisol synthesis. The increased fetal cortisol output is believed to trigger the final, precipitous fall in maternal progestagens levels, thus releasing myometrial quiescence and initiating parturition (Fowden et al. 2008).

Figure 3. Progestagens during equine pregnancy. From Ousey, (2004).



Concomitantly, estradiol concentrations rise steadily throughout the last two to three weeks of gestation, reaching maximal levels immediately prior to foaling. This increase reflects both enhanced placental synthesis and fetal contributions to estrogen production, promoting uteroplacental perfusion, cervical remodeling, and myometrial sensitivity to oxytocin and prostaglandins (Ousey et al., 1984; Haluska and Currie, 1988). The shift in the estradiol-to-progesterone ratio during this period is considered a key endocrine signal triggering the onset of parturition, as estrogen dominance facilitates the transition from uterine quiescence to active contractility, and is a consistent endocrine hallmark, associated with increased uterine activity and cervical softening (Stewart et al., 1984; Haluska and Currie, 1988;). On the day of foaling, plasma progestogen levels were still detectable but rapidly declined thereafter (Noden et al., 1978; Gündüz et al., 2008) remaining low until ovulation, when they increased again, following a pattern similar to that observed in subsequent estrous cycles (Noden et al., 1978). Similarly, estradiol concentrations decline significantly in the immediate postpartum period, with values decreasing steadily over the first week (Gündüz et al., 2008), consistent with earlier reports (Lovell et al., 1975; Hillman & Ganjam, 1979). However, estradiol peaked at the onset of estrus and declined toward ovulation (Nodel et al., 1978). In a study comparing mares that displayed normal maternal behavior with those that rejected their foals, no significant differences were found in oestradiol or progesterone concentrations between the two groups: progesterone declined in both groups during the first three days postpartum, while oestradiol remained stable. The oestradiol-to-progesterone ratio was initially higher in normal mares and increased over time in rejecting mares, suggesting a possible hormonal influence on early maternal behavior (Berlin et al., 2018). Concentrations of steroid hormones during pregnancy, parturition and the postpartum period in the mare are synthesized in Figure 4.

Figure 4. Steroids during late pregnancy and at parturition. In Ginther, (1992) adapted from Lovell et al., (1975).

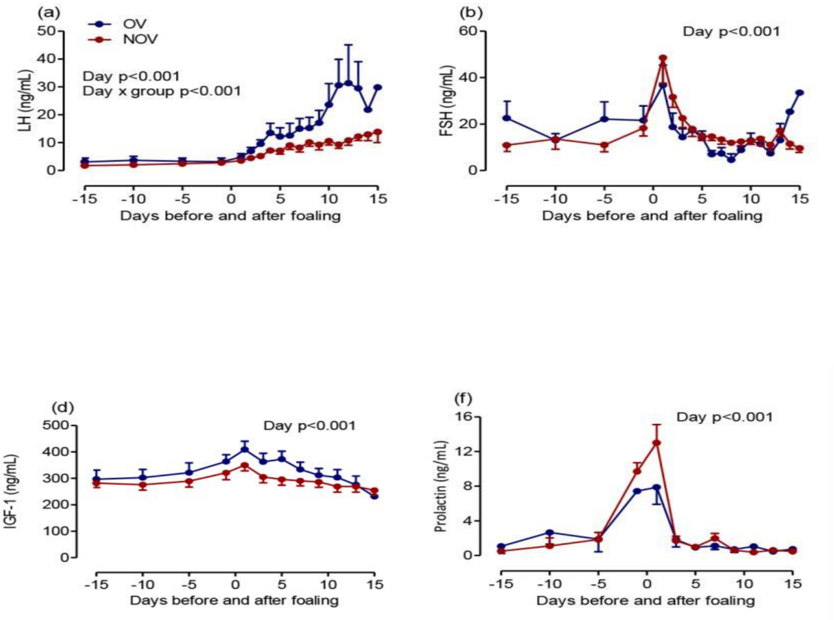


Insulin-like growth factor 1 (IGF-1) plays an important role in the postpartum mare supporting ovarian function. It promotes the proliferation of granulosa and thecal cells and enhances steroidogenesis in combination with gonadotropins (Davidson et al., 2002). In mares, IGF-1 concentrations begin to rise before foaling, reach their highest levels within the first day after parturition, and then gradually decrease over the following months (Hess-Dudan et al., 1994; Heidler et al., 2003; Melchert et al., 2024) (Figure 5). This decline occurs more slowly than in ruminants, which is thought to reflect the lower metabolic demands of horses. By around four months postpartum, IGF-1 levels reach their lowest point. Notably, there are no significant differences in IGF-1 concentrations between lactating and non-lactating mares when compared at similar stages of the estrous cycle, and the highest values are generally observed at the first postpartum estrus (Deichsel et al., 2006).

Throughout most of the gestation, gonadotropin concentration remains at basal levels in mares, with intermittent follicle-stimulating hormone (FSH) surges occurring primarily during the first 60 days of pregnancy and subsequently declining after mid-gestation (Ginther, 1992; Aurich et al., 2001;). A rise in FSH secretion is then observed a few days before foaling, likely associated with the hormonal cascade initiating parturition. LH concentrations remained consistently low before foaling due to endogenous opioids inhibition (Aurich et al., 2001) and increased progressively after parturition during the first two weeks postpartum, which may be necessary for the early resumption of ovulatory oestrous cycles after parturition (Heidler et al., 2003). This post-foaling rise was significantly more pronounced in mares that ovulated within 15 days postpartum compared to those that did not. FSH concentrations showed a transient increase one day after foaling and during the first 5 days, followed by a temporary decline; however, from approximately 12 days postpartum, FSH levels rose again in mares that ovulated early (Melchert et al., 2024) (Figure 5). Postpartum lactating mares consistently exhibited lower FSH and LH concentrations compared to non-postpartum cycling mares. A marked seasonal effect was also observed: postpartum mares that foaled in spring had significantly lower FSH concentrations than both summer-foaled postpartum mares and cycling mares (Gastal et al., 2021; Pastorello et al., 2022). Parturition appeared to have a strong suppressive effect on FSH concentrations in postpartum mares, consistent with earlier observations (Palmer and Driancourt, 1983; Ginther et al., 1994). Increasing levels of IGF-1 and gonadotropins around parturition may facilitate the earlier resumption of ovarian activity in mares compared with other domestic species (Pastorello et al., 2022; Melchert et al., 2024). Lactating mares showed lower basal LH levels and a reduced LH response to GnRH during the early luteal phase compared to non-lactating mares, but not during ovulation (Deichsel et al., 2006).

Prolactin plays a major role in lactogenesis and in the initiation, but not the maintenance of lactation in the mare. During gestation, prolactin secretion in the mare is inhibited by endogenous opioids, but this inhibition ceases after foaling, allowing the pronounced rise in prolactin release (Aurich et al., 1999). Although prolactin alone appears to have no direct effect on follicular growth in mares (Neuschaefer et al., 1991), it may act together with insulin-like growth factor 1 (IGF-1) to stimulate follicular development during the early postpartum period (Heidler et al., 2003). Plasma prolactin concentrations rise markedly during the final days of gestation and reach their highest levels around parturition (Lothrop et al., 1987; Worthy et al., 1987), with peak values observed on the first day after parturition (Melchert et al., 2024) (Figure 5) and two to three days after foaling (Heidler et al., 2003). Then prolactin concentrations decrease substantially within two weeks after foaling but remain higher in lactating mares compared with non-lactating mares for approximately the first 10 weeks, corresponding to the early phase of lactation (Worthy et al., 1987; Heidler et al., 2003). High prolactin concentrations have been observed only shortly before and within 24 hours after foaling, followed by a significant decline by the ninth day postpartum, coinciding with the onset of estrous behavior and post-ovulatory activity (Krakowski et al., 2020). In foal-rejecting mares, prolactin levels declined significantly between days 1 and 3 postpartum, and lower prolactin concentrations may be associated with increased stress or anxiety contributing to abnormal maternal behavior (Berlin et al., 2018).

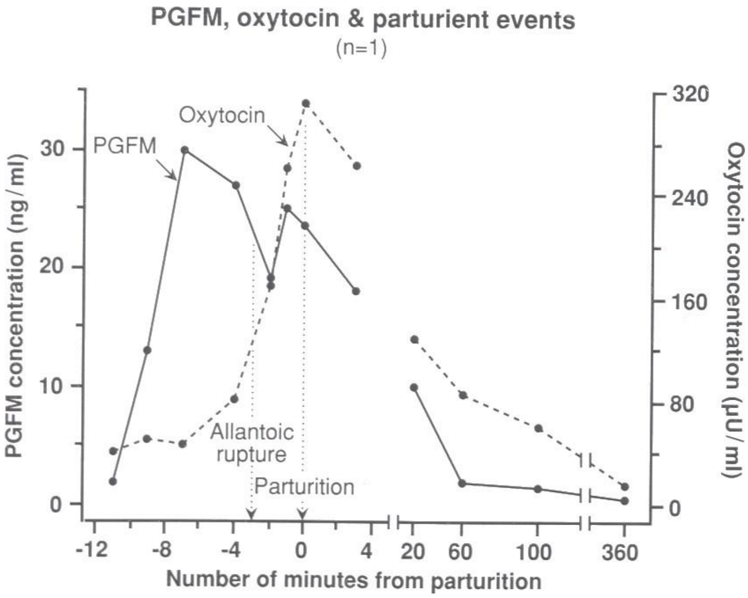
Figure 5. Concentration of LH, FSH, IGF-1, and leptin from late pregnancy to the postpartum period in the mare. Modified from Melchert et al., 2024.



The major prostaglandins of clinical relevance during equine pregnancy are prostaglandin E_2 (PGE_2), prostaglandin $F_2\alpha$ ($PGF_2\alpha$), and its stable metabolite 13,14-dihydro-15-keto-$PGF_2\alpha$ (PGFM). PGE_2 is involved in cervical relaxation and membrane rupture, whereas $PGF_2\alpha$ stimulates myometrial contractions (Leadon et al., 1982). During the second half of pregnancy, PGFM concentration remains low, rising slightly toward term, but during second-stage labor they increase approximately fifty-fold (Haluska and Currie, 1988; Vivrette et al., 2000). Following delivery, as the allantochorion detaches from the uterus, maternal prostaglandin concentrations and uterine electromyographic activity rapidly decline (Haluska et al., 1987; Haluska and Currie, 1988). Within one hour after foaling, prostaglandin levels are typically low (Vivrette et al., 1995). When prostaglandin synthesis was inhibited experimentally by administration of flunixin meglumine prior to labor, placental delivery was delayed, suggesting that uterotonic hormones (particularly prostaglandins) must be present in sufficient quantities postpartum to ensure uterine contractions and placental expulsion (Vivrette et al., 1995). Since prostaglandins are synthesized within the placenta, which begins to detach during delivery, oxytocin is also likely to play a key complementary role in placental expulsion (Ousey and Fowden, 2012). Postpartum studies have shown variable PGFM dynamics. Stewart et al. (1984) reported detectable PGFM levels on the second day after foaling, followed by further $PGF_2\alpha$ release on day 3, whereas Sertich and Watson (1992) observed low PGFM concentrations by the day after parturition and non-detectable levels by day 3, with no apparent correlation to uterine involution.

Plasma oxytocin concentration remains low throughout gestation and rises markedly only during labor. It has been proposed that oxytocin may also act locally in a paracrine manner within the uteroplacental tissues, where an increased density of oxytocin receptors could play a more critical role than oxytocin concentrations (Ousey, 2006). Evidence further suggests that oxytocin secretion from the maternal posterior pituitary begins shortly before or concurrently with the onset of the second stage of labor, while prostaglandin concentrations in the peripheral circulation rise prior to oxytocin release. Additionally, oxytocin release appears to be largely independent of suckling activity in mares (Vivrette et al., 2000). In the study by Ishii et al. (2008), mares with shorter placental expulsion times exhibited significantly higher oxytocin concentrations than those with longer expulsion times. These findings indicate a negative relationship between post-foaling oxytocin levels and the duration of placental delivery. Interestingly, endogenous opioids have been shown to inhibit oxytocin secretion both before and after parturition (Aurich et al., 1996). PGFM and oxytocin concentrations around parturition are resumed in Figure 6.

Figure 6. PGFM and oxytocin concentration at parturition in the mare. In Ginther, (1992) adapted from Haluska and Currie,1984.



Leptin, a hormone primarily secreted by adipocytes, is closely related to fat mass and BCS in horse (Zhang et al., 1997; Buff et al., 2002; Carter et al., 2009), and it reduces food intake through appetite suppression (McManus and Fitzgerald, 2000; Buff et al., 2005). A decrease in serum leptin concentration after foaling has been reported in several studies (Heidler et al., 2003; Berg et al., 2007;

Kędzierski et al., 2011; Arfuso et al., 2016). Berg et al. (2007) reported a significant day effect on mare serum leptin concentrations, with levels peaking 14 days before parturition, declining until day 2 postpartum, and stabilizing thereafter. Similarly, Romagnoli et al. (2007) found that plasma leptin concentrations decreased from two weeks before foaling to ten weeks postpartum, remaining at basal levels until week 21. Subsequently, leptin concentrations increased again, reaching their maximum around week 49. However, serum leptin concentrations did not differ among mares with different body condition scores (BCS) during the postpartum period, up to the second ovulation after foaling (Cavinder et al., 2007). The decline in leptin concentration may be associated with the loss of placental tissue, as the placenta itself is a known source of leptin (Thomas et al., 2001; Jakimiuk et al., 2003). This postpartum decrease in leptin may represent an adaptive physiological mechanism that helps mares avoid negative energy balance by stimulating feed intake (Kędzierski et al., 2011). Enhanced feed consumption during early lactation supports the energetic demands of milk production, allowing the mare to maintain an adequate body condition and sustain milk yield (Heidler et al., 2003; Berg et al., 2007;). Furthermore, during the peripartum period, leptin concentrations appeared significantly lower in pregnant mares compared to non-pregnant mares (Arfuso et al., 2016).

1.3. Uterine involution

After foaling, a rapid uterine involution process begins to reestablish a favorable environment for the development of a new conceptus (Griffin and Ginther, 1991). Uterine involution contributes to the reduction in uterine size, clearance of cellular debris, glandular contraction, apoptosis, and cellular proliferation (Lemes et al., 2017). A sharp decrease in uterine diameter has been documented during the first 4-10 days postpartum followed by a slower rate of involution thereafter (Griffin and Ginther, 1991; Fedorka et al., 2020; Lemes et al., 2017;). Uterine involution was considered complete by three weeks postpartum, as no further significant reductions in diameter were observed afterwards (Lemes et al., 2017; Fedorka et al., 2020). These findings are consistent with previous studies reporting uterine involution to be completed around day 23 postpartum (McKinnon et al., 1988). The abrupt increase in intrauterine fluid observed in the early postpartum days appears to result from a physiological influx involved in the involution process rather than from residual placental fluid (Griffin and Ginther, 1991). Accumulation of intrauterine fluid is not correlated with endometritis or with the recovery of potential bacterial pathogens, with no evident association between intrauterine fluid and uterine inflammation (Blanchard et al., 1991). Malschitzky et al. (2002) demonstrated that intrauterine fluid accumulation during the first postpartum estrus did not negatively affect pregnancy rates in mares. During this period, an increase in uterine echotexture score has been described, reflecting the onset of uterine edema at estrus in most mares (Ginther, 1992). Post-ovulation echotexture at foal heat is greater in postpartum mares than in nonlactating cyclic ones (Griffin and Ginther, 1991), suggesting that this is due to residual postpartum edema. A gradual decrease in uterine tone has been observed during the postpartum period, reaching minimal scores before the first ovulation (Griffin and Ginther, 1991). Cervical tone remained minimal during the first 13 days postpartum (Lemes et al., 2017). This finding is consistent with the low circulating concentrations of progesterone and estradiol until the first postpartum ovulation (Gygax et al., 1979). After that, cervical tone increased in parallel with uterine tone and in accordance with the expected hormonal modifications of a normal estrous cycle (Lemes et al., 2017).

After parturition, the equine endometrium undergoes a rapid yet highly coordinated process of degeneration and repair. Resorption of microcaruncles is complete by day 7 (Gygax et al., 1979; Bailey and Bristol 1983; Jischa et al., 2008; Fedorka et al., 2020) and 9 (Katila, 1988), leaving only deposits of amorphous eosinophilic material (Gómez-Cuetara et al., 1995). Uterine glands, initially dilated and coiled, begin to normalize by days 9-11, and by day 14 the endometrium closely resembles its pre-gravid appearance (Gygax et al., 1979; Fedorka et al., 2020). Oedema, vascular congestion, and neutrophil infiltration characterize the early phase (Katila, 1988a), with neutrophils initially concentrated at the luminal epithelium before migrating to deeper layers (Bailey & Bristol, 1983). As involution progresses, neutrophils decrease markedly, while macrophages, lymphocytes, and siderophages gradually increase, reflecting the transition from acute to chronic inflammatory cell populations (Bailey & Bristol, 1983; Katila, 1988a; Welle et al., 1997; Jischa et al., 2008). By the end of the first postpartum week, the endometrial epithelium is largely restored, and glandular architecture has returned to normal (Gygax et al., 1979; Katila, 1988a). Between days 7 and 10, the endometrium resembles that of a mare in estrus (Gómez-Cuetara et al., 1995), although Sexton and Bristol (1985) reported that in some mares, complete restoration may be delayed until day 12. Isolated inflammatory changes and residual siderophages can persist into the second week (Sertich & Watson, 1992), but these rarely interfere with subsequent fertility. Despite the generally rapid and efficient involution, approximately 20% of mares show delayed uterine recovery beyond 15 days postpartum. In some cases, this is associated with endometritis of variable severity, while in others no identifiable pathology is observed (McKinnon et al., 1988; Gómez-Cuetara et al., 1995;). Steiger et al. (2001) observed a normal uterine involution even in mares with retained fetal membranes and absence of microcaruncular lesions, but disturbed regression of these structures was observed after dystocia. This contrasts with Belz and Glatzel (1995), who described involution as independent of the type of birth. Reduced uterine contractility, possibly due to slower progesterone decline and myometrial fatigue after dystocia (Vandeplassche et al., 1983), may contribute to impaired involution.

Both anti-inflammatory and immunomodulatory cytokines changed throughout the involution process, mostly following similar profiles. Expression of inflammatory-modulating cytokines interleukin (IL)-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF), as well as the anti-inflammatory cytokine IL-1RN, showed a marked decline in endometrial expression from parturition to Day 15, with the most pronounced decrease between Days 10 and 15 for IL-6 and GM-CSF. Parturition involves activation of chemokines, cytokines, and immune cells to degrade cellular adhesions at the feto-maternal interface and coordinate myometrial contractions, explaining early postpartum cytokine elevation (Keelan et al., 2003; Menon and Taylor, 2019; Fedorka et al., 2020). The linear decline in cytokine expression observed during involution aligns with physiological resolution of inflammation. IL-8 expression decreased in parallel with cytologic resolution, and IL-6, which mediates leukocyte adhesion, followed a similar pattern (Zhang et al., 2011; Fedorka et al., 2020). Histologic, cytologic, immunologic resolution of inflammation is complete within 15 days postpartum (Fedorka et al., 2020).

Several strategies have been tested to assess their influence on uterine involution with the aim of improving pregnancy outcomes. Large-volume uterine lavage likely facilitates clearance by eliminating fluid and debris that impair neutrophil function, stimulating uterine contractility through endogenous oxytocin release, and inducing neutrophil influx by mechanical irritation and uterine stretching (Troedsson et al., 1995; Pycock, 2011;). MacPherson and Blanchard (2005) suggested that lavage may be beneficial if uterine involution is delayed or intrauterine fluid persists at the time of breeding. Uterine lavage has been evaluated (Blanchard et al., 1989; McCue and Hughes, 1990; Mitchell et al., 2019), but no significant benefit was observed on uterine cytology, involution, or subsequent fertility. Similarly, the use of hormonal treatments such as progesterone, estradiol-17β, and GnRH analogues has been investigated (Pope et al., 1979; Sexton & Bristol, 1985; Arrott et al., 1994; Bruemmer et al., 2002; Gunduz et al., 2008), yet consistent improvements in endometrial recovery or pregnancy rates have not been demonstrated. The administration of ecbolic agents, including oxytocin and prostaglandin F2α, has also been studied (Blanchard et al., 1991; Gunduz et al., 2008), but results remain inconclusive and of limited clinical relevance. Plasma concentrations of PGFM (13,14-dihydro-15-ketoprostaglandin F2α), a metabolite of prostaglandin F2α, are elevated at parturition but declined by the first day postpartum. In mares, no correlation has been demonstrated between PGFM levels and uterine involution, in contrast to observations in the cow (Lindell et al., 1982; Sertich and Watson 1992). In mares with postpartum metritis, uterine lavage did not significantly affect serum concentrations of sCD14, CCL2, or IL-10, with no differences detected either shortly after treatment or between the end of the first lavage and the start of the second (after 10–23.5 hours), and it was not associated with adverse clinical effects (Tukia et al., 2021). It has long been assumed that foaling results in bacterial contamination of the equine uterus (Purswell et al., 1989). The proportion of bacteriologically positive uterine swabs obtained 1-2 days postpartum is generally lower compared with samples collected at 3-6 days postpartum, when positivity rates rise to 70–90% (Gygax et al. 1979; Katila et al. 1988b; Reilas, 2001). Spontaneous clearance is associated with uterine involution and could explain the decline in positive cultures at subsequent cycles (Purswell et al., 1989). Streptococci and coliform bacteria are the microorganisms most commonly isolated from uterine swabs of postpartum mares (Gygax et al., 1979; Riddle et al., 2007; Mitchell et al., 2019). The degree of bacterial contamination is influenced by mare management, environmental conditions, and foaling events. Mares experiencing puerperal complications, such as dystocia or retained placenta, exhibit higher bacterial growth on days 3, 6, and 9 postpartum compared with mares undergoing normal parturition (Belz and Glatzel, 1995). A marked and precipitous decline in uterine bacterial colonization after foaling has been observed by Fedorka et al. (2020), although previous studies show conflicting evidence. Gygax et al. (1979) reported a similar decrease in positive cultures from Day 7 to Days 12-14, whereas Katila (1987; 1988) found persistent bacterial growth through Day 15. Although uterine swabs collected during foal heat are frequently bacteriologically positive, the incidence is lower than in the immediate postpartum period (Gygax et al., 1979; Bailey and Bristol, 1983).

Polymorphonuclear leukocytes (PMNs) are frequently detected in uterine swabs during the first 6 days postpartum (Katila et al., 1988b) and then declined consistently (Krohn et al., 2019). In foal heat, cytology reveals variable findings: no PMNs, low numbers, moderate to high levels (Shideler et al. 1987; Katila et al. 1988b; Huhtinen et al. 1996). PMNs play a key role in eliminating bacterial contamination acquired at foaling and in the breakdown of microcaruncles (Gygax et al. 1979; Katila 1988), explaining why many mares show PMNs in the absence of bacteria. Mares with puerperal complications, particularly retained placenta, often exhibit high PMN counts in lochial secretions 3-6 days postpartum (Belz and Glatzel 1995). After dystocia, tendency toward higher neutrophil concentrations has been found, likely due to the uterine manipulation and the prolonged second stage of parturition. By day 6 postpartum, a marked reduction in neutrophils was seen regardless of the obstetrical circumstances. However, in mares with retained fetal membranes, persistently higher neutrophil levels and a slower decline compared to those with normal puerperal recovery have been noted (Krohn et al., 2019). Mitchell et al. (2019) observed that mares undergoing routine lavage demonstrated significantly lower endometrial inflammation compared with controls. This contrasts with earlier reports that found no difference between treated and untreated mares (Blanchard et al., 1989; McCue & Hughes, 1990).

1.4. Blood perfusion

In human obstetrics, assessment of uteroplacental and fetal blood flow is an essential component of pregnancy monitoring and color Doppler sonography has become a routine tool for the diagnostic evaluation of high-risk pregnancies (Campbell et al., 1986; Steel et al., 1990; Chan et al., 1995). In equine reproduction, several research groups have investigated uterine and umbilical blood flow during gestation using Doppler techniques (Bollwein et al., 1999; Bollwein et al., 2004; Ousey et al., 2010; Ousey et al., 2012; Bailey et al., 2012a; Bailey et al., 2012b). These studies demonstrated a decrease in peripheral blood flow resistance (pulsatility/resistance index, PI/RI) during early pregnancy and an increase in uterine blood flow volume (BFV), particularly in the last trimester. Although similar uterine arteries' hemodynamics changes have been observed in young and aged mares, a tendency towards reduced uterine blood flow, decreased placental microvillus density and lower foal birth weights in older mares has been noticed (Ousey et al., 2012). Parity plays a major role in shaping uterine hemodynamics, as repeated foalings are associated with progressive increases in uterine artery diameter and BFV. This effect is likely linked to incomplete uterine involution after foaling and contributes to enhanced feto-maternal exchange through increased microcotyledon density and surface area. Indeed, the uterus appears to require "priming" by the first pregnancy to achieve full microcotyledon development (Klewitz et al., 2015). In contrast, age predominantly influences vascular resistance. Older mares display higher PI values (Blaich et al., 1999; Bollwein et al., 2008; Klewitz et al., 2015), which reflect degenerative changes such as endometrial fibrosis and angiosclerosis that impair vascular elasticity. Maternal age and parity may also act as confounding factors, as often nulliparous mares are young, while older mares are mostly multiparous; studies including older maiden mares might help to distinguish the effect of age and parity. In fact, both diameter and cross-sectional area appeared to be influenced by age and parity, with lower values observed in young primiparous mares compared to old/young multiparous and old maiden ones (Perina et al., 2024). This suggests that increasing age and/or parity leads to structural and functional degeneration in the reproductive tract, impacting embryo, placenta, fetal intrauterine growth and foal health (Derisoud et al., 2021). However, in young mares, parity does not appear to influence endometrial structure (Tunòn et al., 1995). In contrast, old maiden mares develop degenerative endometrial changes, suggesting that maternal aging, rather than parity, is primarily responsible for the degenerative alterations observed in the endometrium (Ricketts and Alonso, 1991). On the day following parturition, mares show a significant reduction in uterine blood flow, uterine artery diameter, and vascular indices compared with prepartum measurements, indicating that the uterus no longer needs to sustain a pregnancy and can return to a nonpregnant state. Overall, vascular perfusion decreased during the first week after foaling (Mortensen et al., 2011). Even though there is a general decrease in blood perfusion after foaling, local increases in the vascularization of the endometrium (days 1–4) and mesometrium (days 1–2) have been detected (Lemes et al., 2017). The increased endometrial vascularization in the first 13 days postpartum is important for supporting the uterine repair processes (Lemes et al., 2017). Uterine blood flow postpartum (Lemes et al., 2017) and the RI on the first day postpartum (Krueger et al., 2009) appeared to be similar between the two uterine horns. However, in another study, differences between the gravid horn and nongravid horn had been reported (Mortensen et al., 2011).

1.5. Postpartum complications

The postpartum period is a particularly delicate phase for the mare, who requires careful monitoring just as much as the newborn foal. Even after an apparently normal parturition, complications may still arise. Clinical signs may not always be immediately evident, can present later or appear nonspecific, making veterinary evaluation of the mare crucial in the postpartum period. Reproductive problems after foaling can negatively affect a mare's future fertility and, in severe cases, may even become life-threatening. Potential abnormalities include uterine artery or uterine hemorrhage, retained fetal membranes (RFM), postpartum metritis (PPM), rectovaginal injuries, uterine rupture, uterine prolapse. Beyond reproductive disorders, gastrointestinal and urinary tract conditions should also be taken into account. Dystocia, defined as any impediment to the normal progression of foaling due to maternal, fetal, or fetal membrane origin (Lanci et al., 2022), is one of the most common causes of postpartum complications in the mare. In a retrospective study conducted by our research group on Standardbred mares, postpartum complications were reported with an incidence of 44% after dystocic delivery, which was statistically higher compared to the group with eutocic foaling. In mares with dystocia, postpartum complications included RFM (52%), vaginal hematoma (8%), PPM (8%), cecal rupture (8%), hemorrhage (4%), cervical laceration (4%), uterine prolapse (4%), mastitis (4%), and constipation (4%) (Lanci et al., 2022).

Incidence of these peripartum diseases in a referral hospital has been reported by Dolente et al., (2005) (Figure 7). Recently, a retrospective study on Thoroughbred in Australia, showed that 30% of mares were diagnosed with gastrointestinal problems, 50% with urogenital trauma, and 20% with periparturient hemorrhage (Offer et al., 2022).

Figure 7. Postpartum problems in the mare. In McKinnon, (2013) adapted from Dolente et al., 2005.

Reason for admission	% of diagnoses	Comment
Urogenital hemorrhage	16.6% (27/163)	11 Broad ligament haemorrhage, 5 Intraluminal 6 haemoperitoneum
Large colon Volvulus	16.6% (27/163)	
Metritis	8% (13/163)	70% had dystocia
Small intestinal disease	7.4% (12/163)	10 SI Volvulus
Uterine tear	5.5% (9/163)	Luekopaenia (P<0.001)
Caecal disease	6.1% (10/163)	6 Rupture, impaction
Nephrosplenic entrapment	5.5% (9/163)	
Small colon disease	3.1% (5/163)	4 trauma
Colitis	3.1% (5/163)	
Large colon displacement	3.1% (5/163)	
Rectovaginal trauma	2.5% (4/163)	Mostly prolpases
No diagnosis	17.8% (29/163)	

1.5.1. Retained fetal membranes

RFM, the most common post-partum problem in mares (Frazer, 2002) is defined as a failure to expel all or a part of the fetal membranes within a specific period of time after delivery of the foal (Threlfall, 1992). Normally, fetal membranes are expelled within 30 minutes to 3 hours after parturition (Blanchard and Varner, 1993). After this short time frame potentially life-threatening consequences can occur, such as PPM, laminitis, septicemia, endotoxemia and death (Canisso et al., 2013; Warnakulasooriya et al., 2018). The reported incidence of RFM in mares varies from 2 to 10% in non-draft mares (Vandeplassche, 1971), although a study conducted by Sevinga et al. (2001) found that Friesians had an incidence of 54%. The same study showed that mares with a previous episode of RFM had a 2.9-fold increase in the chance of developing the condition again. The exact cause of RFM in mares remains uncertain; however, several predisposing factors have been identified. Uterine inertia, often linked to hypocalcemia, overstretching of the myometrium in cases of twin pregnancy, myometrial degeneration due to infection, exhaustion following dystocia (Threlfall, 2011), hormonal imbalances (Rapacz-Leonard et al., 2015a) have been proposed. Additional risk factors include disturbances in calcium and phosphate homeostasis, dysregulation of extracellular matrix remodeling, placental infection or edema, trauma to endometrial tissue, and uterine infections (Canisso et al., 2013; Rapacz-Leonard et al., 2015b). In draft breeds, especially Friesians, RFM has been associated with both breed predisposition and inbreeding (Sevinga et al., 2001; Sevinga et al., 2004). Other factors reported include advanced maternal age (>15 years), a previous history of RFM, systemic or uterine infections before or during pregnancy, poor body condition, dystocia, prolonged gestation, and surgical interventions such as cesarean section (Provencher et al., 1988; Leblanc, 2008; Canisso et al., 2013). Maternal age and gestational length remain controversial (Sevinga et al., 2001). The management of RFM in mares remains one of the most debated topics in equine reproduction, with treatment strategies differing between clinicians and literature. The main therapeutic goals are to eliminate toxic and inflammatory products from the uterus, control systemic complications, and prevent laminitis (Canisso et al., 2013). Antibiotics are widely recommended to prevent uterine infection, septicemia, and endotoxemia. A broad-spectrum approach provides effective coverage against most uterine pathogens (Canisso et al., 2013). Uterine lavage is another key component as it assists in eliminating bacterial contamination, debris, and small tissue fragments while stimulating contractility and neutrophil recruitment (Canisso et al., 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) are recommended to control inflammation, pain, and endotoxemia (Perkins, 1999). Several techniques have been described in case of RFM, including the administration of oxytocin, the Burns technique, umbilical vessel catheterization, and manual removal of the fetal membranes. Oxytocin therapy is considered the first-line treatment and is the most frequently administered drug (Perkins, 1999). Various administration routes are possible, including intravenous (IV) bolus or slow drip and intramuscular (IM) injections, with dosages ranging from 2 to 120 IU. Treatment is usually limited to the first 24 h postpartum (Threlfall, 2011). The concurrent use of calcium borogluconate has been suggested (Sevinga et al., 2001). The manual removal of membranes remains the most controversial treatment. While some clinicians advocate for immediate removal to expedite resolution (Sevinga et al., 2002; Cuervo-Arango and Newcombe, 2009;), others warn against it due to risks of hemorrhage, uterine damage, retained microvilli, uterine horn eversion/intussusception or prolapse., embolism, and delayed involution (Leblanc, 2008; Threlfall, 2011; Vandeplassche et al., 1971; Blanchard and Varner, 1993; Hooper et al., 1993). The Burns technique involves filling the allantoic cavity with a large volume of fluid while manually occluding the cervix for 10–15 minutes. This procedure facilitates the detachment of microvilli from the endometrium and promotes placental release. However, it is only effective when the allantochorion remains intact. (Burns et al., 1978) (Figure 8-9). The technique of infusing water into the umbilical vessels of the fetal membranes (Dutch Technique) consists of infusion of 0,5-1 liter of liquid through the umbilical vessels to distend the allantochorion, facilitating detachment from the endometrium (Meijer et al., 2015) (Figure 10). This technique is particularly useful when fetal membranes have been retained for less than eight hours. After dystocia or prolonged retention, the membranes often undergo autolysis or tissue damage, which compromises vascular integrity and limits their ability to expand. Nevertheless, the technique is simple, inexpensive, and poses minimal risk to the mare when performed correctly (Burden et al., 2019). This technique is an effective and safe method for the treatment of RFM also after C-section in mares (Neto et al., 2025). With timely and appropriate treatment, prognosis for both survival and future fertility in mares with RFM is generally excellent.

Figure 8. Burns technique. From Brinsko, (2011).

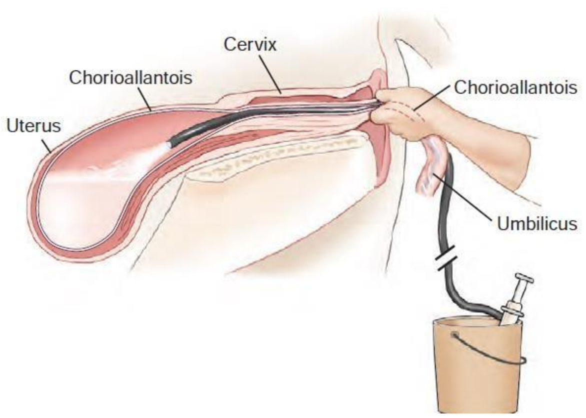


Figure 9. Burns technique. From Pozor, (2016).

Figure 10. Dutch technique. From Pozor, (2016).

1.5.2. Postpartum metritis

PPM is among the most common postpartum complications in the mare (Dolente et al., 2005). It occurs most frequently within 24-72 hours after foaling, although cases can present up to 10 days post-partum. The condition is more often associated with dystocia and RFM (Dolente et al., 2005; Frazer, 2002), but can also develop after abortion, uterine trauma, uterine inertia and even normal foaling, especially in poor hygiene conditions (Le Blanc, 2008). Breed predisposition has been reported, particularly in draft horses such as Belgians (Aoki et al., 2014), and in Friesians, largely due to their increased risk of RFM (Sevinga et al., 2002). The pathogenesis includes bacterial contamination of the uterus, poor uterine clearance, and disruption of the endometrial barrier (Frazer, 2002; Le Blanc, 2008). Mixed bacterial infections are common, with *Escherichia coli* and *Streptococcus equi* subsp. *zooepidemicus* being the most frequent isolates, while anaerobic bacteria are rarely involved (Blanchard and Macpherson, 2007; Blanchard, 2011; Ferrer and Palomares, 2018). Clinical signs range from mild to severe. Early manifestations include depression, anorexia, fever, tachycardia, tachypnea, congested mucous membranes, and increased digital pulses. Other signs include purulent vulvar discharge, colic-like symptoms, laminitis, or abdominal discomfort. In severe cases, systemic inflammatory response syndrome (SIRS), laminitis, peritonitis, or death may occur (Blanchard et al., 1987; Frazer, 2002; Dolente et al., 2005; Le Blanc, 2008; Blanchard, 2011). Treatment involves systemic stabilization and uterine therapy. Broad-spectrum antimicrobials are the first line of therapy, usually administered empirically before culture results are available. Common choices include β-lactam/aminoglycoside combinations or trimethoprim/sulfonamides (Frazer, 2002; Dolente et al., 2005; Card and Lopate, 2007; Blanchard and McPherson, 2007; Le Blanc, 2008; Blanchard, 2011), with metronidazole added in severe cases or when anaerobic infection is suspected (Card and Lopate, 2007). One study found penicillin G procaine/gentamicin effective against bacteria in 65% of mares, while trimethoprim/sulfonamide in 48.8% (Ferrer and Palomares, 2018). Uterine lavage remains the pivotal component of treatment, as mechanical removal and dilution of uterine contents reduce bacterial load and inflammatory mediators (Blanchard et al., 1990). Ecbolic such as oxytocin support clearance. Supportive therapy may include intravenous fluids when systemic compromise is present. NSAIDs and anti-endotoxic drugs such as flunixin meglumine should be administered to mitigate the effects of endotoxemia (Perkins and Frazer 1994).

1.6. Impact of Assisted Reproductive Techniques

Assisted reproductive techniques (ARTs) are defined as procedures involving the in vitro manipulation of oocytes, semen, and embryos with the purpose of establishing a pregnancy. They are applied to obtain offspring from infertile or subfertile animals, to enhance the reproductive efficiency of animals with high genetic or sporting value, and to preserve the genetic potential of individuals that have died. However, the use of ARTs has also been associated with an increased risk of adverse perinatal outcomes in several species. In equine species, the application of ARTs is relatively recent compared to their widespread use in other domestic animals such as cattle and pigs (Galli et al., 2007). These procedures include ovarian stimulation, semen collection and preservation, in vitro fertilization, intracytoplasmic sperm injection (ICSI), embryo preservation and transfer (ET), and cloning. Such interventions may significantly affect both gametes (Galli et al., 2007; Watkins et al., 2008) and the embryonic microenvironment (Fleming et al., 2015; Hansen, 2015; Hansen et al., 2016). Since early embryonic development is highly sensitive to environmental conditions, with potential long-term consequences on fetal development, neonatal health, and adult physiology (Chavatte-Palmer et al., 2016; Hansen and Siqueira, 2018; Duranthon and Chavatte-Palmer, 2018), it is essential to assess whether ARTs in horses could influence pregnancy outcomes, fetal membranes, umbilical cord morphology, dystocia incidence, and the overall health of mares and foals, as previously demonstrated in bovine and ovine species (Kruip & Den Daas, 1997; Sinclair et al., 1997; Van Wagtendonk-de Leeuw et al., 2000; Lazzari et al., 2002; Miles et al., 2004; Lopes et al., 2020). In equine pregnancies obtained through somatic cell nuclear transfer (SCNT), alterations in fetal membranes and umbilical cord, hydroamnios, hydroallantois, abortion, neonatal maladjustment, enlarged umbilical remnants, and angular limb deformities have been described (Lagutina et al., 2005;

Wilcox et al., 2009; Johnson et al., 2010; Johnson & Hinrichs, 2015; Pozor et al., 2016; Malin et al., 2022). However, only a few studies have investigated the effects of ARTs on foal health and outcomes. (Hunka et al., 2017; Fonte et al., 2023; Lanci et al., 2024). Recent findings showed a significantly different occurrence of dystocia between artificial insemination (AI) and both ICSI and ET mares. Macroscopic alterations of fetal membranes had lower incidence in AI compared to ET and ICSI pregnancies. In addition, umbilical cord length was significantly shorter in ICSI pregnancies compared to AI and ET. Nevertheless, no significant differences were observed among groups in the incidence of neonatal disease or foal survival (Lanci et al., 2024). Regarding neonatal viability at birth, no significant differences were observed between ET and ICSI foals (Fonte et al., 2023). Morphometric and molecular evaluations performed on foals and placentas from naturally conceived, ET, and ICSI groups revealed no significant differences in most parameters (Valenzuela et al., 2018).

Chapter 2. Uterine Vascularization in the Peripartum Mare

Oral presentation “Interaction of parity and age on uterine arteries in peripartурient healthy mares: preliminary study” for the Young Scientist Competition at the 1st TIAR (Turkish & Italian Joint International Congress of Animal Reproduction) Congress, 10-13 October 2024, Antalya (Turkey)

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Interaction of parity and age on uterine arteries in periparturient healthy mares: preliminary study

Author
Francesca Perina – Department of Veterinary Medical Sciences, Bologna University, Via Tolara di Sopra 50, Ozzano dell'Emilia 40064, Italy
Jole Mariella – Department of Veterinary Medical Sciences, Bologna University, Via Tolara di Sopra 50, Ozzano dell'Emilia 40064, Italy
Aliai Lanci – Department of Veterinary Medical Sciences, Bologna University, Via Tolara di Sopra 50, Ozzano dell'Emilia 40064, Italy
Carolina Castagnetti – Department of Veterinary Medical Sciences, Bologna University, Via Tolara di Sopra 50, Ozzano dell'Emilia 40064, Italy
Francesca Freccero – Department of Veterinary Medical Sciences, Bologna University, Via Tolara di Sopra 50, Ozzano dell'Emilia 40064, Italy

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Introduction and aim

Doppler ultrasonography of the uterine arteries is a common diagnostic method to evaluate uterine blood flow and vascularization in the equine species. Maternal age and parity are known to affect horse reproduction; but they may also act as confounding factors, as nulliparous mares are usually young, while older mares are mostly multiparous. Unlike maternal age, parity appears to have a non-linear effect [1]. This study aims to evaluate whether the interaction of parity and age influence the diameter, cross-sectional area and resistance index of the uterine arteries, in pre- and post-partum mares with normal pregnancies and eutocic deliveries.

Methods

Two-dimensional pulsed-wave (PW) Doppler flow ultrasonography of both uterine arteries was performed, using Esaote Mylab Alpha with a 5-10MHz linear probe. This study included healthy mares hospitalized for attended parturition at a Veterinary University Hospital. Transrectal US was performed at various time points: every 5-10 days from admission to parturition (Tpre −30, -20, -15, -10, -5, 0) and within 72h post-partum (Tpost). Diameter (mm), cross-sectional area (cm^2), and resistance index, expressing uterine impedance, of the gravid uterine artery (GUA) and non-gravid uterine artery (NGUA). Mares were divided in four groups according to age (≤10 years Young-Y; >10 years Old-O) and parity (primiparous-P; multiparous-M): YP, YM, OP, OM. Pre-partum time points were then paired for data analysis: -30/-20, -15/-10, -5/0. Differences in all parameters between times (Tpre) within each group, between groups at each time (Tpre, Tpost) and between GUA and NGUA at each time were estimated using non-parametric tests.

Results

Sixty light breed mares were included: 10/60 Italian Saddlebred, 2/60 PSA, 3/60 Quarter Horse, 45/60 Standardbred. YP (n=11) had an average age of 6 (4-9) years, YM (n=19) had an average age of 8 (5-10) years and an average parity of 3 (2-4), OP (n=6) had an average age of 16 (12-22) years, OM (n=24) had an average age of 14 (11-24) years and an average parity of 6 (2-12). For the NGUA, there were no differences in diameter and area across different time points within each group. However, the resistance index was significantly lower in the YM ($p<0.001$), OP ($p=0.018$), and OM ($p<0.001$) groups at Tpre-30/-20, -15/-10, -5/0 compared to Tpost. In the YP, the resistance index did not differ across time points. For the GUA, no differences in diameter were found, but the area was higher at T-5/0 than at Tpost in the YM group ($p=0.004$). The resistance index was significantly at Tpre-30/-20, -15/-10, -5/0 than at Tpost in the YP ($p=0.002$), YM ($p<0.001$), OP ($p=0.028$), and OM ($p<0.002$) groups. Analyzing all parameters among the groups at each time point for the GUA, no differences in diameter and resistance index were observed, while the area was significantly lower at Tpre-5/0 in YP compared to YM ($p=0.011$) and OM ($p=0.015$). For the NGUA: at Tpre-5/0 the diameter and area were significantly lower in YP compared to YM (p=0.026

and p=0.026, respectively) and OM (p=0.003 and p=0.005). At Tpre-15/-10, diameter and area were significantly lower in YP compared to OP (p=0.022 and p=0.032, respectively) and OM (p=0.004 and p<0.001), with area also lower in YP compared to YM (p=0.035). At Tpre-30/-20, the area resulted lower in YP compared to OP (p=0.037) and OM (p=0.008). No differences in all parameters were found between GUA and NGUA except for resistance index in OM, which was significantly lower in GUA (p=0.024).

Discussion and conclusions

In the present study, resistance index declined as expected after parturition. Both diameter and cross-sectional area were influenced by age and parity, with lower values observed in the YP group. This suggests that increasing age and/or parity leads to structural and functional degeneration in the reproductive tract, impacting embryo, placenta, fetal intrauterine growth and foal health [1]. Previous studies have shown that diameter of the uterine artery is affected by parity, while age influenced resistance index during pregnancy [2]. Our findings indicate no differences between GUA and NGUA, suggesting that non-gravid horn, not holding fetal hind limbs, is essential for full fetal development, especially in late pregnancy [3]. After parturition, a rapid uterine involution and a decrease of uterine artery blood flow occur, with an increase in the resistance index [4] that was also observed in this study. Since the OP group is the smallest, but still the most particular and the least studied, future goals will be to increase the number of animals in this group, evaluating whether the parameters of the uterine arteries can give indications for the use of a reproductive assisted technique such as ovum pick-up or embryo transfer in these mares instead of a pregnancy. Furthermore, this preliminary study will allow to have normal reference values, which can be compared with those of mares with different periparturient pathologies, in order to be able to discriminate between these and to be able to intervene promptly.

Keywords: Mares, peripartum, uterine arteries, color doppler, uterine blood flow, parity, age

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Chapter 3. Endocrine and Ovarian Dynamics in the Postpartum Period

Oral presentation "The resumption of ovarian activity in the postpartum mare: what really matters?" for the Young Scientist Competition at the 28th ESDAR (European Society for Domestic Animal Reproduction) Congress, 11-13 September 2025, Albena (Bulgaria).

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ABSTRACTS

Young Scientist Competition

YSC2 | The resumption of ovarian activity in the postpartum mare: What really matters?

F. Perina; A. Lanci; C. Castagnetti; J. Mariella
Department of Veterinary Medical Sciences, University of Bologna, Italy

After parturition mares ovulate within 14 days (d), but alternative patterns can occur. The aim was to evaluate if season, age, parity, type of postpartum (pp) and lactation, affect the resumption of ovarian activity pp. After foaling, follicular dynamics were assessed in 38 light-breed mares every other day until the onset of a dominant follicle (DF> 28 mm), then daily until ovulation (OV). Mean mares' age was 11 years (y) (3–22), 11/38 (29%) were primiparous and 27/38 (71%) multiparous. A DF occurred in 34/38 (89%) mares 5d pp (1–9 d), in 2/38 (5%) 17d pp; the latter two developed haemorrhagic anovulatory follicles. In this study, 2/38 (5%) mares exhibited anoestrous after severe dystocia, C-section, stillbirth, pp metritis; one also developed necrotic vaginitis. OV occurred in 34/38 mares (89%) (OVm): in 32/34 (94%) 11d pp (5–15 d) and in 2/34 (6%) 17d pp. Considering OVm, 10/34 (29%) foaled early (Feb–Mar; 11d) and 22/34 (64%) late in the season (Apr–Aug; 11d); 3/4 (75%) mares that did not ovulate foaled early. The chi-squared test showed that OV occurred more frequently in mares with physiological pp (29/30, 97%; 11d) than with pathological pp (5/8, 63%; 11d) ($p = 0.005$), in multiparous (26/27, 97%; 11d) than in primiparous (7/10, 70%; 10d) ($p = 0.03$), in lactating (32/33, 97%; 11d) than non-lactating (2/5, 40%; 14d) ($p = 0.00011$), and that age (≤10: 15/17, 88%; >10y: 19/21, 90%) and foaling month did not affect OV. Although OV occurred in most mares, it was influenced by parity, type of pp and lactation, as previously described. The resumption of ovarian activity might be disrupted by severe peripartum conditions, warranting further investigation, particularly regarding possible hormonal dysregulation.

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"Exploring the postpartum period in mares: hormonal dynamics and ovarian activity under physiological and pathological conditions"

Manuscript in preparation

Francesca Perina[1], Aliai Lanci[1], Diana Fanelli[2], Saverio Maltinti[2], Carolina Castagnetti[1], Jole Mariella[1]

[1] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, 40064 Bologna, Italy

[2] Department of Veterinary Sciences, University of Pisa, Via Livornese (SP-22), San Piero a Grado, 56122, Pisa, Italy

Introduction

Mares have an early resumption of ovarian activity after parturition, and the first ovulation usually occurs, despite ongoing lactation, within 9-20 days after foaling, during the so-called foal heat (Nagy et al., 1998; Ginther 1992; Sharma et al., 2010; Melchert et al., 2024). It has been observed that mares are at different stages of follicular development at the time of foaling, with those ovulating early postpartum showing more advanced follicular growth soon after parturition (Lemes et al., 2017). Follicular growth resumes prior to foaling, with follicles larger than 10 mm increasing in number during the last two weeks of gestation and the dominant follicle nearly doubling in size; moreover, already before foaling, follicular growth was more pronounced in mares that ovulated within 15 days postpartum than in mares with delayed ovulation (Melchert et al., 2024).

Although ovarian activity continues regularly after the first postpartum ovulation in most mares, alternative patterns have also been described such as continuous ovarian inactivity (anestrous phase) and ovulation at foal heat followed by ovarian inactivity (Palmer and Driancourt 1983; Sharma et al., 2010; Gastal et al., 2021; Gastal et al., 2022; Pastorello et al., 2022).

Mares foaling early in the year experience opposing influences: the endocrine stimulus of parturition, which promotes ovulation, and the suppressive effect of the anovulatory season. A delayed onset of foal heat in early foaling mares has been reported (Heidler et al., 2004) and is attributed to the photoperiod-dependent reduction in LH secretion during winter (Palmer and Driancourt, 1983). However, in most mares the stimulatory endocrine effect of parturition appears sufficient to override the seasonal inhibition. (Nagy et al., 1998; Heidler et al., 2004; Melchert et al., 2024).

The resumption of ovarian activity in postpartum mares is regulated by complex endocrine mechanisms and influenced by seasonal, metabolic, and nutritional factors. Several studies have examined the role of somatotrophic and gonadotropic hormones, follicular dynamics, and physiological conditions during lactation. A strong seasonal effect has been extensively described as negatively affecting the resumption of postpartum ovarian activity (Palmer and Driancourt 1983; Nagy et al., 1998; Sharma et al., 2010; Gastal et al., 2021; Gastal et al., 2022), while parity seems to negatively affect cyclicity, particularly in primiparous mares foaling early in the season (Nagy et al., 1998). Body condition score (BCS), lactational status, and breed do not appear to significantly affect postpartum cyclicity (Nagy et al., 1998; Sharma et al., 2010) although other studies suggested that lactation (Ginther et al., 1994) and low BCS (Henneke et al., 1984; Gastal et al., 2021) may affect the resumption of cyclic activity.

After foaling, cortisol plasma levels decrease, suggesting a reduced HPA response to stress. This adaptation may help the mare to conserve energy required for lactation, protect against stress-associated inhibition of lactation, reduce psychological stress, and support immune function (Piccione et al., 2017; Arfuso et al., 2021).

Plasma concentration of oestrogens and progestogens decrease markedly during the 48 hours preceding parturition. In one study, mean progesterone values were 3.3 ± 0.9 ng/ml on the day of foaling, subsequently falling to < 1 ng/ml until ovulation; oestradiol concentrations ranged from 18 to 30 pg/ml and showed no significant changes during the first five days postpartum (Noden et al., 1978). Gündüz et al. (2008) showed that mean serum progesterone and oestradiol concentrations declined significantly within the first 48 h following parturition. Progesterone levels averaged 2.8 ± 0.26 ng/ml on the day of foaling and decreased to < 1 ng/ml by day 4 postpartum, with a continued decline until the occurrence of the first postpartum oestrus, in agreement with previous findings (Lovell et al., 1975; Hillman & Ganjam, 1979); mean oestradiol concentrations were 420 ± 31.7 pg/ml on day 1 postpartum and decreased steadily to < 300 pg/ml by day 4, continuing to decline until day 8 postpartum.

Leptin, a hormone involved in the regulation of energy balance, is mainly secreted by adipocytes (Zhang et al., 1997). In the horse, leptin concentration is related to fat mass and BCS (Buff et al., 2002; Carter et al., 2009) and it reduces food intake suppressing appetite (McManus and Fitzgerald, 2000; Buff et al., 2005). A decrease in leptin postpartum has been observed in mares (Heidler et al., 2003; Arfuso et al., 2016; Berg et al., 2007;). The decrease in serum leptin may serve to protect mares against negative energy balance by encouraging feed intake (Kedzierski et al., 2011) during early lactation (Arfuso et al., 2016).

Most studies regarding the different patterns of resumption of cyclic activity have been conducted in the Southern Hemisphere or under subtropical climate conditions, focusing on mares with a normal postpartum or on non-lactating cycling mares (Sharma et al., 2010; Gastal et al., 2021; Gastal et al., 2022; Pastorello et al., 2022).

The aims of the present study were: 1) to evaluate whether season, age, parity, type of postpartum, and lactation could affect the resumption of ovarian activity in the early postpartum period, 2) and to determine how circulating hormone concentrations vary among mares with normal postpartum, pathological postpartum, and cyclic non-lactating mares. The study was conducted in the Northern Hemisphere and included lactating postpartum mares and non-lactating cycling mares.

Materials and methods

This investigation was designed as a prospective comparative observational study. All the postpartum mares, hospitalized at the Veterinary Teaching Hospital of the University of Bologna between 2023 and 2024 for attended parturition or within 48h after foaling for postpartum/neonatal diseases, were evaluated. Mares were included when postpartum reproductive activity could be monitored for a sufficient period to evaluate estrous function. Mares were considered to have a normal postpartum period when no signs of retained fetal membranes, postpartum metritis, trauma to the reproductive tract, or other systemic diseases were observed. In mares with a normal postpartum period, only those that could be followed through the first ovulation were included, excluding those discharged soon after foaling. Mares with a pathological postpartum period were also included, regardless of whether ovulation or follicular activity occurred, as long as they could be followed long enough to allow reproductive monitoring. Dystocia was defined as any impediment to the normal progression of foaling due to maternal, fetal, or fetal membrane origin (Lanci et al., 2022). Clinical records for postpartum mares included: age (≤10/>10 years), parity (primiparous/multiparous), breed, BCS (Henneke et al., 1983), foaling month (early: February-March; late: April-August), type of parturition (dystocic/eutocic), type of postpartum (normal/pathological), lactation status (yes/no). The control group consisted of non-lactating recipient mares hospitalized at the University of Pisa for oestrous cycle monitoring. For these mares, clinical records included: age, parity, breed, BCS. For hormonal analysis mares were divided into three groups: Physiological postpartum group (PhyG), Pathological Postpartum Group (PatG), Control Group (CG). For ultrasonographic evaluation, mares were divided into two groups: Postpartum Groups (PPG) and Control Group (CG).

Ultrasonographic evaluation

Follicular activity in PPG mares was assessed with Esaote Mylab Alpha ultrasound (Esaote Lab s.p.A, Genova, Italy) using a 3-9 MHz transrectal transducer. Transrectal ultrasonographic evaluation started soon after parturition or admission (Tpp/Ta: within 48h after foaling) and was performed by one or two experienced veterinarian every other day until the occurrence of a dominant follicle (Td), then every day until ovulation (To). The dominant follicle was defined as the first follicle that reached a diameter >28 mm (Ginther et al., 2004). In postpartum mares with abnormal ovarian activity, ultrasonographic examinations were performed at intervals determined by clinical requirements, typically every other day or less frequently as clinically indicated. The monitoring aimed to assess uterine involution, the presence of intrauterine fluid, other ultrasonographic findings, and to follow follicular dynamics, even when ovulation did not occur. For the CG mares, transrectal ultrasonography was performed with Mindray Dp-50 vet ultrasound (Mindray, Shenzen, China) using a 4-8 MHz transrectal transducer every other day until Td then every day until To.

Hormonal concentrations

Blood was sampled from postpartum mares at Tpp/Ta, Td, and To, and from CG mares at Td and To, to determine concentrations of progesterone, oestradiol-17β, leptin, and cortisol. For mares not exhibiting normal cyclicity (anestrous/anovulatory cycles), the second and third samples were obtained during routine clinical or hematological monitoring, and aliquots were also stored for hormonal analyses, rather than being collected at specific reproductive stages. Blood was collected from the jugular vein into serum tubes (S-Monovette®, Sarstedt). Blood samples were allowed to clot for approximately 30 minutes at 37° and then centrifuged in a refrigerated centrifuge (4°C) at 3000× g for 10 min and stored at −20 ◦C until analysis.

Serum oestradiol (E2) concentrations were measured using Horse Estradiol (E2) ELISA Kit (MyBiosource). Sensitivity is typically less than 40 pg/ml, and the detection range 50 pg/ml-1200 pg/ml. Intra-assay CV%<15%, inter-assay CV%<15%.

Serum cortisol concentrations were measured using Horse Cortisol (COR) ELISA Kit (MyBiosource) Sensitivity was determined to be 0.4 ng/ml (mean of 6 independent assays), detection range 0.4 ng/ml - 80 ng/ml. Intra-assay: CV <8%, inter-assay: CV <12%.

Serum leptin concentrations were measured using Equine Leptin (LEP) ELISA Kit (MyBiosource) sandwich enzyme immunoassay. Sensitivity is typically less than 0.245 ng/ml, detection range 0.625-40 ng/ml. Intra-assay CV <10%, inter-assay: CV<12%.

Hormonal analysis of estradiol, cortisol and leptin were performed at the IRET Foundation (Ozzano dell'Emilia, Bologna, Italy).

Progesterone (P4) concentrations were determined by solid-phase, competitive chemiluminescent enzyme immunoassay using an Immulite 2000 analyser (Siemens). The analytical sensitivity is 0.1 ng/ml. For progesterone concentrations in mares, the sensitivity and specificity were determined at 94% and 95%, respectively, with an intra-assay coefficient of variance (CV) of 9% (Relave et al., 2007).

This study was reviewed and approved by the Animal Care and Use Committee of the University of Bologna (Protocol No. 333764; Approval number: 333764; Approval date: 13 November 2023).

Statistical analysis

Data distribution was analyzed using the Kolmogorov–Smirnov test. Since data were not normally distributed, non-parametric tests were used. Data regarding hormone concentrations are expressed as medians, interquartile ranges and min-max values. To evaluate hormonal changes between the two time points (Td and To) in the CG, the non-parametric Wilcoxon signed-rank test was used. The Mann-Whitney U test was used to compare pathological and physiological postpartum mares groups at each time point. To assess differences among all groups (physiological, pathological, and control) in hormonal concentrations at three time points, the Kruskal-Wallis test was used. Differences in hormone concentrations between groups (age: ≤10 vs. >10 years; primiparous vs. multiparous; early vs. late foaling month) at each time points were analyzed using the Mann–Whitney U test. Associations between ovulation in mares (yes/no) and influencing factors including parity, lactation status (yes/no), foaling month (early/late), mare's age, and type of postpartum (pathological vs. normal) were evaluated using chi-square analysis. Age, parity, BCS, day of dominant follicle, and day of ovulation were expressed as mean, (median; minimum–maximum) values. All analyses were performed using SPSS v.30, with significance set at $p<0.05$.

Results

Thirty-eight postpartum mares were included. Mean mares' age was 11 years (median 11; 3-22 years), 17/38 (45%) had ≤10 years, and 21/38 (55%) had >10 years; 11/38 (29%) were primiparous and 27/38 (71%) multiparous. Twenty/38 (52%) mares experienced eutocic delivery, 12/38 (31%) dystocic delivery, and for 6 out of 38 (15%) mares, parturition was no assisted or information was lacking due to poor anamnesis. Mares had a mean BCS of 6 (median 6; range 4-8). Regarding breed, 25/38 (65%) were Standardbred, 5/38 (13%) were Quarter Horse, 4/38 (10%) were Italian Saddlebred, 1/38 (2%)

was a Thoroughbred, 3/38 (7%) were mixed breed mares. Twenty-five/38 (66%) mares foaled late (April-August) and 13/38 (34%) foaled early (February-March) during the breeding season. Moreover, 30/38 (79%) mares had a physiological postpartum, while 8/38 (21%) had a pathological postpartum period. Mares with pathological postpartum had postpartum metritis (PPM) (n = 7), retained fetal membranes (RFM) (n = 4) mastitis (n = 1) after parturition. These mares also experienced dystocia (n = 5), controlled vaginal delivery (n = 1), C-section (n = 3). The postpartum mares included 33/38 (87%) lactating mares and 5/38 (13%) non-lactating mares. Non-lactating postpartum mares lost their foals due to abortion (n=1), dystocia (n=3) or neonatal problems (n=1).

A total of 28 CG mares were included in the study, and for 11 of them, two consecutive cycles were ultrasonographic monitored, resulting in a total of 39 cycles. Mean mares' age was 10 years (median 11; 4-20 years); 18/28 (64%) were multiparous, 6/28 (21%) primiparous and 4/28 (14%) maiden and the mean BCS was 5 (median 5; range 4-6). Ovulation was pharmacologically induced in 7/39 (17%) cycles with a GnRH agonist (Decapeptyl), and double ovulations occurred in 7/39 (17%) cycles. Ovulation was confirmed in all the cycles evaluated (39/39; 100%). In the 11 mares monitored over two consecutive cycles, the mean inter-ovulatory interval was 18 days (median 19 days, 15-25 days).

Ultrasonographic evaluation

A dominant follicle occurred in 34/38 (90%) mares on mean of 5 days postpartum (median 4; 1-9 days), while in 2/38 (5%) 17 days postpartum and they both developed hemorrhagic anovulatory follicles; one of these two mares had a severe dystocia, controlled vaginal delivery, stillbirth, RFM, PPM and she ovulated only 111 days postpartum, while the other had a mild dystocia, and a sick foal (unable to nurse for orthopedic conditions, euthanized 10 days after birth) and she ovulated 43 days after foaling. Other 2/38 (5%) mares had anestrous after severe dystocia, C-section, stillbirth, PPM, and one of them also developed necrotic vaginitis; the latter one only resumes ovarian activity 100 days postpartum, while the other one was discharged 23 days after foaling without any sign of ovarian cyclic activity. Ovulation occurred in 34/38 (90%) mares while 4/38 (10%) mares did not ovulate. In 33/34 (97%) mares ovulation occurred with a mean of 11 days postpartum (median 10.5; 5-15 days) and in 1/34 (3%) 16 days after foaling. The χ^2 test showed that ovulation occurred more frequently in mares with a physiological postpartum period ($p = 0.005$), in multiparous ($p = 0.03$) and lactating mares ($p = 0.00011$) (Table 1). Age and the foaling month did not statistically affect ovulation).

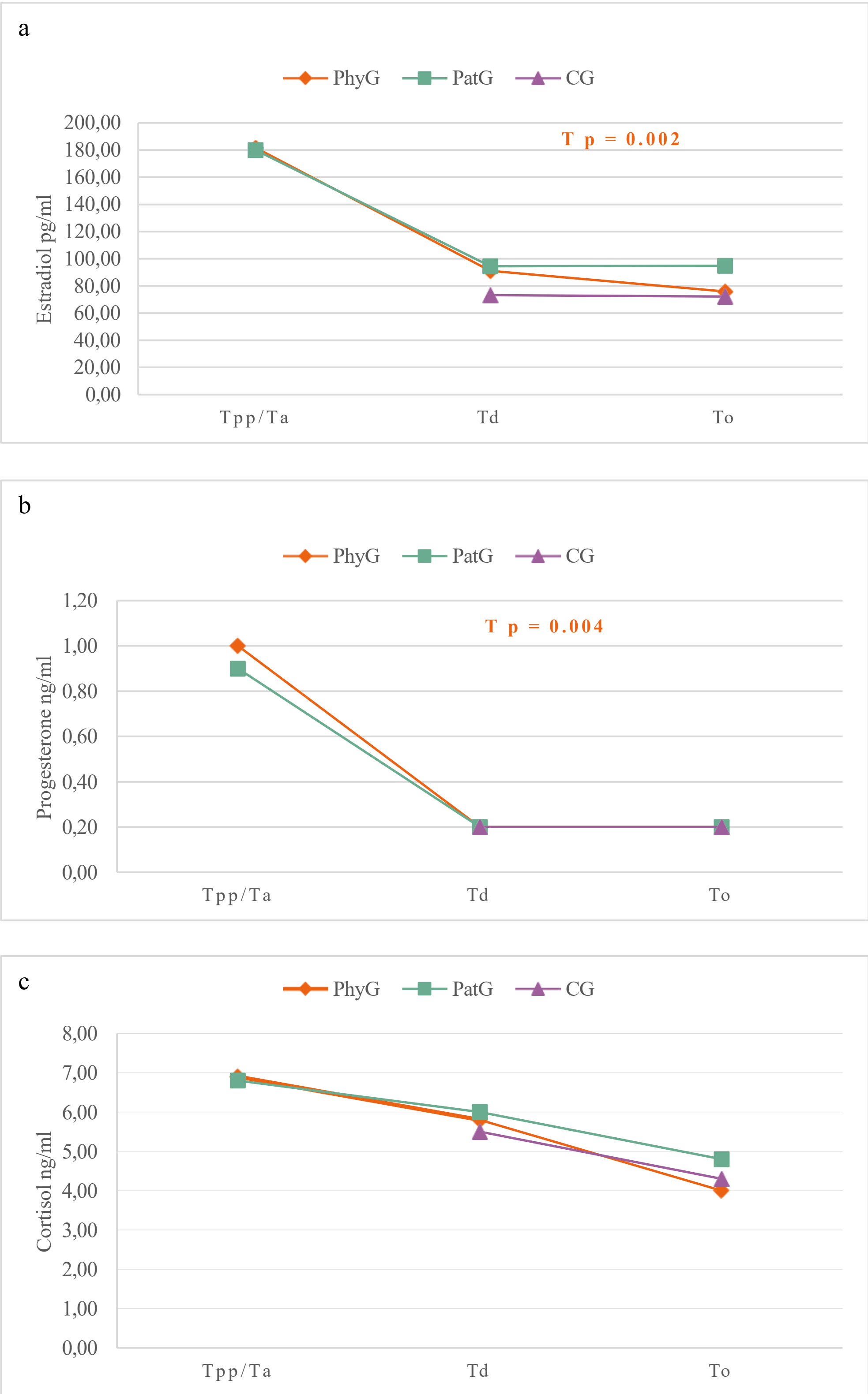
Table 1 Influence of postpartum type, age, parity, lactation status, and foaling month (early: Feb-March; late: Apr-Aug) on ovulation in physiological postpartum (PhyG) and pathological postpartum (PatG) mares. Data are presented as percentages of ovulating and non-ovulating mares, mean; median ovulation days, range (minimum–maximum).

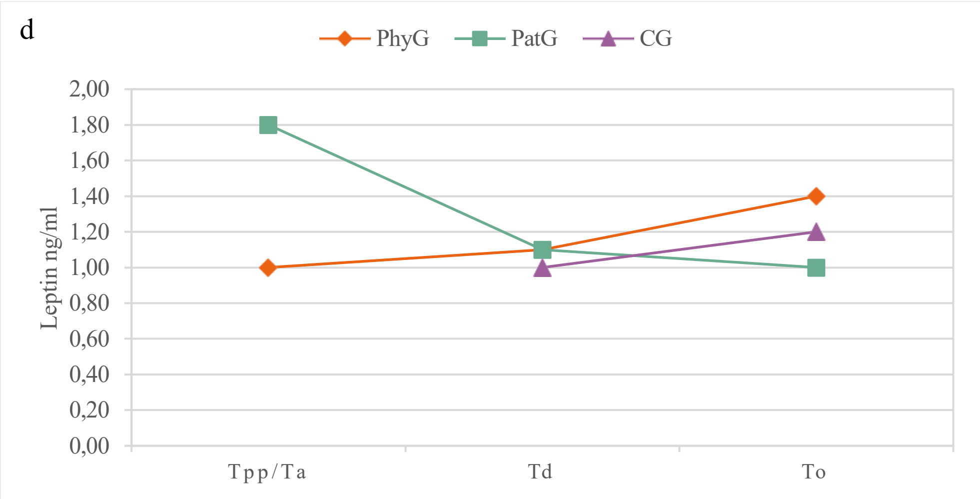
Factors	Groups	Ovulation Mares (%)	Non-Ovulation Mares (%)	Ovulation days
Type of postpartum	PhyG n = 30	29 (97%)	1 (3%)	11;10 (5-16)
	PatG n = 8	5 (63%)	3 (37%)	11;10 (10-14)
Age	≤10 years n = 17	15/17 (88%)	2 (12%)	11;11.5 (5-16)
	>10 years n = 21	19/21 (90%)	2 (10%)	11;14.5 (7-15)
Parity	Primiparous n = 11	7/11 (64%)	4 (36%)	10;13 (5-15)
	Pluriparous n = 27	26/27 (97%)	1 (3%)	11;11 (7-15)
Lactation status	Yes n = 33	32/33 (97%)	1 (3%)	11;10 (5-16)
	No n = 5	2/5 (40%)	3 (60%)	14;14 (13-15)
Foaling month	Early n = 13	10/13 (77%)	3 (23%)	11;11 (8-17)
	Late n = 25	24/25 (96%)	1 (3%)	11;10 (5-16)

Hormonal concentrations

Only considering mares with physiological postpartum, the repeated measures general linear model analysis show that estradiol and progesterone exhibit a significant decrease over time ($p = 0.002$; $p = 0.004$ respectively) (Figure 1 a-b); leptin and cortisol remain stable over time, without significant variation (Figure 1 c-d). For mares with pathological postpartum, the repeated measures general linear model analysis reveals that: cortisol, leptin, progesterone and estradiol levels do not significantly change over time. (Figure 1 a-d). In the control group (CG), no statistically significant differences were observed between the two time points for any of the hormones considered (Figure 1 a-d)

Figure 1 a-d. Hormone concentrations of (a) estradiol, (b) progesterone, (c) cortisol, and (d) leptin at different time points in physiological postpartum (PhyG), pathological postpartum (PatG), and control (CG) mares. Tpp/Ta = postpartum/admission time point; Td = dominant follicle time point; To = ovulation time point.





No significant differences in hormone concentrations were observed across time points, either between PatG and PhyG or among PatG, PhyG, and CG (Table 2).

Table 2. Hormone concentrations of estradiol, progesterone, leptin, and cortisol at different time points in physiological postpartum (PhyG), pathological postpartum (PatG), and control (CG) mares. Data are expressed as mean [interquartile range], min–max. Tpp/Ta = postpartum/admission time point; Td = dominant follicle time point; To = ovulation time point. (*) indicates a significant difference among time points. (#) indicates a significant difference among groups.

Hormone	Time point	PhyG	PatG	CG
Estradiol pg/ml	Tpp/Ta	181.7* [89.9-271] 55.4-584.9	180 [102.7-502.6] 72.9-1200	-
	Td	91* [64.7-127.7] 57.6-532.9	94.5 [52-127.2] 41.8-176.7	73.2 [56.5-121.3] 27.8-1200
	To	75.9* [53.5-109.9] 42.6-355.9	94.9 [46.4-134.4] 46.3-165	72.2 [46.4-114.7] 30.2-1200
Progesterone ng/ml	Tpp/Ta	1* [0.4-2.4] 0.2-3	0.9 [0.3-1.8] 0.2-1.8	-
	Td	0.2* [0.2-0.3] 0.2-0.6	0.2 [0.2-0.3] 0.2-0.7	0.2 [0.2-0.2] 0.2-1
	To	0.2* [0.2-0.4] 0.2-5.9	0.2 [0.2-0.4] 0.2 5.6	0.2 [0.2-0.2] 0.2 5.9
Cortisol ng/ml	Tpp/Ta	6.9 [4.9-9.7] 2.3-33.3	6.8 [5.1-7.9] 3-8.5	-
	Td	5.8 [3.9-7.8] 2.04-16.9	6 [4-10.3] 0.5-11.5	5.5 [3.9-7.8] 0.03-25.7
	To	4 [3.2-6.1] 0.6-23.6	4.8 [4-8] 3.9-10	4.3 [3.4-6] 0.6-23.6
Leptin ng/ml	Tpp/Ta	1 [1-1.8] 0.7-2.6	1.8 [1.3-2.7] 0.5-4.1	-
	Td	1.1 [0.9-2.2] 0.8-2.3	1.3 [0.5-2.2] 0.3-3.2	1 [0.8-1.8] 0.3-3.2
	To	1.4 [1.2-1.8] 0.7-3.8	1 [0.8-1.3] 0.7-1.4	1.2 [0.9-1.6] 0.2-3.8

When hormonal levels at each time point were analyzed according to age, parity, and foaling month, no significant differences were found, except for progesterone at Tpp/Ta, which was lower in primiparous than in multiparous mares (p = 0.048). Although not statistically significant (p = 0.052), a trend toward higher cortisol concentrations at Tpp/Ta was observed in multiparous mares compared with primiparous ones (Table 3).

Table 3. Hormone concentrations (mean [interquartile range], minimum–maximum) of estradiol, progesterone, leptin, and cortisol at different time points in mares, according to age, parity, and foaling month (early: Feb-March; late: Apr-Aug). Tpp/Ta = postpartum/admission time point; Td = dominant follicle time point; To = ovulation time point. (*) indicates a significant difference between groups for the same variable.

Hormone	Variables	Group	Tpp/Ta	Td	To
Estradiol pg/ml	Age	Age ≤10	159.8 [121.2-340.5] 65.1-1200	92.9 [62.7-114.0] 55.5-176.7	87.3 [61.6-119.8] 46.3-135.4
		Age >10	202.7 [86.2-270.3] 55.4-584.9	77.7 [60.8-130.2] 41.8-532.9	73.8 [46.4-113.5] 42.6-355.9
	Parity	Primiparous	112.6 [72.9-181.7] 65.0-1200	94.9 [59.1-110.7] 55.5-176.7	70.3 [46.5-119.6] 46.3-135.4
		Pluriparous	203.6 [112.1-271] 55.4-584.9	86.5 [61.1-127.7] 41.8-532.9	75.9 [50.1-119.9] 42.6-355.9
	Foaling month	Early	203.6 [114.6-440.7] 65-1200	96.0 [72.8-339.1] 55.5-532.9	109.9 [52.6-129.8] 46.3-355.9
		Late	146.8 [97.3-225.2] 55.4-393.5	69.0 [60.5-110.2] 41.8-137.9	75.4 [48.2-87.3] 42.6-165.0
Progesterone ng/ml	Age	Age ≤10	0.9 [0.3-1.8] 0.2-3	0.2 [0.2-0.2] 0.2-0.7	0.2 [0.2-4.9] 0.2-5.9
		Age >10	0.8 [0.4-2.3] 0.2-2.8	0.2 [0.2-0.3] 0.2-0.6	0.2 [0.2-0.3] 0.2-1.5
	Parity	Primiparous	0.4* [0.3-1.2] 0.2-1.8	0.2 [0.2-0.2] 0.2-0.7	0.2 [0.2-2.6] 0.2-5.6
		Pluriparous	1.6* [0.5-2.4] 0.2-3	0.2 [0.2-0.3] 0.2-0.7	0.2 [0.2-0.4] 0.2-5.9
	Foaling month	Early	1.2 [0.4-2.1] 0.3-2.8	0.2 [0.2-0.4] 0.2-0.7	0.2 [0.2-0.2] 0.2-2.6
		Late	0.6 [0.3-2.2] 0.2-3	0.2 [0.2-0.2] 0.2-0.4	0.2 [0.2-1.1] 0.2-5.9
Leptin ng/ml	Age	Age ≤10	1.7 [1.1-2.1] 0.8-2.2	1.4 [0.7-2.2] 0.3-2.3	1.3 [1.1-3.0] 0.7-3.8
		Age >10	1.0 [0.9-1.4] 0.5-4.1	1.1 [0.9-1.6] 0.5-3.2	1.2 [0.9-1.5] 0.7-1.8
	Parity	Primiparous	1.5 [1.0-1.9] 0.5-2.0	0.9 [0.5-1.9] 0.3-2.2	1.2 [0.9-1.4] 0.7-2.4
		Pluriparous	1.0 [0.9-1.8] 0.8-4.1	1.2 [1-2.2] 0.8-3.2	1.3 [1-1.6] 0.7-3.8

	Foaling month	Early	1.3 [0.9-2] 0.5-2.2	1.1 [0.7-2.0] 0.3-3.2	1.2 [0.9-1.5] 0.7-2.4
		Late	1.2 [0.9-1.8] 0.8-4.1	1.1 [0.8-2.0] 0.7-2.3	1.2 [1.1-1.7] 0.7-3.8
Cortisol ng/ml	Age	Age ≤10	6.9 [5.2-7.7] 4.6-20.5	4.6 [2.2-9.4] 0.5-16.9	3.6 [2.7-4.7] 0.6-23.6
		Age >10	7.1 [4.3-9.2] 0.8-33.3	6.2 [4.6-7.8] 3.1-11.5	4.7 [3.9-7.2] 0.6-10.0
	Parity	Primiparous	4.9 [4.6-7.7] 3.0-7.7	4.3 [2.7-5.5] 0.5-9.9	4.0 [3.4-4.9] 0.6-7.4
		Pluriparous	8.1 [5.9-9.7] 0.8-33.3	6.8 [4.7-7.8] 2.0-16.9	4.7 [3.2-7.2] 0.6-23.6
	Foaling month	Early	5.9 [4.7-7.9] 3.0-9.7	5.1 [3.3-6.2] 0.5-7.8	4.0 [3.7-4.0] 0.6-7.4
		Late	6.9 [5.1-10.3] 0.8-33.3	7.4 [4.5-9.0] 2.0-16.9	4.2 [3.0-7.6] 0.6-23.6

Discussion

This study aims to evaluate factors that may influence the resumption of cyclic activity after foaling in the mare and, for the first time, to investigate the specific effect of the type of postpartum period. In particular, mares with pathological postpartum have been directly compared with those experiencing normal postpartum and with non-postpartum cycling mares, not only to assess the impact on the return to cyclicity but also to explore how pathological postpartum may influence the concentrations of specific reproductive hormones and their possible interrelationships.

In the present study, lactation did not negatively affect the resumption of cyclic activity. In fact, lactating postpartum mares tended to ovulate more frequently than non-lactating mares. This is in contrast with Nagy et al. (1998), who reported that ovarian activity in mares that lost their foal within the first two days postpartum was similar to that of suckling mares, indicating that suckling per se does not delay postpartum ovarian activity. Although our results differ in terms of the direction of the effect, both studies suggest that lactation or suckling alone does not inhibit ovarian activity in mares. However, since most non-lactating mares have experienced severe peripartum complications, these apparent positive effects of lactation may have been confounded by the nature and severity of postpartum complications affecting the non-lactating mares, rather than by lactation itself. Among the non-lactating mares, three of four did not ovulate and had foaled out of season. While not statistically significant, seasonality may have contributed to delayed cyclic activity alongside postpartum complications. Under adequate nutritional management, lactation does not generally impair fertility or cyclic resumption in mares (Heidler et al., 2004; Neuschaefer, 1990). Plasma glucose levels in lactating mares are slightly reduced during early postpartum but remain within physiological limits, suggesting that increased feed intake compensates for energy demands (Heidler et al., 2004). Therefore, lactation per se does not appear to inhibit ovarian activity, if energy balance and health are maintained. In our population, BCS values were within normal ranges, and all mares maintained good condition, which likely supported timely ovulation. Previous studies have shown that mares with BCS < 5.0 exhibit delayed estrus, lower conception rates, and longer intervals between foaling and first ovulation compared with mares of moderate or high condition (Henneke et al., 1984; Gastal et al., 2022).

In our study, multiparous mares were more likely to ovulate postpartum than primiparous mares, highlighting a clear effect of parity on the resumption of ovarian activity. This observation is consistent with Nagy et al. (1998), who reported delayed first and second ovulations in primiparous mares, particularly when foaling early in the breeding season, likely due to the metabolic and physiological stress associated with the first lactation. Similarly, Sharma et al. (2010) found that primiparous mares were less likely to display postpartum estrous behavior, although parity did not affect the occurrence of the foal-heat ovulation. While parity and age are often interrelated under field conditions, this relationship is not always strict. Increasingly, older mares enter reproduction for the first time, resulting in a population of "older primiparous" mares. In our dataset, the correlation between age and parity was moderate, indicating partially independent effects. As Sharma et al. (2010) noted, although parity is often confounded by age, observed differences in postpartum ovarian activity are likely attributable to parity itself rather than age, consistent with tropical field studies showing no effect of age on the interval from foaling to first ovulation (Winter et al., 2007). In the present study, although not statistically significant, multiparous mares showed a tendency toward higher cortisol concentrations at Tpp/Ta compared with primiparous mares. This finding is consistent with Leitner et al. (2020), who reported significantly lower maternal cortisol concentrations and a delayed prepartum increase in primiparous mares and, although not significant, lower cortisol concentrations during the postpartum period. Higher cortisol levels in multiparous mares may reflect an earlier activation of the fetal-maternal HPA axis before foaling and a more advanced endocrine preparation for foaling (Leitner et al.,2020), Consequently, our findings, may reflect a less pronounced activation of the fetal-maternal HPA axis in primiparous mares. In our study, progestin concentrations at Tpp/Ta were significantly higher in multiparous mares compared with primiparous mares. This difference may be related to placental size, as larger placentas in multiparous mares could produce more progestins, consistent with observations in Warmblood versus pony mares, where higher relative placental weights are associated with higher plasma progestin concentrations (Nagel et al., 2020). Conversely, Leitner et al. (2020) did not observe significant differences in maternal progestin concentrations between primiparous and multiparous mares either pre or postpartum. These contrasting findings indicate that the influence of parity on maternal progestin levels is not yet fully understood and may depend on different factors.

Although the foaling month did not have a statistically significant effect in our study, a trend toward lower ovulation rates during the early breeding season was observed. Notably, three of the four mares that did not ovulate within 15 days postpartum foaled before March 18. In ovulating mares, ovulation occurred within 15 days after parturition, indicating that the resumption of ovarian activity was generally rapid. Among the mares that failed to ovulate early postpartum, two ovulated at 43 and 110 days postpartum, while the remaining two mares resumed cycling activity at approximately 23 and 100 days postpartum; however, subsequent ovulations could not be monitored as both mares were discharged before ovulation was observed. Photoperiod strongly influences reproduction in horses: short days suppress gonadotropins, while increasing day length stimulates LH pulses and ovarian activity (Palmer and Driancourt, 1983; Ginther, 1992). Consequently, foal heat is often delayed in early-foaling mares (late winter/spring) in the Northern Hemisphere (Koskinen, 1991; Nagy et al., 1998; Palmer and Driancourt, 1983). Mares that failed to ovulate within 15 days postpartum foaled about one month earlier than those that did, likely reflecting the stronger stimulatory effect of increasing day length later in the season (Melchert et al., 2024). Nevertheless, parturition itself is a potent stimulus that can induce ovulation even during the anovulatory season (Palmer and Driancourt, 1983; Loy, 1980). According to two recent studies by Gastal et al. (2021, 2022), spring-foaled mares had longer parturition ovulation intervals and lower LH levels than mares foaling later in the season. Moreover, regular ovarian cycles resulted more common in mares foaling after the spring equinox than before it (Gastal et al., 2021). In contrast, mares kept under subtropical or tropical conditions show little or no seasonal variation in postpartum reproductive activity, likely due to the smaller monthly changes in photoperiod (Sharma et al., 2010). Overall, these findings suggest that, while there may be a subtle trend toward delayed ovulation in early season foaling mares, most mares exhibit rapid postpartum ovarian recovery, and parturition acts as a strong stimulator of ovulation. However, since subsequent cycles were not systematically monitored in this study, the long-term re-establishment of normal cyclicity in all mares remains uncertain.

A key finding of this study is the association between pathological postpartum events and delayed or absent ovulation. Mares with such complications often develop hemorrhagic anovulatory follicles or prolonged anestrus. Although systemic hormone concentrations did not differ significantly, ovarian function was clearly impaired. To the best of our knowledge, no studies have evaluated ovarian function or the resumption of cyclic activity in mares affected by postpartum disorders. In contrast, in dairy cows, several studies have investigated the impact of uterine disease on ovarian dynamics and reproductive performance. Metritis has been associated with a reduction in follicle diameter and progesterone concentrations and a tendency to decrease plasma estradiol concentrations (Seekford et al., 2025). Previous studies have shown that uterine disease or infection reduces follicle growth and estradiol secretion during the first estrous cycle following parturition (Sheldon et al., 2002; Williams et al., 2007; Williams et al., 2008; Strüve et al., 2013). Another important consequence of uterine inflammation is the suppression of luteinizing hormone (LH) secretion from the anterior pituitary, as inflammatory cytokines released during uterine infections interfere with LH release, thereby disrupting the hormonal balance required for ovarian follicle maturation and ovulation (Peter et al., 1989; Huszenicza et al., 1999; Sheldon et al., 2002). Cows with a higher uterine bacterial load on the day of calving show slower ovarian follicle growth (Cheong et al., 2017), and greater uterine bacterial contamination is associated with reduced follicular growth and decreased estradiol production (Sheldon et al., 2002). Bacterial endotoxins can directly inhibit estradiol production by granulosa cells in vitro (Williams et al., 2008). Impaired estradiol synthesis represents a key difference between non-ovulatory and ovulatory follicles during the early postpartum period (Cheong et al., 2015). Collectively, these studies suggest that uterine diseases may impair ovarian function, which could contribute to the reduced fertility commonly observed in cows following the resolution of uterine disorders. Another possible explanation for the relatively high percentage of non-ovulation observed in the pathological group could be related to the fact that almost all mares with postpartum disorders, except one, were affected by metritis and therefore received non-steroidal anti-inflammatory treatment (flunixin meglumine, 1.1 mg/kg intravenously twice daily). It has been reported that the administration of high dose of flunixin meglumine within the preovulatory time window can inhibit ovulation (Cuervo-Arango and Domingo Ortiz, 2011), although another study has found no such effect (Cuervo-Arango, 2011). Nevertheless, two studies reported a high incidence of hemorrhagic anovulatory follicles (HAFs) in mares treated with flunixin meglumine (Cuervo-Arango and Domingo Ortiz, 2011; Martinez-Bovì et al., 2023). It should also be noted that, even in mares with a normal postpartum period, different ovarian reproductive patterns have been described after foaling. While most mares ovulate and subsequently resume regular cyclic activity, alternative patterns such as continuous ovarian inactivity (anestrous phase) and ovulation followed by ovarian inactivity have been observed (Palmer and Driancourt 1983; Sharma et al., 2010; Gastal et al., 2021; Gastal et al., 2022; Pastorello et al., 2022). Therefore, even though such alternative patterns can naturally occur during the postpartum period, the absence or delay of ovulation observed in some mares of the pathological group may reflect factors beyond normal variability, highlighting that the potential influence of postpartum disorders should not be underestimated.

In the present study, no significant differences in leptin concentrations were observed among the different postpartum groups, nor when compared with the group of non-lactating cycling mares. This finding contrasts with the results reported by Heideler et al. (2003), who observed markedly lower leptin concentrations between 2 and 4 weeks after foaling in postpartum mares compared with non-postpartum mares. According to those authors, the decrease in leptin levels likely reflects an adaptive response aimed at increasing feed intake to meet the elevated energy demands associated with lactation. The discrepancy between the two studies may be related to differences in the sampling protocol: while Heideler et al. collected samples weekly, in the present study blood samples were obtained only at three specific time points, and in most cases, ovulation occurred around day 11 postpartum. Therefore, the timing of sampling may have coincided with a period in which leptin levels were still elevated, masking the subsequent decrease reported in previous studies.

Cortisol concentrations, similarly, showed no significant effect of time or group, with comparable values observed in the control and postpartum mares. Similarly, Fazio et al. (2019) found no significant differences between non-pregnant/non-lactating mares and pre- or postpartum mares, consistent with our findings. Conversely, Arfuso et al. (2021) reported higher cortisol levels in postpartum mares compared to non-lactating controls, although no significant temporal variations were detected in their study, similar to our findings. Another study reported that peripartum mares showed a statistically significant decrease in cortisol levels during the postpartum period compared to prepartum but not difference between postpartum and non-lactating mare, except for the first sampling after parturition, and no effect of time in the postpartum period (Piccione et al., 2017). At foaling, cortisol concentrations in mares are markedly increased compared to the already elevated prepartum values (Nagel et al., 2012); however, this rise is limited to the short expulsive phase of foaling, and then returns to baseline values within two hours after the birth of the foal (Melchert et al., 2019). The reduction in cortisol concentrations observed after foaling may indicate an attenuated stress response to physical and/or psychological stress during the postpartum period (Ousey, 2004). Studies conducted in humans have suggested that HPA axis attenuation in postpartum females plays a crucial role in reducing anxiety and optimizing maternal care (Ousey, 2004; Bosch et al., 2007). This physiological adaptation could help the mare conserve the energy required for lactation, protect against stress-associated inhibition of milk production, alleviate psychological stress, and enhance immune function (Piccione et al., 2017).

Both estrogens and progestogens show a sharp decline during the first 48 hours after parturition, followed by a slower decrease until the first ovulation (Gunduz et al., 2008). According to Noden et al. (1978), plasma progesterone concentration remains low until ovulation and increase again toward the end of the foal heat, whereas estrogens are basal for several days after foaling, rise at the onset of estrus, and decrease again at ovulation. Similarly, Nagel et al. (2012) reported a continuous decrease in progestin concentrations from the late prepartum phase through the first postpartum days. In the present study, although no significant differences among groups or over time were detected, the hormonal patterns observed were consistent with these general trends. Mares in the normal postpartum group showed a tendency toward decreasing progesterone and estradiol concentrations from foaling to ovulation, whereas hormone levels remained relatively stable in mares with pathological postpartum conditions and in non-lactating mares. In non-lactating mares, progesterone remained low, as expected during estrus, and estradiol levels appeared stable, or at least did not show statistically significant variations, likely because both sampling points occurred at times when estrogen concentrations were similar, either before the preovulatory peak or after its decline.

Conclusion

This study provides new insights into the factors influencing the resumption of ovarian cyclicity after foaling in mares, with particular emphasis on the impact of postpartum complications.
Lactation, when supported by good nutritional and management conditions, did not affect the return to cyclicity, confirming that energy balance plays a crucial role in postpartum fertility. Parity emerged as a relevant factor, as multiparous mares showed a greater likelihood of early ovulation and higher progestin concentrations compared with primiparous mares, possibly reflecting a more advanced endocrine preparedness for foaling and cyclic resumption. Although no significant hormonal differences were detected between groups, mares experiencing pathological postpartum events exhibited a markedly delayed or absent ovulation, indicating impaired ovarian function. This finding, together with evidence from other species, suggests that postpartum complications, particularly uterine inflammation, may negatively affect follicular development and steroidogenesis and highlight the importance of considering the potential long-lasting effects of postpartum health problems on fertility, rather than focusing only on the early return to cyclicity. Overall, these results highlight that the physiological recovery of reproductive function after foaling depends on a complex interplay of physiological, environmental, management, and health-related factors. Future research should include a larger number of animals and apply multivariate analyses to simultaneously evaluate the influence of multiple interacting factors. Such an approach would help clarify the relative contributions of environmental, physiological, and pathological conditions to reproductive recovery, ultimately improving management strategies and fertility outcomes in the postpartum mare.

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Chapter 4. Postpartum Implications of Assisted Reproductive Technologies

Poster presentation "Could assisted reproductive techniques affect equine fetal membranes and neonatal outcome" at the 1st ESAR (European Symposium on Animal Reproduction) Congress, 21-23 September 2023, Nantes (France).

Could assisted reproductive techniques affect equine fetal membranes and neonatal outcome?

ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA
DEPARTMENT OF VETERINARY MEDICAL SCIENCES

Perina F. [1], Armani S. [2], Mariella J. [1], Iacono E. [1], Merlo B. [1], Castagnetti C. [1] and Lanci A. [1]

[1]Department of Veterinary Medical Sciences, University of Bologna, [2]Practitioner

: francesca.perina2@unibo.it

Introduction

Assisted reproductive techniques (ARTs) has been defined as procedures that involve in vitro manipulation of oocytes, semen and embryos with the aim of establishing a pregnancy; embryo transfer (ET) and intracytoplasmic sperm injection (ICSI) are widely used in equine species, but their effects have not been investigated yet.

Aim

The aim of this study was to retrospectively evaluate whether the production of in- vivo or in- vitro embryos may be associated with the presence of macroscopic alterations of fetal membranes (FM) and umbilical cord (UC) and whether they could influence neonatal outcome.

Materials and Methods

Clinical reports of 66 mares of mixed medium to long size breeds and their foals obtained by ET, ICSI and artificial insemination (AI) hospitalized for attended delivery at the Equine Perinatology and Reproduction Unit (Equine Clinical Service, Veterinary Teaching Hospital of the Department of Veterinary Medical Sciences, University of Bologna, Italy) from 2014 to 2022 were reviewed.

Results

Mares were divided into AI (32/66, 48.5%), ET (12/66, 18.2%) and ICSI (22/66, 33.3%) group. Macroscopic examination of FM revealed alterations in 30/66 mares (45.5%): 8/32 in AI (25%), 7/12 in ET (58.3%) and 15/22 in ICSI (68.2%) with significant differences between ET and AI ($p = 0.04$), ICSI and AI ($p = 0.002$). Alterations reported: chorionic villi hypoplasia, chorioallantois edema, allantois cysts, necrotic areas and greenish-gray concretions. Total length of UC resulted significantly shorter in ICSI (49.3±8.6; $p < 0.03$) compared with AI (60.2±16.7) and ET (59.2±14.8). The chi-square test did not show differences in the incidence of foals' diseases at birth and foals' survival among groups.

Discussion and conclusion

Equine embryos production may result in a higher frequency of FM alterations which do not seem to be associated with a higher incidence of neonatal morbidity and mortality. Alterations resulting from the use of ART are well documented in cattle and ovine species and they are mainly related to embryonic culture techniques, in horses the most significant alterations are observed in cloning techniques. It would be interesting to further investigate the possible consequences resulting from the use of ART, involving a larger number of animals and histologically evaluating fetal membranes and placental gene expression.

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Original Research Article

Could assisted reproductive techniques affect equine fetal membranes and neonatal outcome?

Aliai Lanci [a,*], Francesca Perina [a], Sabrina Armani [a], Barbara Merlo [a,b], Eleonora Iacono [a,b], Carolina Castagnetti [a,b], Jole Mariella [a]

[a] Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50- 40064-Ozzano Dell' Emilia (BO), Italy

[b] Health Science and Technologies Interdepartmental Center for Industrial Research (HST-ICIR), University of Bologna, Bologna, Italy

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ABSTRACT

Embryo transfer (ET) and intracytoplasmic sperm injection (ICSI) are widely used in equine species, but their effects on fetal adnexa and neonates have not been investigated yet. The aim of this study was to retrospectively evaluate whether pregnancies obtained by ET or ICSI could be associated with the presence of macroscopic alterations of fetal membranes (FM) and umbilical cord (UC) and if the use of these techniques could influence neonatal outcome. Sixty-six light breed mares hospitalized at the Veterinary Teaching Hospital, University of Bologna, for attending delivery were included in the study. Mares were divided into Artificial Insemination (AI; 32/66 mares, 48 %), Embryo Transfer (ET; 12/66 mares, 18.2 %) and Intracytoplasmic Sperm Injection (ICSI; 22/66 mares, 33 %) groups. All the medical reports of mares and their foals were reviewed and data about mare, pregnancy, foaling, fetal membranes, umbilical cord and foal were recorded. The occurrence of dystocia resulted statistically different between AI group and ICSI group ($p = 0.0066$), and between AI group and ET group ($p = 0.044$). Macroscopic examination of FM revealed alterations in 30/66 mares (46 %): 8/32 in AI (25 %), 7/12 in ET (58 %) and 15/22 in ICSI (68 %) with significant lower incidence in AI compared to ET ($p = 0.04$) and ICSI ($p = 0.002$) groups. Alterations reported were chorionic villi hypoplasia, chorioallantois edema, allantois cysts, necrotic areas and greenish-grey concretions. Total length of UC resulted significantly shorter in ICSI group (49 $\pm$ 9 cm; $p < 0.03$) compared to AI (60 $\pm$ 17 cm) and ET (59 $\pm$ 15 cm). However, there were no differences in the incidence of foals' diseases at birth and in foals' survival among groups ($p > 0.05$). The results demonstrate that transfer of *in vivo* or *in vitro* produced embryos may lead to alterations of placental development, as observed in other species, without being associated with a higher incidence of neonatal morbidity and mortality. Further studies about trophoblast development, FM histological evaluation, and placental gene expression should be carried out to clarify the mechanisms underlying the placental alterations.

1. Introduction

Assisted reproductive techniques (ARTs) has been defined as procedures that involve *in vitro* manipulation of oocytes, semen and embryos with the aim of establishing a pregnancy. In equine species, the use of these techniques is relatively recent compared to other domestic species, such as cattle and pigs [1]. These techniques include ovarian stimulation, semen collection and preservation, *in vitro* fertilization, intracytoplasmic sperm injection, embryo preservation and transfer, and cloning procedures, which can induce significant modifications in gametes [2,3] and in embryonic microenvironment [4–6]. The early stages of embryo development are known to be strongly affected by environmental conditions, with long-term effects on fetus, newborn foal and adult health [7–9]. It is therefore essential to evaluate whether the use of ARTs in equine species could affect pregnancy, fetal membranes and umbilical cord, the onset of dystocia and the health of mare and foals as extensively reported in bovine and ovine [10–15].

Although it is known that pregnancies produced by somatic cell nuclear transfer (SCNT) in the mare could present fetal membranes alterations, umbilical cord abnormalities, hydroamnios, hydroallantois, abortion, maladjustment, enlarged umbilical remnant, and angular deformity of the forelimbs [16–21], only few studies have focused on the potential of postnatal consequences of ARTs [22–24].

The aim of this study was to retrospectively evaluate whether the

* Corresponding author. Department of Veterinary Medical Sciences, University of Bologna, via Tolara di Sopra 50, 40064 Ozzano Emilia (BO), Italy.
E-mail address: aliai.lanci2@unibo.it (A. Lanci).

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transfer of *in vivo* or *in vitro* produced embryos may be associated with the presence of macroscopic alterations of fetal membranes and umbilical cord and whether the use of these techniques could influence pregnancy and the neonatal outcome.

2. Materials and methods

2.1. Population and data collection

All the medical records of mares and their foals obtained by ET, ICSI and artificial insemination (AI) and hospitalized for attending delivery at the Equine Perinatology and Reproduction Unit (Department of Veterinary Medical Sciences, University of Bologna, Italy) from 2014 to 2022 were reviewed. Mares with pregnancy obtained with artificial insemination that gave birth during 2019 were considered the control group. Data collected at admission and during hospitalization were obtained from clinical records and from the hospital veterinary medical information system (FeniceVET®, ZakSoft srl, Bologna, Italy).

Mares hospitalized for attending delivery were admitted at approximately 310 days of gestation and remained under around-the-clock observation until at least 7 days post-partum. The mares were housed in separate wide straw-bedded boxes with night vision cameras, fed hay ad libitum and concentrates twice a day, and were allowed to go to pasture during the day. All mares received a complete physical examination twice a day during hospitalization and a complete blood cell count and blood chemistry at admission. Additionally, transrectal palpation and ultrasonographic examination were performed to evaluate the combined thickness of the uterus and placenta (CTUP), fetal presentation and vitality, and quality of fetal fluids at admission and every ten days until parturition. In case of suspected high-risk pregnancy, a transabdominal ultrasonographic examination was performed to evaluate CTUP and fetal biophysical profile. The reference ranges of CTUP were considered in relation to gestational age, as reported elsewhere [25]. High-risk pregnancy was defined by the occurrence of a history of premature udder development/lactation, increase in combined thickness of the uterus and placenta, purulent/serosanguineous vulvar discharge and/or systemic illness of the mare [26]. All parturitions were attended to promptly intervene in case of dystocia. Dystocia was defined as any impediment to normal delivery due to complications of maternal, fetal and/or fetal membrane origin [27,28].

The following data were recorded for all mares: breed, age, parity, weight at admission (kg), ARTs conception method (AI, ET, ICSI), gestation length (days), type of pregnancy (normal or high-risk), duration of stages II and III (min, h respectively), dystocia resolution procedure (assisted vaginal delivery - AVD, controlled vaginal delivery - CVD, fetotomy or cesarean section) [28], gross fetal membranes alterations [29,30], placental total weight (kg), chorioallantoic weight (kg), fetal membranes/foal weight ratio (%) [31], gross umbilical cord (UC) alterations, total UC length (cm), total umbilical coil number, umbilical coiling index (UCI: umbilical coils divided by umbilical cord length) [32], length and number of coils of the allantoic and amniotic portion of the UC, site of UC insertion, placental vascularization pattern (type 1, type 2, type 3) [33], postpartum complications, outcome (discharge, death or euthanasia).

The following data were collected from all foals at birth: weight (kg), sex, APGAR score [34], temperature (°C), time of onset of suckling reflex (min from birth), time to acquire sternal recumbency (min from birth), standing position (min from birth) [35], first intake of colostrum (min from birth), IgG concentration at 12–24 h of life (via DVM Rapid Test II immunoturbidimetric test, MAI Animal Health, Elmwood, WI), neonatal diseases, outcome (discharged, death or euthanasia). Stillbirth was recorded.

Mares and their respective foals were divided into three groups accordingly to the conception method: AI group, ET group, and ICSI group.

2.2. Statistical analysis

Data were analyzed for normality with the Kolmogorov-Smirnov test. Given the non-normal distribution of data, nonparametric tests were used for statistical analysis. Data were expressed as mean, standard deviation, median, minimum and maximum values. The chi-squared test (χ^2) was used to evaluate the presence of a significant difference between the AI, ET and ICSI Groups for categorical variables. The Kruskall-Wallis test was used to evaluate the presence of a significant difference between the AI, ET and ICSI groups for numerical variables. If a significant difference was found, groups were subsequently compared with the Mann-Whitney test. A $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using SPSS software (IBM® SPSS® Statistics).

3. Results

Sixty-six mares were included in this study and divided into three groups: AI group (32/66 mares; 48 %) as control; ET group (12/66 mares; 18 %); ICSI group (22/66 mares; 33 %).

Regarding the breed, 52/66 mares were Standardbred (78.8 %), 9/66 Italian Saddlebred (13.6 %), 3/66 Quarter Horse (4 %) and 2/66 Thoroughbred (3 %). In the ET group, 8/12 were Standardbred with a fetus of the same breed (67 %), 2/12 Standardbred with Italian Saddlebred fetuses (17 %), 1/12 Standardbred with a Quarter Horse fetus (8 %), and 1/12 Thoroughbred with an Italian Saddlebred fetus (8 %). In the ICSI group, 19/22 were Standardbred mares with the fetus of the same breed (87 %), 1/22 Standardbred with an Italian Saddlebred fetus (5 %), 1/22 Standardbred with a Quarter Horse fetus (5 %), and 1/22 Italian Saddlebred with a fetus of the same breed (5 %).

Data regarding mares, pregnancy, foaling and postpartum are reported in Table 1. Regarding the high-risk pregnancies in AI group, 5/32 mares (16 %) had placental edema, 3/32 (9 %) had suspected placentitis, 1/32 (3 %) had abdominal hernia and 1/32 (3 %) had rupture of the prepubic tendon. Of these mares, 7/10 (70 %) had dystocic delivery. In ET group, 1/12 (8 %) had placental edema and had dystocia.

Regarding dystocia, some mares had more than one cause of dystocia in all groups. They included: maternal origin such as uterine inertia, ineffective abdominal contractions and reduced size of the birth canal, fetal origin such as abnormal attitudes, positions and presentations and fetal membranes origin, such as premature placental separation. Details regarding the clinical description of the dystocic parturition and foals and mares' outcome are summarized in Table 2.

Regarding sick foals born from dystocia, in AI group 3 stillborn were born with CVD due to fetal malposition; one stillborn with AVD due to premature placental separation and fetal malposition. One foal experienced AVD due to uterine inertia and had congenital flexural limb deformities. In ET group, 2 foals with perinatal asphyxia syndrome (PAS) and 1 foal with congenital flexural limb deformities were born with AVD due to fetal malposition. In ICSI group, 2 foals with congenital flexural limb deformities and 2 foals with umbilical remnant diseases were born with AVD due to fetal malposition. Additionally, 3 foals with PAS were born with AVD resulting from maternal causes of dystocia.

The chi-square test did not show any difference in the incidence of dystocia between groups ($p > 0.05$). Considering the total population, dystocia occurred in 36/66 mares (54.5 %). Only considering mares with a normal pregnancy (55/66), dystocia occurred in 22/55 mares (40 %): significant differences were found between AI group and ICSI group ($p = 0.0066$), and between AI group and ET group ($p = 0.044$); no significant differences were found between ET group and ICSI group ($p > 0.05$).

Post-partum complications occurred in 24/66 mares (36 %) without differences between groups ($p > 0.05$): 13/24 (54 %) in AI group, 3/24 (13 %) in ET group and 8/24 (33 %) in ICSI group. In all groups, some mares had more than one complication.

Data regarding macroscopic evaluation of the fetal membranes and

Table 3
Macroscopic characteristics of fetal membranes and umbilical cord and fetal membranes/foal weight ratio (%) in AI, ET and ICSI Groups. Data are expressed as mean ± standard deviation, and median (minimum - maximum values).

	AI Group (N = 32)	ET Group (N = 12)	ICSI Group (N = 22)
Fetal membranes weight (kg)	5.3 ± 1.1 5.4 (3.2–7.7) (n = 32)	5.2 ± 1.1 5.3 (3.9–6.7) (n = 12)	4.8 ± 0.9 4.8 (3.1–6) (n = 22)
Chorioallantoic membrane weight (kg)	3.5 ± 0.8 3.3 (2.2–5.9) (n = 32)	3.5 ± 0.9 3.5 (2.3–5.2) (n = 12)	3.4 ± 0.7 3.3 (2.4–4.8) (n = 22)
Vascular pattern	16/19 type I (84 %) 3/19 type II (16 %) (n = 19)	4/6 type I (67 %) 1/6 type II (17 %) 1/6 type III (17 %) (n = 6)	6/8 type I (75 %) 2/8 type III (25 %) (n = 8)
Fetal membrane alterations (n)	8/32 (25 %) (n = 32)	7/12 (58 %) * (n = 12)	15/22 (68 %) * (p = 0.002) (n = 22)
Fetal membranes/ foal weight ratio (%)	11 ± 2 11 (7–17) (n = 32)	11 ± 2 11 (8–17) (n = 12)	11 ± 1 11 (8–14) (n = 22)
Total umbilical cord length (cm)	60 ± 17 58 (30–103) (n = 32)	59 ± 15 55 (43–85) (n = 11)	49 ± 9 *(p < 0.03) 50 (34–65) (n = 21)
Allantoic umbilical cord length (cm)	31 ± 11 29 (10–54) (n = 32)	31 ± 12 30 (15–55) (n = 11)	23 ± 7 23 (12–37) (n = 21)
Amniotic umbilical cord length (cm)	30 ± 10 30 (13–60) (n = 31)	28 ± 10 26 (20–55) (n = 11)	26 ± 8 25 (16–39) (n = 21)
Total umbilical cord coils (n)	6 ± 2 5 (3–10) (n = 32)	5 ± 2 5 (2–9) (n = 11)	5 ± 1 5 (2–9) (n = 22)
Allantoic umbilical cord coils (n)	3 ± 1 3 (1–8) (n = 32)	3 ± 1 3 (1–3) (n = 11)	2 ± 1 2 (1–6) (n = 22)
Amniotic umbilical cord coils (n)	3 ± 1 3 (1–6) (n = 31)	2 ± 1 2 (1–6) (n = 11)	2 ± 1 3 (1–4) (n = 22)
Umbilical coiling index (UCI)	0.10 ± 0.03 0.10 (0.06–0.19) (n = 32)	0.09 ± 0.02 0.08 (0.05–0.14) (n = 11)	0.09 ± 0.02 0.09 (0.06–0.15) (n = 21)
Umbilical cord insertion	4/30 base of pregnant horn (13 %) 5/30 base of non-pregnant horn (17 %) 21/30 between two horns (70 %) (n = 30)	2/10 base of pregnant horn (20 %) 8/10 between two horns (80 %) (n = 10)	8/19 base of pregnant horn (42 %) 11/19 between two horns (58 %) (n = 19)
Umbilical cord alterations (n)	5/32 (16 %) (n = 32)	2/12 (17 %) (n = 12)	3/22 (14 %) (n = 22)

The presence of (*) indicates a significant difference between the groups in the row.

4. Discussion

This study aimed to evaluate if pregnancies achieved by *in vivo*- or *in vitro*-produced embryos may be associated with the presence of macroscopic fetal membranes and umbilical cord alterations. In addition, it was evaluated if the use of these techniques may affect the course of pregnancy and the occurrence of neonatal diseases.

In the present study, most of the recipients included were Standardbred mares in both ET and ICSI groups. However, other morphologically similar breeds have been used and due to morphology and size similarity, no abnormalities were found in neonatal growth, as reported by Valenzuela et al. [24]. Conversely, many abnormalities have been observed when embryos and mare's breed were not similar [36–41]. In the equine species, the development of these anomalies depends on the type of placentation, since the nutritional supply to the fetus, which depends on the contact surface between the placenta and the endometrium, is determined by uterus and mare's size [39]. Fetal development is therefore correlated with the size of the recipient, the weight of the placenta, the gross placental area, and the microcotyledonary density [37,41]. If a recipient is smaller than the breed of the embryo, intrauterine growth retardation (IUGR) may occur, as it happens in other conditions that interfere with functional placental area, such as in twin pregnancies, placentitis, or in case of severe atrophy of chorionic villi [42].

In the present study, no difference was found between foal's weight at birth in the ET and ICSI groups compared to AI group. This is in agreement with literature, since in the equine species, unlike in bovine and ovine species, the production of *in vivo* and *in vitro* embryos is not associated with the development of Large Offspring Syndrome (LOS). In the equine species, excessive fetal growth is extremely rare, as the size of the mare and placenta influences fetal size [37,41]. In cattle and sheep, LOS can occur *in vitro* embryo production (IVEP) [43,44] and more frequently in SCNT [45]. It can occur in late gestation and involves a variety of fetal, placental and neonatal developmental defects [46]. Numerous studies have shown that abnormal fetal and placental development in ruminants is due to the presence of serum in the culture medium and the use of co-cultures [44,47]. Notwithstanding, during *in vitro* maturation of equine oocytes 10 % calf serum is utilized, while on day 6 after ICSI it is replaced with 10 % of a mixture (1:1) of fetal calf serum and serum replacement [48].

The high incidence of high-risk pregnancies in the present study in the AI group may be due to the fact that AI mares are predominantly chosen for their genetic value and sports performance, while recipient mares were younger, with parity ranging from 1 to 3 and less likely to develop a high-risk pregnancy [28]. In this study, a very high incidence of dystocia (54.5 %) was detected, even considering only mares with a normal pregnancy and admitted exclusively for attending delivery (40 %). This high percentage is partially related to the fact that this study was conducted in an equine hospital facility where mares with high-risk pregnancy and fetal posterior presentation were referred, as reported in a previous retrospective study about dystocia [28]. In the literature, dystocia's rates are decidedly lower, ranging from 2.7 % to 16 % in Thoroughbred, Standardbred and Quarter Horse [28,49,50]. The higher incidence of dystocia was observed in the ICSI group where, on one hand, there were no high-risk pregnancies, but on the other hand the maternal causes of dystocia were the most prevalent. This can be explained by the fact that all ICSI mares that had dystocia were primiparous, and dystocia is more common in primiparous mares than in multiparous ones [28,49,50]. The same observation can be done for the mares in the ET group.

In the post-partum period, the most frequent complications in mares were retention of fetal membranes (RFM) and reproductive tract trauma, which were more frequent following dystocia. According to literature, the incidence of RFM increases of about 50 % after dystocia [51], while obstetric manipulation led to increased injuries [52] and infections of the genital tract [53]. Despite the high incidence of dystocia, the ET and ICSI groups had a lower incidence of postpartum complications compared to the AI group, probably because in the last group there were older and multiparous mares.

Macroscopic alterations of fetal membranes were more prevalent in ET and ICSI groups, as preliminary observed by Lanci et al. [23]. However, in other studies, these alterations were not observed in IVEP pregnancies [22,24], but were present in SCNT ones, where placental alterations were very common and have been observed in apparently healthy foals [20,21]. In SCNT derived pregnancies, chorionallantoic edema, hypoplasia, atrophy and necrosis of villi, areas of placental hemorrhage and necrosis, placentitis, enlarged allantoic vessels with thrombi and signs of vasculopathy have been observed [16–18,20].

At macroscopic observation of the umbilical cord, UC length was shorter in ICSI group. The possible consequences of a reduced UC length

Fig. 1. Chorioallantois edema (a); chorionic areas of necrosis (b–c); a short umbilical cord (d); multiple umbilical cord cysts (e–f); allantois cysts (g); chorionic villi hypoplasia (h–i).

in horses are still unknown. However, Whitehead et al. [33] have shown that it was not associated with abortion or other placental alterations, whereas in humans a reduced cord length is known to be associated with reduced bone mineralization in the newborn due to limitations in fetal movements during intrauterine life [54,55]. Macroscopic alterations of the UC are very frequent in equine pregnancies derived from SCNT and include dilated umbilical vessels with thrombi and signs of vasculopathy, excessive length, thickening and coiling of the cord and the presence of cystic formations [15–19–21].

Unlike in cattle, where some authors report a higher frequency of male calves compared to females in pregnancies obtained from IVEP and SCNT [12,43,56,57], while others have observed a similar proportion [15,58,59], in this study the foal's sex had a similar distribution.

Although significant differences in the presence of macroscopic alterations of fetal membranes were detected in the ET and ICSI groups, these did not result in a higher frequency of sick foals at birth. In fact, in the ET and ICSI groups there are no stillbirth foals, whereas they were 12.5 % in the AI group, involving foals born from high-risk pregnancies and dystocic delivery; only about half of them also showed macroscopic alterations of fetal membranes. These observations are in agreement with the literature for foals obtained by ET and ICSI, indicating no adverse effects from ARTs on neonatal health in the perinatal period [22, 60]. Conversely, SCNT pregnancies often result in abortions, perinatal death, or birth of sick foals, which may present neonatal maladjustment syndrome, dysmaturity, pneumonia, umbilical cord swelling, omphalitis, umbilical hernias, blood clots in the urinary bladder, angular

Table 4
Foals' sex, weight, clinical findings and serum IgG concentration in AI, ET and ICSI groups. Data are expressed as mean ± standard deviation, and median (minimum - maximum values). Neonatal encephalopathy (NE), umbilical remnant diseases (URD), congenital flexural limb deformities (CFLD), prematurity (Prem), meconium impaction (MecImp), congenital inguinal hernia (CIH), perinatal asphyxia syndrome (PAS).

Parameter	AI group (N = 32)	ET group (N = 12)	ICSI group (N = 22)
Foal sex (M: male; F: female)	20 M (63 %) 12 F (38 %) (n = 32)	7 M (58 %) 5 F (42 %) (n = 12)	13 M (59 %) 9 F (41 %) (n = 22)
Foal weight (kg)	48 ± 7 49 (28–59) (n = 32)	46 ± 6 45 (38–59) (n = 12)	44 ± 4 44 (38–54) (n = 22)
APGAR score	9 ± 1 9 (8–10) (n = 28)	9 ± 1 9 (7–10) (n = 12)	9 ± 1 9 (7–10) (n = 22)
Rectal temperature (°C)	37.6 ± 0.4 37.6 (36.4–38.2) (n = 28)	37.6 ± 0.3 37.7 (37.1–38.2) (n = 12)	37.4 ± 0.3 37.5 (36.7–38) (n = 22)
Time to sternal recumbency (min)	4 ± 3 4 (0–12) (n = 28)	4 ± 3 4 (0–10) (n = 11)	4 ± 4 3 (0–15) (n = 22)
Time to standing position (min)	67 ± 26 60 (26–150) (n = 28)	89 ± 47 82 (47–205) (n = 11)	72 ± 18 67 (18–111) (n = 22)
Time to suckling reflex (min)	42 ± 29 29 (10–104) (n = 28)	46 ± 49 38 (5–180) (n = 11)	41 ± 48 25 (2–255) (n = 22)
Time to first intake of colostrum (min)	76 ± 26 73 (30–120) (n = 28)	91 ± 35 90 (31–142) (n = 12)	109 ± 49 106 (41–267) (n = 22)
IgG (mg/dL)	1265 ± 608 1157 (396–3681) (n = 27)	1658 ± 505 1854 (777–2315) (n = 11)	1684 ± 632 1600 (700–3000) (n = 22)
Neonatal diseases (n)	18/28 healthy (64 %) 10/28 sick (36 %) (n = 28)	8/12 healthy (67 %) 4/12 sick (33 %) (n = 12)	15/22 healthy (68 %) 7/22 sick (32 %) (n = 22)
Pathological condition (n)	NE 3/10 (30 %) URD 2/10 (20 %) CFLD 2/10 (20 %) Prem 1/10 (10 %) MecImp 1/10 (10 %) CIH 1/10 (10 %) (n = 10)	PAS 2/4 (50 %) URD 1/4 (25 %) CFLD 1/4 (25 %) (n = 4)	PAS 3/7 (43 %) URD 3/7 (43 %) CFLD 2/7 (29 %) MecImp 1/7 (14 %) (n = 7)

deviations and flexural limb deformities, incomplete calcification of carpal bones, multiple rib fractures, and brachygnathism [18,20,21].

5. Conclusions

In the equine species, the production of *in vivo* and *in vitro* embryos may result in a higher frequency of dystocic delivery and fetal membranes alterations. Although these findings, ARTs do not seem to be associated with a higher incidence of neonatal morbidity and mortality; it is well known that the type of parturition and placental insufficiency can affect fetal development and foal's health. Even though they are not considered high-risk foals, like those obtained with SCNT, it would be recommendable a closely monitoring during the perinatal period to promptly detect any problems and appropriately intervene. Despite alterations resulting from the use of ARTs are well-documented in ruminants and they are mainly related to embryonic culture techniques, in horses the most significant alterations are observed in cloning techniques. It would be interesting to further understand the possible consequences resulting from the production of *in vivo* and *in vitro* embryos in the equine species investigating the hormonal parturition pathway of the recipient mare, and histologically evaluating fetal membranes and placental gene expression, in order to highlight the possible mechanisms responsible for placental abnormalities.

CRediT authorship contribution statement

Aliai Lanci: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Francesca Perina:** Writing – review & editing, Visualization, Data curation. **Sabrina Armani:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Barbara Merlo:** Writing – review & editing, Visualization, Formal analysis, Conceptualization. **Eleonora Iacono:** Writing – review & editing, Visualization, Conceptualization. **Carolina Castagnetti:** Writing – review & editing, Visualization, Data curation, Conceptualization. **Jole Mariella:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization.

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Chapter 5. Postpartum Disorders in the Mare

Retained fetal membranes in light breed mares

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Retained fetal membranes in the light breed mare: A retrospective study from 2017 to 2024

Author

Laura Fischetti, Francesca Perina, Jole Mariella, Carolina Castagnetti, Aliai Lanci – Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Ozzano dell'Emilia, Bologna, Italy

Citation

Fischetti, L., Perina, F., Mariella, J., Castagnetti, C., Lanci, A. Retained fetal membranes in the light breed mare: A retrospective study from 2017 to 2024.

Introduction and Aim

Although the reported incidence in non-draft breeds is as low as 2–10%, Retained Fetal Membranes (RFM) is considered one of the most common peripartum problems in mares (1). Predisposing factors include advanced age, uterine or systemic infections before or during pregnancy, dystocia or prolonged gestation (2). However, previous studies are scarce, dated, and with controversial results. Therefore, the aim of this study was to retrospectively describe the incidence, predisposing factors, consequences and treatment of RFM in light breed mares hospitalized at the Veterinary Teaching Hospital (VTH) of the University of Bologna.

Methods

The medical records of 243 mares admitted at the VTH from 2017 to 2024 were reviewed. Normal and high-risk pregnant mares hospitalized for attended delivery were included. High-risk pregnancy was defined by the history of premature lactation, increased combined thickness of the uterus and placenta, vulvar discharge and/or systemic illness of the mare. Dystocia was defined as any impediment to normal delivery due to complications of maternal, fetal and/or fetal membrane origin. RFM was defined as failed expulsion (partial or complete) of fetal membranes > 3h after parturition (3). The following data were collected from all mares: age, parity, assisted reproduction technique (ART) used, type of pregnancy (normal or high-risk), gestational length, NSAIDs administration during pregnancy, type of foaling (eutocia or dystocia) and occurrence of PPM. Treatments of RFM were also recorded. Mares without RFM were included in the C group and mares with RFM in the R group. Categorical variables (mare's age: $\geq$10 y or <10 y, parity, ART: ET/ICSI or AI, type of pregnancy and foaling, NSAIDs treatment and PPM) were tested with the χ^2 test. Differences between groups (C vs R) were analyzed using Student's t-test for the quantitative variables (mare's age and gestational length). A $p < 0.05$ was considered statistically significant.

Results

Group C included 195/243 mares (80%), and group R included 48/243 mares (20%). Considering only normal pregnancies, the incidence of RFM was 17% (32/191 mares). High-risk pregnant ($_{hr}$) mares were diagnosed with placentitis (14/52, 27%), placental edema (17/52, 33%), hydrops (3/52, 6%) and systemic illness (18/52, 35%). The incidence of RFM was higher in high-risk pregnant mares (16/52 mares, 31%). In high-risk pregnant mares (n=52), the incidence of RFM was reduced when NSAIDs were administered during pregnancy (p=0.0001) (C_{hr} group 30/36 mares, 83% vs R_{hr} group 6/16 mares, 37%). The incidence of RFM was not related to parity, mare's age (≥ 10 y) and ART. No difference was found between the two groups for mare's age (C group 11±6 y; R group 11±5 y) and gestational length (C group 339±11 d; R group 337±13 d). Premature deliveries (<320 d) were 8/243 (3%), 5/8 in C group and 3/8 in R group. The incidence of RFM was higher in mares with dystocia (24/58 mares, 41%) than in normal foaling (24/185 mares, 13%). The incidence of PPM was higher (p<0.0001) in R group (29/48 mares, 60%) compared to the C group (5/196 mares, 2%). In the R group, 21/48 (44%) mares partially retained fetal membranes and 18/21(86%) developed PPM, while 3/21 (14%) did not. Regarding RFM treatments, 13/48 (27%) mares received 10-20 IU oxytocin (ox) IV boluses, 2/48 (4%) a combination of 10-20 IU ox IV boluses and ox IV infusion, 31/48 (65%) a combination of 10-20 IU ox IV boluses and uterine lavages and 1/48 (2%) all treatments. All mares were treated successfully and completely recovered.

Discussion and Conclusions

In the present study, the incidence of RFM in light-breed mares was higher compared to literature, but the previously reported 2–10% incidence (1) did not evaluate the type of pregnancy. The higher incidence of RFM in high-risk pregnancies found in the present study agrees with data reported (2, 4). In contrast, RFM was not related to advanced age (4) nor parity. For the first time, NSAIDs administration during late-term high-risk pregnancies was correlated to a lower incidence of RFM but more research is needed to confirm it. In conclusion, the pathophysiology of RFM is multifactorial and not yet completely elucidated (5). Although not considered in this study, serum calcium and phosphate imbalances have been reported among risk factors for RFM in draft mares (6), but further investigations are needed in other breeds.

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Cytokines expression in mares with postpartum metritis

Poster presentation "Cytokines' profile of the low-volume lavage in mares with normal postpartum and septic metritis" at the 13th ISER (International Symposium on Equine Reproduction) Congress, 10-14 July 2023, Foz do Iguaçu, (Brazil)

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Cytokines' profile of the low-volume uterine lavage in mares with normal *postpartum* and septic metritis

Jole Mariella[1,*], Francesca Perina[1], Alice Caneschi[1], Maurizio Scozzoli[2], Maura Cescatti[3], Carolina Castagnetti[1], Anna Zaghini[1], Aliai Lanci[1]

[1] *Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy*
[2] *APA-CT Srl, Forlì, Italy*
[3] *Fondazione IRET – Onlus, Bologna, Italy*

Even though metritis is one of the most common puerperal diseases in mares, there is a lack of information with regard to the condition. The aim of this study was to evaluate cytokines' profile in uterine fluid collected with low volume lavage (LVL) from mares with normal *postpartum* period (PPP) (group 1; N=11) and from mares with septic metritis (group 2; N=12). Septic metritis was defined as the presence of hyperthermia (>38.5°C), depression, an enlarged and an excessive amount of variably echogenic uterine fluid. Mares were treated with oxytetracycline IV q12 h for 5 days, high volume uterine lavage with saline q12 h until necessary. In group 1, LVL was performed 36-48 hours after parturition (T1) and then after 2 days (T2). In group 2, LVL was performed when evidence of septic metritis appeared and then after 2 days of treatment. The samples were centrifuged, the supernatant was removed and stored at -80°C until analysis. Cytokines were measured simultaneously, using Equine Cytokine/Chemokine Panel (Merck-Millipore), based on xMAP technology. Analyzed cytokines were monocyte chemoattractant protein-1 (MCP-1), fibroblast growth factor-2 (FGF-2), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (INF)-γ, tumor necrosis factor (TNF)-α, interleukins (IL)-1β, 1α, 2, 4, 5, 6, 8, 10, 13, 18. Data are given as median in pg/mL. Differences between groups were analyzed by Mann-Whitney test and between sample times by Wilcoxon test. In group 2, the concentration of most cytokines was larger than in group 1: IL-6 (2256.3 vs 188.2; $p<0.01$), IL-8 (94.8 vs 72.1; $p<0.05$), IL-1β (13738.7 vs 16.5; $p<0.01$), TNF-α (393.2 vs 0.4; $p<0.05$), INF-γ (224.5 vs 31.6; $p<0.05$), IL-10 (39.5 vs 7.9; $p<0.05$). The concentrations of IL-18, IL-13, and FGF-2 did not differ between groups, while IL-1α, 2, 4, 5, MCP-1 and GM-CSF were not detectable. In group 1 there was an increase ($p<0.05$) in IL-10, IL-5 and IL-18, while in group 2 there was no difference between T1 and T2. All mares with septic metritis recovered well and without any complications. Results suggest that, although an inflammatory response is already present in mares with normal PPP to ensure a proper endometrial regeneration and uterine involution, it is stronger in mares with septic metritis. In both groups, a high percentage (>60%) of neutrophils was detected, suggesting that in physiologic uterine involution, an inflammatory response is required. In agreement with findings from cattle, more neutrophils than monocytes and macrophages are recruited. An intense inflammatory response persists for at least 4 days. It should be noted that metritis was treated without any empirical use of intrauterine antibiotics as suggested by the WHO.

Chapter 6. Clinical Case Report

Oral presentation "It is not always what it seems: Chorioallantois cervical pole necrosis" at the 1st ESAR (European Symposium on Animal Reproduction) Congress, 21-23 September 2023, Nantes (France).

CCO 7 | **It is not always what it seems: Chorioallantois cervical pole necrosis**

J. Mariella; L. Fischetti; N. Ellero; F. Freccero; G. Sarli; C. Castagnetti; F. Perina
Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia, Italy

A mare at 286 days of pregnancy was presented for premature udder development and ventral oedema. The ultrasound exams revealed

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a slightly increased CTUP (1 cm) and a viable fetus with heart rate of 86 bpm and oligohydramnios. No vulvar discharge was present and the cervical swab culture was negative. Progesterone concentrations measured every 2 days were 5.64, 6.86 and 11.70 ng/mL. Eight days later, the mare aborted a moderately autolyzed fetus. The amniotic portion of the umbilical cord was 135 cm long and excessively twisted with segmental dilatations of the urachus. The chorioallantois showed a brown discoloration and rough surface from the cervical pole up to the base of the horns. The amniotic vessels were distended with engorgement and haemorrhage. *E. coli* was isolated from fetal membranes, fluids and tissues. Histologically, rare neutrophils, degeneration and necrosis with dystrophic calcification of the chorial villi were localized at the cervical pole. The final diagnosis was abortion due to placental infarction with secondary placentitis. Cervical pole necrosis (CPN) is a poorly understood and probably a misdiagnosed disease of the allantochorion, since the macroscopic lesions resemble an ascending placentitis. The histological exam is necessary to have a definitive diagnosis in cases of macroscopical abnormalities of fetal membranes. This case of CPN is associated with secondary bacterial infection of fetus and placenta which likely developed due to bacterial invasion through the devitalized allantochorion. The increase in progesterone is suggestive of chronic placental damage and/or activation of fetal hypothalamic-hypophysis-adrenal axis.

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Case Report

It is not always what it seems: allantochorion cervical pole necrosis

L. Fischetti [a], F. Perina [a,*], G. Sarli [a], N. Ellero [a], F. Freccero [a], C. Castagnetti [a,b], J. Mariella [a]

[a] Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, Bologna 40064, Italy
[b] Health Sciences and Technologies Interdepartmental Center for Industrial Research (CIRI-SDV), Bologna University, 40100, Italy

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ABSTRACT

A 286-day pregnant mare was referred for signs of impending abortion. The ultrasonographic evaluation revealed a high normal combined thickness of the utero-placental unit (CTUP), oligohydroallantois and a living fetus. No vulvar discharge was observed, and the cervical swab culture was negative. After a choke, the mare received NSAIDs and antibiotics and then developed systemic inflammatory response syndrome (SIRS) and aborted. The amniotic portion of the umbilical cord was excessively long and twisted and the chorionic side of the placenta showed a brown discoloration and rough surface area. *E. coli* was isolated from all the fetal membranes, fetal fluids and tissues. Histologically, degeneration and necrosis with dystrophic calcification of the chorial villi at the cervical pole was found. The final diagnosis was abortion due to placental infarction with secondary placentitis. Cervical pole necrosis is a poorly understood condition of the allantochorion, and a histological exam is necessary to obtain a definitive diagnosis.

1. Introduction

Pregnancy loss in mares may occur at any stage of gestation from fertilization to parturition and is a major problem due to its economic and emotional losses [1–3]. Causes of abortion, stillbirth and neonatal death in horses may vary over time and according to the geographical region [4–7]; non-infectious and infectious causes are involved [3].

Non-infectious abortions have been described as accounting for 10.5 %–72 % of the total number of abortions and may occur at any stage of pregnancy in horses; causes include abnormalities of gestation and fetal membranes, i.e. umbilical cord (UC) and placenta, as well as maternal and fetal factors [8]. The pathological conditions of the UC represent the major cause of non-infectious abortion in the mare. In most cases, the cord is too long and twists to such an extent that UC flow of blood and urine is impeded or even completely obstructed, resulting in fetal death. This anomaly is known as UC torsion (UCT) and is responsible for more than half of all non-infectious abortions [9–13]. Cervical pole necrosis, also known as placental necrosis or placental infarction, is a poorly understood disease of the allantochorion and an infrequent cause of non-infectious abortion in horses. Currently, the pathogenesis of this unique lesion is not definitively known and only theories have been proposed to explain the presence of necrosis [14].

Placentitis is a common cause of abortion, stillbirth and neonatal death in horses [1,7,9,10,15,16]. Four morphologic types of placentitis have been described according to the type of lesions and potential pathogenesis: ascending, focal mucoid (nocardioform), diffuse (hematogenous), and multifocal [17]. Ascending placentitis, which is caused by bacteria or fungi ascending through the cervical canal and infecting the placenta, is the most prevalent form of placentitis [18–21]. This condition is clinically characterized by premature udder development, increased uteroplacental thickness at the level of the cervical pole and mucopurulent vaginal discharge. If placentitis is untreated or not early treated, placental function is compromised, placental separation ensues and pathogens can spread to the fetus, resulting in fetal death and expulsion or the birth of a premature and/or septic compromised foal [22]. In chronic placentitis, the fetus may show intrauterine growth retardation. In this report, abortion in a 15-year-old Warmblood mare at 293-day gestation due to UCT, and cervical pole necrosis with secondary placentitis is described.

2. Case presentation

2.1. Ethical Statement

The owner provided informed consent, as part of the hospital consent form, for the use of the horse's data in the study.

* Corresponding author.
E-mail address: francesca.perina2@unibo.it (F. Perina).

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2.2. Presenting complaint and history

In March 2023, a 15-year- old multiparous Warmblood mare at 286 days of pregnancy was admitted to the Equine Perinatology and Reproduction Unit (University of Bologna, Italy) for signs of impending abortion and abdominal discomfort. The owners reported that she had four previous normal pregnancies without complications. The mare had been housed with other horses, daily fed with hay and concentrates but was not regularly vaccinated nor dewormed. At the breeding soundness examination prior to artificial insemination, the uterine swab cultured positive for *E. coli*. Endometrial cytology was not performed. The mare did not receive any treatment. Due to a poor perineal conformation, a Caslick vulvoplasty has been performed.

2.3. Clinical findings and follow up

On presentation, the mare was in moderate body condition (BCS 5/9), with mild colic signs (constantly kicking and watching the flank), moderate tachypnea (36 breaths/min), normal body temperature (37.7°C), and normal heart rate (40 bpm). She showed laxity of pelvic ligaments, premature udder development, premature lactation and ventral edema. No vaginal discharge was observed, and a poor perineal conformation was confirmed. Since there were signs of impending parturition, the Caslick vulvoplasty was promptly opened. Vaginal examination with a speculum revealed no obvious abnormalities. A cervical swab was taken for bacteriological examination: microorganisms were not cultured. Complete blood cell count (CBC) and blood chemistry parameters were within normal limits (ADVIA 2120, Siemens Healthcare srl, Milan, Italy; Chemistry Analyzer AU400; Olympus Diagnostica GmbH, Lismeehan, Ireland) and serum progesterone (P4) was 5.64 ng/mL (Immulite 2000, Siemens) which was physiological for this gestational age (normal range 3–6 ng/mL at 110-300 days of pregnancy) [13]. Transrectal ultrasound (US) showed a high normal combined thickness of the utero-placental unit (CTUP) of 10.3 mm [23,24]. The fetus was in anterior presentation, and fetal fluids had a normal echogenic appearance. Transabdominal US showed a physiological fetal heart rate at rest of 83 bpm (82–98 bpm at 10 months of pregnancy) [24, 25] and moderate fetal activity. The fetal aortic diameter was 18.7 mm which was lower than the normal values (22.25 ± 1.5 at 10 months of pregnancy) [24]. The CTUP in the mid-caudal and right-caudal areas measured 5.4 and 6.1 mm respectively and fluid's depth was subjectively reduced. No placental detachment was observed. One day after, at 288 days of gestation, US examination confirmed the reduced volume of fetal fluids except for the mid-cranial area of the abdomen. The fetal hearth rate remained within normal limits. At 289 days of pregnancy, serum P4 increased to 6.86 ng/mL.

2.4. Treatment and outcome

At 290 days of pregnancy, the mare accidentally injured the left masseter muscle that became edematous and painful by the night and dysphagia with a concurrent transitory episode of esophageal obstruction occurred the following day. On US of the thorax, multiple confluent B-lines associated with initial focal consolidation of lung parenchyma in the left cranio-ventral quadrant were visible. Parameters of CBC were within normal limits except for a slight neutrophilia ($9.3 \times 10^6/\mu L$; normal ranges $2.2–8.5 \times 10^6/\mu L$) and serum amyloid A (SAA) level increased exponentially to 311 µg/dL (reference range 0–10 µg/dL); therefore, flunixin meglumine (1.1 mg/kg IV BID), broad-spectrum antibiotic treatment with procaine benzylpenicillin and dihydrostreptomycin (15000 UI/kg IM SID) and gastric protection with sucralfate (20 mg/kg PO TID) were started associated with a soft food diet. P4 rapidly peaked at 11.70 ng/mL.

At 292 days of gestation, the mare was depressed with poor appetite, hyperthermia (39.1 °C), tachycardia (56 bpm) and mild tachypnea (24 bpm), and she developed profuse, watery and malodorous diarrhea. Total CBC revealed an increased PCV (40 %), severe leukopenia ($2.4 \times 10^3/\mu L$ WBC; normal ranges $5.5–12.5 \times 10^3/\mu L$) and further increase of SAA (316 µg/mL). Microbiological fecal exam and blood culture tested negative. On transabdominal US, the fetus had a normal heart rate of 90-100 bpm at rest, and subsequently fetal viability was monitored every 4 to 6 h. Considering the clinical signs and collateral exams, the mare was developing systemic inflammatory response syndrome (SIRS) associated with multiple organ dysfunction syndrome (MODS) [26,27]; therefore flunixin meglumine and sucralfate treatments were continued, maintenance fluid therapy (2 mL/kg/h) with 1 % glucose combined with constant rate infusion of lidocaine (0.05 mg/kg/h), paracetamol (20 mg/kg PO BID) and pentoxifylline (10 mg/kg PO BID) were added and procaine benzylpenicillin and dihydrostreptomycin was replaced by ampicillin (20 mg/kg IV TID) and gentamicin (6.6 mg/kg IV SID). During the night, the first signs of laminitis developed, hence cryotherapy was started.

At 293 days of gestation, there had been no clinical improvement, and the US demonstrated the death of the fetus. Transvaginal palpation was performed, and it was possible to feel the head of the fetus due to a dilated cervix, although no evident uterine contractions were found. Dystocia due to fetal malposition developed: the fetus was in anterior presentation, dorso-pubic position, with lateral head deviation and bilateral shoulder flexion. The fetus was extracted via obstetric manipulations *per vaginam*2 h after it was found dead. The fetus was a 30 kg male, in good body condition and moderately autolyzed. The UC was macroscopically abnormal, 145 cm long (125 cm the intra-amniotic portion with 9 coils, 20 cm the intra-allantoic portion with 2 coils), excessively twisted, edematous, congested, inserted between the two horns (Fig. 1a) and the urachus showed segmental fluid-filled dilatations in the amniotic portion and petechiae in its inner walls (Fig. 1b). Placenta passed 2 h after abortion: the allantochorion weighed 5 kg (16 % fetal body weight) with a type III pattern of vascularization and the gravid horn was the right one. Macroscopically, on the chorionic side there was a brownish lesion with firmly adherent calcified materials bordered by congestion, distended vessels and diffuse edema originating from the cervical pole and ascending to the base of the non-gravid horn (Fig. 1c); near the cervical star necrotic areas with transmural perforations were present (Fig. 1d) and on the allantoic side diffuse congestion. The amnion had focal necrotic areas and distended vessels (Fig. 1e). Portions of 1 × 1 cm were collected from fetal membranes (pregnant horn, non-pregnant horn, body, cervical pole and amnion) for microbiological and histopathological examination. The gastric contents of the fetus were collected and submitted for bacteriological analysis. The fetus was sent to the Experimental Zooprophylactic Institute of Lombardia and Emilia Romagna for necropsy and microbiological examination. After stage III parturition, the mare became brighter and alert, the appetite improved, and diarrhea declined. On the same day, the lidocaine infusion was discontinued.

The next day, the mare showed mild hyperthermia (38.7 °C) and stiff gait, she was still leukopenic ($3.9 \times 10^3/\mu L$ WBC) and SAA was still elevated (249 µg/mL). A transrectal US revealed > 6 cm hypoechoic fluid in the uterine body and right horn; therefore, a uterine lavage was performed with 25 L of sterile isotonic NaCl solution: the recovered fluid was hemorrhagic with abundant fibrinous debris and a 50 mL sample was collected for bacteriological culturing; 10 IU oxytocin IV was administered at the end of the procedure. Together, these signs were indicative of septic puerperal metritis [28–30]. Therapies remained unchanged, and cryotherapy was continued. Uterine lavages were performed every other day until no more fluid was visible on US. Since the submitted samples of fetal membranes, fetal gastric contents and uterine fluid resulted positive for *E. coli* on culture (VELABAC) a Kirby-Bauer susceptibility test was performed, and gentamicin was replaced by amikacin (10 mg/kg IV SID).

Five days after abortion, diarrhea and leukopenia resolved ($7.4 \times 10^3/\mu L$), the consolidation was no longer detectable on US of the thorax, but SAA was unchanged. Vaginal examination with a speculum revealed

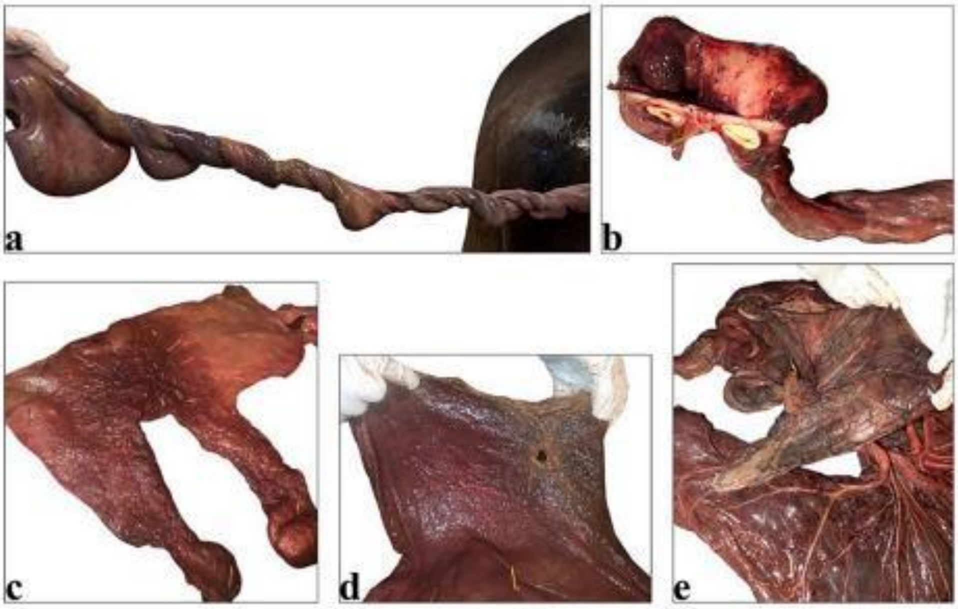

Fig. 1. Umbilical cord (a): 145 cm long (125 cm the intramniotic portion with 9 spires, 20 cm the intrallantoic part with 2 spires), excessively twisted, segmentally edematous, congested and implanted between the two horns. **Urachus (b):** The urachus showed segmental fluid-filled dilatations in the amniotic portion and in its inner surface petechiae were visible. **Allantochorion (c):** Brownish lesion with firmly adherent calcified materials originating from the cervical pole and ascending up to the base of the non-gravid horn. It was surrounded by congestion and diffuse edema. **Cervical star (d):** In proximity of the cervical star, necrotic and devitalized areas were present characterized by fenestrations of the placenta. **Amnion (e):** Diffusely congested, with focal necrotic areas and distended blood vessels.

no obvious abnormalities but on US, an inhomogeneous 3 cm long area with a hyperechoic center was visualized ventral to the cervix suggestive of a uterine wall abscess or hematoma. A low volume uterine lavage was performed, and a 50 mL uterine fluid sample was sent for microbiological examination. Eleven days after abortion, uterine involution was proceeding normally [31] and US demonstrated a 30 mm follicle on the left ovary which ovulated four days later.

Thirteen days after abortion, all therapies were stopped except for antibiotics as the culture of the low volume lavage was still positive for *E. coli* and also cultured positive for *Staphylococcus aureus*. Based on antibiogram results, ampicillin and amikacin were substituted with sulfadiazine/trimethoprim (30 mg/kg PO BID) that was continued for

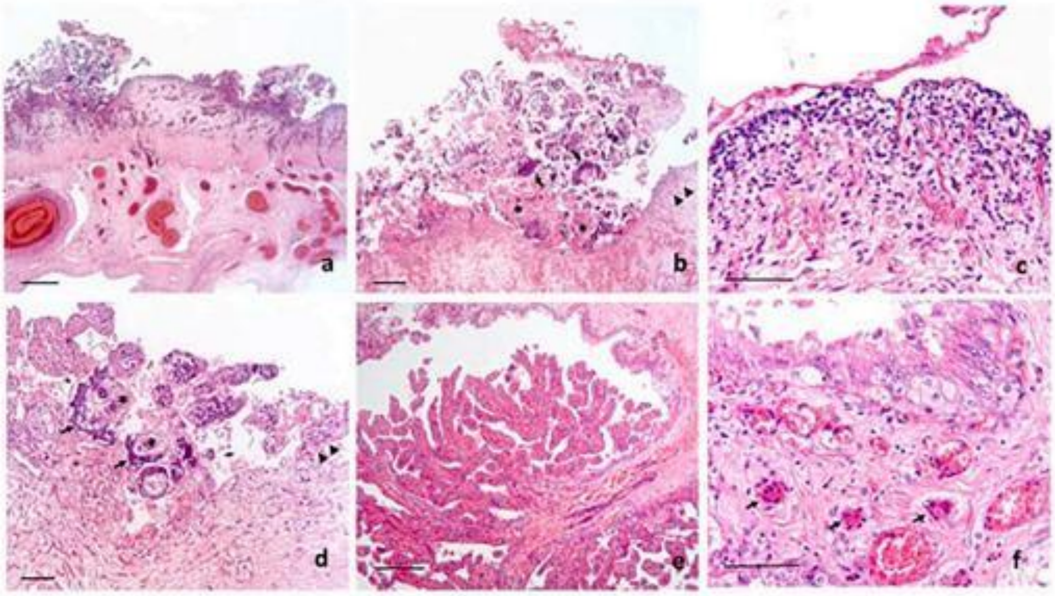

Fig. 2. Placenta at the level of the cervical star (a), (b) and (c). Diffuse congestion of the chorion **(a)** showing necrosis (asterisk) and calcification (arrow) of the chorionic villi and scant inflammatory infiltrate (arrowheads) **(b)**; degenerated neutrophils in the superficial layer of the chorion **(c)**. **Placenta at the level of the uterine body (d)** atrophy and necrosis (asterisk) of chorionic villi and trophoblasts associated with dystrophic calcification (arrow) and mild inflammation (arrowheads). **Placenta at the level of the gravid horn (e), (f).** Placenta of the gravid horn shows normotrophic villi **(e)** but marked dilation and hyperemia of blood vessels and focal microthrombosis (arrow) **(f)**. Scale bar (μm): (a) 800; (b) 500; (c) 200; (d) 100; (e) 300; (f) 200.

10 days; on US, the uterine wall lesion was still present and unchanged. The following day, SAA normalized, and the patient was discharged. Based on the referring veterinarian examination, the mare fully recovered.

2.5. Gross and histopathological diagnosis

At postmortem exam, fetal septicemia was demonstrated by isolation of *E. coli* from the fetal spleen and liver accompanied by subcutaneous icterus, subendocardial petechiae and hepatic necrosis. A real time PCR abortion panel (EHV, EAV, Leptospira; Experimental Zooprophylactic Institute of Lombardia and Emilia Romagna) performed on fetal membranes and tissues was negative. Histological examination of the cervical pole revealed necrosis and calcification of the villi, with sparse degenerated neutrophils in the superficial layer of the chorion (Fig. 2a–c). At the level of the body of the allantochorion severe hyperemia of chorionic vessels and edema were present; moreover, atrophy and necrosis of chorionic villi and trophoblasts associated with dystrophic calcifications and minimal cellular inflammation were the predominant findings (Fig. 2d). Normal villi were scattered but the overlying epithelium and trophoblasts were atrophic and vacuolised. The allantochorion villi of the non-gravid horn were necrotic and focal calcifications of vessel walls and trophoblasts associated with scant neutrophils were further findings. The allantochorion at the level of the gravid horn showed normotrophic and normo-plastic chorionic villi (Fig. 2e) but, in the chorion, marked congestion of blood vessels, focal thrombosis in small vessels (Fig. 2f) and rare perivascular neutrophils were also evident.

3. Discussion

At admission, the mare presented in this report showed typical signs of a high-risk pregnancy and impending abortion, i.e. colic symptoms and premature udder development/lactation [32]; additionally, she had poor perineal conformation and a history of a positive uterine swab before breeding which are predisposing factors for an ascending infection of the reproductive tract due to fecal contamination and/or air aspiration in the vagina and cervix [33,34]. However, no vulvar discharge was present and the cervical swab at admission cultured negative for microorganisms. The transrectal US showed reduced fluids depth and a high normal CTUP [23,24]: decreased allantoic fluid's depth is an indication of fetal distress and is associated with a negative outcome in human beings and horses [35]. In women, it usually develops due to decreased fetal urine production and is a good indicator of fetal chronic hypoxia [36], associated with intrauterine growth retardation and a poor perinatal outcome [37]. In mares, reduced amounts of allantoic fluid have been associated with a poor fetal outcome [35]. Collectively, none of the findings recorded at admission were indicative of an ascending placentitis but the mare was, despite that, closely monitored to detect any potential changes.

Due to the suspected aspiration pneumonia following the mandibular injury and a choking episode, the mare was administered procaine benzylpenicillin. Subsequently, she developed SIRS associated with MODS [26,27,38]; in addition, P4 peaked, indicating fetal stress due to progestogens synthesis from the fetal adrenal gland [24,39,40–42] and on US no more fetal heart activity was detectable. Experimental studies on neonatal leukocytes indicate that killing *E. coli* bacteria with b-lactam antimicrobial agents leads to a greater release of endotoxin and increased cellular synthesis of tumor necrosis factor compared to amikacin alone or the combination of amikacin and ampicillin [43]. In our case, it could be hypothesized that the stress of the choke induced cortisol and prostaglandins release stimulating parturition or that the administration of the antimicrobial drug may have caused a massive release of endotoxins from the fetoplacental unit and/or from the maternal gastro-intestinal tract leading to abortion, or a combination of both. Moreover, the mare had signs of impending delivery at presentation and the timing of abortion may simply reflect progression of the underlying pathology.

UC length (UCL) in normal gestation can be quite variable and twisting is a physiological phenomenon that affects all its structures, sometimes with pathological implications [44,45]. Coiling is considered pathologic when it is excessive, causing vascular obstruction, edema, hemorrhage, thrombosis of umbilical vessels and/or obstruction of the urine flow [45–48]. UCL is affected by mare parity, age [49,50], fetal sex [49,51] and to date, the only known risk factor associated with UCT in equine fetus is an UCL > 80 cm [45,52–58]. The development of an excessive UCL could be potentially influenced by the site of UC insertion and the type of vascular pattern of the allantoic surface [59] with approximately half of the UCT cases associated with type II or III pattern [56]. More recently, some authors suggested that genetics may explain this condition, but more research is needed to confirm this [60,61]. Another possible explanation may be the association with the amount of fetal movement [62] with the excessive UCL that develops secondary as a response to stretching of the UC because of tension arising from the twisting of the UC [61,63]. The fetal-amniotic unit is highly mobile within the first five months of gestation, hereafter its mobility decreases and ceases by approximately the 7th month [64], mainly because of decreased space within the uterine lumen as the fetus grows [44,64]. Increased length of the amniotic part of the UC has been associated with UCT, and the risk of abortion due to UCT increased by 7.3-fold when the amniotic: allantoic UCL ratio was >1 [58]. These findings combined with the high fetal mobility up to 7th month of gestation and the exponential growth of the amniotic UC in the same time period support the hypothesis that may explain the high prevalence of abortion associated with UCT in the 6th to 8th month of gestation and that UCT is more likely to occur in the amniotic UC part rather than the allantoic one [13,45,52,58,63]. Altogether, these data correspond with those of this study: in fact, the total UCL was longer than normal (145 cm) with a more twisted amniotic portion (125 cm long, 9 coils) compared to the allantoic one (20 cm long, 2 coils), and a vascular type III pattern. However, not all mares with a long UC will abort due to UCT and they may even deliver a clinically normal foal at term [6]. In our case, UCT was macroscopically characterized by pathological twisting with tension and compressive forces on the affected portion of the cord, blanched constricted areas, edema, hemorrhage, aneurysms, tearing of the intima of vessels, thrombosis, and urachal dilatations of varying sizes forming urine-filled sacculations. Moreover, in cases of UCT, as observed in the present study, the fetus is fully developed, with good BCS and slightly to moderately autolyzed consistently with fetal death prior to abortion [45,47,56]. The most consistent histopathological finding related to UCT is the evidence of necrotic changes with secondary deposition of calcified material in the blood vessels of the allantochorion [6,45]. Mineralization due to hypoxia may be caused by the decreased integrity of damaged endothelial cell membranes, allowing extracellular calcium to enter cells and combine with phosphate to produce the vascular endothelial mineralization observed with UC compression [65]. In a recent study by Roach et al (2023), histological assessment of the allantochorion identified three features associated with UCT diagnosis such as autolysis, villous mineralization and villous karyorrhexis but no UCT cases exhibited a significant inflammatory infiltrate in the allantochorion compared to cases without UCT [58]. Overall, these findings are similar with those of the present study since the lesion was mainly characterized by necrosis, mineralization and scarce inflammatory clusters. The presence of karyorrhexis and mineralization likely reflects ischemic injury resulting from poor perfusion of the chorion and, in the case of UCT, as a response to episodes of venous stasis following increased vascular resistance associated with the increase in UCL and/or vascular obstruction. All this suggests a more chronic pathophysiology involving lengthening of the amniotic, not allantoic, UC [58]. Identification of these features enables evidence-based refinement of diagnostic criteria for UCT, which can now undergo consultation to reach a generally accepted consensus [58].

Apart from UCT, cervical pole necrosis was confirmed in the present case. It has been reported that inadequate perfusion can cause intravascular thrombosis in the peripheral tissues of the allantochorion and possible localized ischemic necrosis in placental portions distant from UC insertion, such as the cervical pole [52,62,66]. In fact, cervical pole necrosis has been theorized to occur either secondarily to placental detachment at the cervical pole or by ischemic insult related to hemodynamic abnormalities associated with long UCs and/or vascular occlusion [14,56]. Grossly, this condition appears as a sharply delineated area of brown discoloration without an increase in thickness and random foci of ischemic necrosis that may produce fenestrations in the allantochorion. Loynachan (2023) reported that 33/57 cases of cervical pole necrosis had no signs of impending abortion while 22 cases presented signs of placentitis and were treated; in 44 cases the outcome was abortion and in one case was also present UCT; UCL was on average 89.4 cm long and fetuses age averaged 272 days of pregnancy [14]. These observations were also noted in the current report. Although the macroscopic lesion on the chorionic side of placenta ascending from the cervical star to the base of non-gravid horn and the isolation of *E. coli* in fetal membranes and tissues could give reason to suspect an ascending placentitis alone as cause of abortion [33,67], a microscopic examination of fetal membranes was needed to reach a final diagnosis. The histological changes described in the present report were suggestive of a chronic lesion of the allantochorion characterized by degeneration, necrosis and dystrophic calcification with concurrent low to moderate inflammatory infiltrate localized mainly at the cervical pole. The differential diagnosis should include placental infarction with subsequent secondary placentitis or primary chronic infectious placentitis. Both diagnostic conclusions are possible but considering the chronicity of the lesion characterized by low grade of inflammation, its main localization at the cervical pole, and the degeneration and necrosis, which are typical consequences of ischemia, the final diagnosis is most likely the former according to previous studies [56,68]. *E. coli* is one of the most common causes of cervical pole placentitis and an abortive agent in the equine species [8,69]. In this report, primary necrosis and devitalization of the cervical pole region, due to chronic ischemia associated with UCT, may have allowed this opportunistic pathogen to access the fetal membranes and consequently/secondarily instigating the placentitis [14,67]. Abortion due to bacterial infections generally is more frequent from the 9th month of gestation [8] as observed in this report.

4. Conclusions

Cervical pole necrosis is an idiopathic, non-infectious, placental condition that can result in abortion, premature birth, delivery of a weak foal or delivery of a viable foal. A histopathological exam is of paramount importance to reach a final diagnosis of this condition, but future studies are needed to better understand this unique cause of equine abortion.

CRediT authorship contribution statement

L. Fischetti: Writing – review & editing, Writing – original draft, Investigation, Data curation. **F. Perina:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Data curation. **G. Sarli:** Writing – review & editing. **N. Ellero:** Writing – review & editing. **F. Freccero:** Writing – review & editing. **C. Castagnetti:** Writing – review & editing. **J. Mariella:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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General Discussion and Conclusion

The postpartum period represents a critical and complex phase in the reproductive life of the mare, encompassing extensive and coordinated adaptations across endocrine, vascular, anatomical, and immune systems. These changes are essential for the restoration of normal reproductive function and are subject to considerable variability due to both physiological and pathological factors. The present thesis sought to comprehensively explore these dynamics, aiming to elucidate how various physiological, pathological, and management-related factors influence postpartum recovery and reproductive outcomes.

Doppler ultrasonography provided valuable insights into the hemodynamic changes of the uterine arteries during the peripartum period. Uterine artery parameters were influenced by age and parity, reflecting how successive pregnancies and age-related vascular alterations can shape uterine perfusion. These findings suggest that uterine artery parameters should be interpreted in the context of the mare's age and parity to identify potential hemodynamic alterations during pregnancy. In future studies, assessing these parameters in high-risk pregnancies could provide valuable insights, as uterine artery hemodynamics are widely studied in human obstetrics to detect women at risk for conditions such as preeclampsia and fetal growth restriction.

The resumption of ovarian cyclicity postpartum is influenced by a combination of physiological and pathological factors. By integrating assessments of follicular dynamics with endocrine profiling, it was possible to evaluate the relative contributions of the type of postpartum, lactation, parity, age, and the foaling month on the return to cyclicity. Mares experiencing pathological postpartum events exhibited markedly delayed or absent ovulation, indicating impaired ovarian function. This observation, consistent with evidence from other species, suggests that postpartum complications, particularly those involving uterine inflammation, can negatively affect follicular development and steroid hormone production. In contrast, increasing parity and progression through the reproductive season appeared to facilitate the restoration of ovarian activity, while lactation per se did not exert a measurable influence. Hormonal patterns were generally consistent across groups, showing no major differences, although their analysis provided additional insight into the coordination of follicular and endocrine dynamics. These findings highlight how multiple factors—including hormonal cues, ovarian physiology, and reproductive history—interact to shape the timing and quality of ovarian cyclicity resumption postpartum.

Alongside physiological changes, pathological conditions were investigated to understand their impact on postpartum recovery. RFM were characterized retrospectively in light-breed mares, assessing overall incidence and associations with factors including age, parity, high-risk pregnancies, dystocia, the use of ARTs, postpartum complications, treatment, and outcomes. The overall incidence was higher than previously reported in the literature, and even higher when considering only high-risk pregnancies. Placental pathologies and systemic illness were associated with an increased likelihood of RFM, as previously described. Consistent with previous literature, RFM incidence was not influenced by parity, mare age, or the use of ARTs, whereas mares experiencing dystocia had a threefold higher incidence of RFM compared to those with normal foaling, and mares with RFM were also much more likely to develop PPM. Most RFM cases in this study were successfully managed with standard therapeutic approaches, with all mares achieving complete recovery. These results indicate that specific mare populations, such as those experiencing dystocia or high-risk pregnancies, should be carefully monitored and managed, as they are at higher risk of developing RFM and related postpartum complications.

In line with these observations, obstetric complications such as dystocia are strongly associated with an increased risk of RFM, and mares experiencing RFM are, in turn, more likely to develop PPM. These interconnected events highlight a continuum of postpartum disorders in which parturition difficulties can predispose to incomplete placental expulsion and subsequent uterine and/or systemic inflammation. Mares with postpartum metritis exhibited a markedly stronger inflammatory response compared to those with a normal postpartum period. In physiologic uterine involution, a controlled inflammatory process, characterized by neutrophil recruitment, is necessary to ensure proper endometrial regeneration and uterine clearance. In mares with PPM, this response was more intense and sustained, persisting for several days. These findings emphasize that while moderate inflammatory reactions are essential for normal uterine healing, excessive or prolonged inflammation can compromise reproductive recovery. All affected mares were treated successfully with systemic antibiotics and high-volume uterine lavages, which not only facilitated removal of residual debris and reduction of bacterial load, but proved highly effective in enhancing uterine clearance and promoting complete recovery.

The use of ARTs, including embryo transfer and intracytoplasmic sperm injection, was investigated for its effects on both mares and foals. ART procedures were found to influence the macroscopic morphology of fetal membranes and were associated with an increased incidence of dystocia. This higher risk was likely related to the younger age and primiparity of recipient mares, reflecting a demographic trend inherent to ART programs. In a retrospective study on dystocia in Standardbred mares conducted in 2022, we observed that dystocia occurred more frequently in primiparous mares. Importantly, neonatal outcomes were not compromised, suggesting that while ART can modify aspects of the maternal–fetal interface, overall foal viability remains largely unaffected. Nevertheless, when ARTs are employed, careful obstetric monitoring is warranted, particularly for primiparous mares, who may be more predisposed to dystocia and the sequelae of postpartum complications that may follow.

In summary, postpartum recovery in mares reflects a delicate balance between physiological adaptation and the potential impact of pathological conditions. Understanding the intricate interplay among the various aspects explored in the present study is crucial, not only to deepen our comprehension of reproductive physiology, but also to recognize the factors that can compromise, to varying degrees, both fertility and the overall health of the mare. Such awareness is essential for developing preventive strategies and ensuring timely intervention when complications arise, helping to preserve fertility and support the mare's full recovery.

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Abstract

The postpartum period in the mare represents a complex and dynamic phase, characterized by endocrine, vascular, anatomical, and immune adaptations that work together to restore normal reproductive function. However, careful attention must be paid to potential postpartum complications, which can affect short- and long-term fertility. This thesis aimed to characterize physiological and pathological postpartum dynamics and to evaluate the factors influencing reproductive recovery and clinical outcomes. Through Doppler ultrasonography, it was possible to observe that uterine artery parameters were shaped by the combined effects of age and parity, highlighting how successive pregnancies and age-related vascular alterations interact to modulate uterine hemodynamics. By analyzing follicular dynamics alongside endocrine profiles, it was possible to assess the factors, influencing the resumption of postpartum cyclicity and the associated hormonal patterns in mares with physiological and pathological postpartum conditions. Alongside the physiological postpartum changes, pathological conditions were also investigated. Retained fetal membranes were retrospectively characterized in light-breed mares in terms of overall incidence and their association with factors such as age, parity, high-risk pregnancies, dystocia, assisted reproductive techniques (ARTs), postpartum complications, treatment, and outcomes. Postpartum metritis was examined through cytokine profiling, revealing that although some immune activation is always present after foaling, it becomes markedly exaggerated in affected mares, contributing to impaired uterine recovery. The use of ARTs was investigated, revealing their influence on the macroscopic morphology of fetal membranes and their association with a higher incidence of dystocia, but do not appear to affect neonatal outcomes. Finally, the case report provided a practical case in which several of the previously discussed aspects were illustrated, demonstrating how timely intervention and monitoring can support the restoration of normal ovarian function even in high-risk situations. In summary, postpartum recovery in mares reflects a delicate balance of physiological and pathological factors, emphasizing the importance of optimized management strategies.