



Postpartum uterine and ovarian condition affect the foal estrus fertility in Mangalarga Marchador mares?

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ABSTRACT. This study was conducted to evaluate the follicle development, uterine morphology, and fertility of Mangalarga Marchador mares submitted to natural breeding in the first postpartum estrous cycle. Eight mares were evaluated with ultrasound equipment every three days, from the second to the 42nd day after delivery. During this period, follicular diameter, corpus luteum persistence, appearance and diameter of the horns and uterine body in addition to weight and body condition were evaluated. Postpartum reproductive efficiency was verified through conception and estrous repetition rates. Data were submitted to Pearson's correlation analysis, chi-square test, linear and quadratic regression. Ovulation occurred between days 14.5 ± 2.5 postpartum. The mean range of the follicular wave was 11.75 ± 2.5 days, with a daily follicular growth rate of 3.6 ± 0.6 mm. There was reduction of the uterine body of 20.6 ± 1.4 mm, characterizing uterine involution in mares. High conception rate (75%) was found during foal heat and variation in body weight and body condition score after parturition did not present significant interference in the other parameters. Therefore, follicular dynamic, uterine involution, weight, and body condition variation were compatible with the postpartum estrus onset in Mangalarga Marchador mares and did not interfere in conception rates.

Keywords: equine; puerperium; uterine involution; ovulation; reproduction.

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Introduction

The mare's puerperium is characterized by rapid uterine involution accompanied by the manifestation of postpartum estrus, termed 'foal estrus', which typically occurs between days 5 and 12 post-parturition. Conception during this interval shortens the inter-foaling interval and permits one foal per mare per year (Gibson et al., 2018). In competitive contexts, this efficiency allows early pedigree registration of foals, conferring an advantage over those born later in the season (Barros & Oliveira, 2017).

Although mares are fertile during foal estrus, conception viability remains contentious: pregnancy rates are roughly 20% lower than in subsequent cycles (Lemes et al., 2017a), likely due to a suboptimal uterine environment from incomplete endometrial involution. This may impair folliculogenesis, compromise fertility, hinder embryogenesis and increase early embryonic loss (Lemes et al., 2017b).

Complete uterine involution occurs by days 30–32 postpartum (Sharma et al., 2010), and endometrial recovery to pre-pregnancy morphology by days 23–24 (Lemes et al., 2017a). In advanced-age mares or those with inadequate body condition, involution is slower, adversely affecting conception at first estrus (Davies Morel et al., 2009). Additional factors reducing fertility include poor general or reproductive health, suboptimal breeding management and seasonal anestrus (Zúccari et al., 2013).

Although postpartum follicular dynamics have been studied in other Brazilian breeds (Buratini et al., 1997), data on Mangalarga Marchador mares remain scarce. This study therefore aimed to evaluate follicular development and corpus luteum formation, uterine morphology over 42 days postpartum, and their association with fertility under natural mating during foal estrus.

Material and methods

The study was approved by the Animal Use Ethics Committee (protocol no. 142/2019) and conducted at the Hamilton Abreu Navarro Experimental Farm of the Federal University of Minas Gerais (UFMG), Montes Claros, Brazil. According to Köppen's classification, the area has a tropical 'As' climate (Alvares et al., 2013).

Eight clinically healthy Mangalarga Marchador mares, aged five to eleven years, were kept under natural photoperiod in extensive *Panicum maximum* cv. Mombaça pastures with mineral supplement and water ad libitum. One week prior to parturition they were moved to a maternity paddock and fed chopped *Pennisetum purpureum* cv. Green twice daily, in addition to mineral supplement and water ad libitum. The trial was conducted from September to November (spring), when mean precipitation was 284 mm and temperatures ranged from 17–30°C. Mares and paddock conditions were monitored twice daily. All foalings were unassisted and occurred mostly at night.

Ultrasound evaluations

The ovaries, uterine horns, and uterine body were scanned with a Mindray DP 10 Vet® linear transrectal probe (5 MHz, B-mode) every three days from days 2 to 42 postpartum. Follicles were measured in two planes and classified as:

- small: 6–10.9 mm
- medium: 11–25.9 mm
- large: 26–35.9 mm
- ovulatory: > 35.9 mm

The follicular growth rate (FGR) for the first postpartum cycle was calculated as:

$$\text{FGR (mm day}^{-1}\text{)} = \frac{\text{largest pre-ovulatory diameter} - \text{smallest diameter}}{\text{follicular wave duration (days)}}$$

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Corpus luteum development during foal heat was monitored similarly. For mares returning to estrus, corpus luteum persistence (PPCL) was calculated as:

$$\text{PPCL (mm day}^{-1}\text{)} = \frac{\text{maximum diameter} - \text{minimum diameter}}{\text{regression time}}$$

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Transverse diameters of uterine horns and body, and thickness of the uterine mucosa, were measured at midpoints. Qualitative traits—echogenicity, hypertrophy, and endometrial folding—were scored 1–3. Echogenicity: 1 = hyperechoic; 2 = hypoechoic; 3 = anechoic. Hypertrophy and folding: 1 = absent; 2 = moderate; 3 = intense. Uterine tone (via rectal palpation) was scored: 1 = flaccid; 2 = intermediate; 3 = turgid.

Reproductive indices

Estrus was detected by teasing with a stallion and observing urination, vulvar contractions, and tail raising, corroborated by ultrasonography. Once in heat, mares were naturally mated twice daily until service refusal, using a healthy, fertile, eight-year-old stallion. Pregnancy was confirmed ultrasonographically on day 16 post-last mating.

Conception rate (CR) and postpartum estrus repetition rate (PPHR) were calculated as:

$$\text{CR (\%)} = \frac{\text{number of pregnant mares}}{\text{number of bred mares}} \times 100$$

$$\text{PPHR (\%)} = \frac{\text{number of non-pregnant mares}}{\text{number of bred mares}} \times 100$$

Body condition

Body weight was measured using a Filizola® mechanical platform scale (0.1 Kg precision) one week before the expected foaling date and at 15, 30, and 60 days postpartum. Body condition score (BCS) was rated 1–9 (Henneke et al., 1983) on the same days.

Statistical analysis

Means and standard deviations were calculated in Microsoft Excel®. Quantitative variables (ovarian and uterine parameters, body condition, body weight, age) were analyzed by Pearson correlation; estrus occurrence versus qualitative uterine variables was assessed by chi-square test. Continuous quantitative variables over the 42-day period were evaluated by linear and quadratic regression using SAS University Edition 9.4.

Results and discussion

Follicular dynamics

Most small (6–10.9 mm) and medium (11–25.9 mm) follicles were identified on days 2 and 4 postpartum (Figure 1), indicating onset of follicular emergence in the first estrous cycle (Ginther et al., 2004). Between

days 4 and 7, the presence of large follicles (26–35.9 mm) was accompanied by a reduction in the number of small and medium follicles, characterizing follicular selection. Follicular dominance—defined by ovulatory follicles > 35 mm in diameter—was observed between days 11 and 14 postpartum (Figure 1). Signs of estrus in these mares were detected during the same interval. Follicular development fluctuated throughout the remainder of the postpartum period, with small and medium follicles predominating, as most mares conceived when bred during foal estrus.

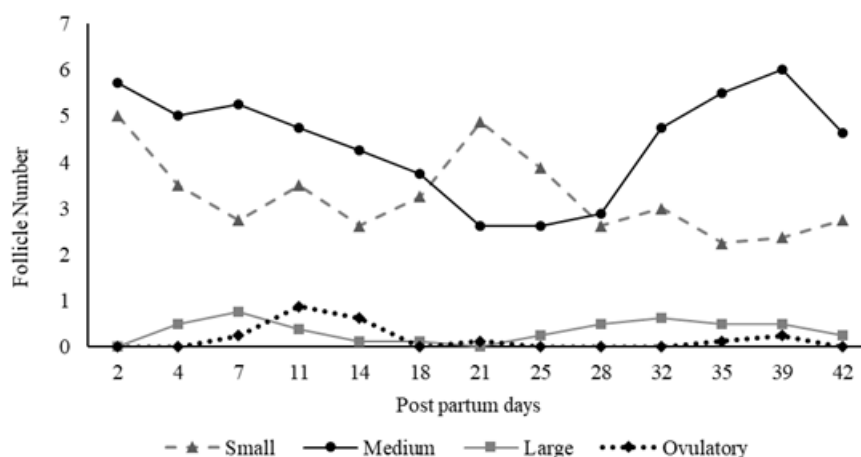


Figure 1. The number of follicles in both ovaries of Mangalarga Marchador mares classified as small from 6 to 10.9 mm, medium from 11 to 25.9 mm, large from 26 a 35.9 mm, and ovulatory, greater than 35.9 mm in diameter.

Maximum follicular diameter (mm) was observed on days 11 and 14 postpartum, with mean $\pm$ standard deviation of 40.96 ± 5.76 and 39.08 ± 16.77 , respectively (Figure 2). Ovulation occurred at 14.5 ± 2.5 days postpartum. In Mangalarga Marchador mares, Buratini et al. (1997) reported that the mean diameter ($\pm$ s.d.) of the dominant follicle in the first wave ranged from 39.0 ± 3.9 mm in mares with a single major follicular wave to 34.7 ± 2.5 mm in mares exhibiting two waves. The maximum diameter of the dominant follicle in the second wave was 34.3 ± 11.0 mm.

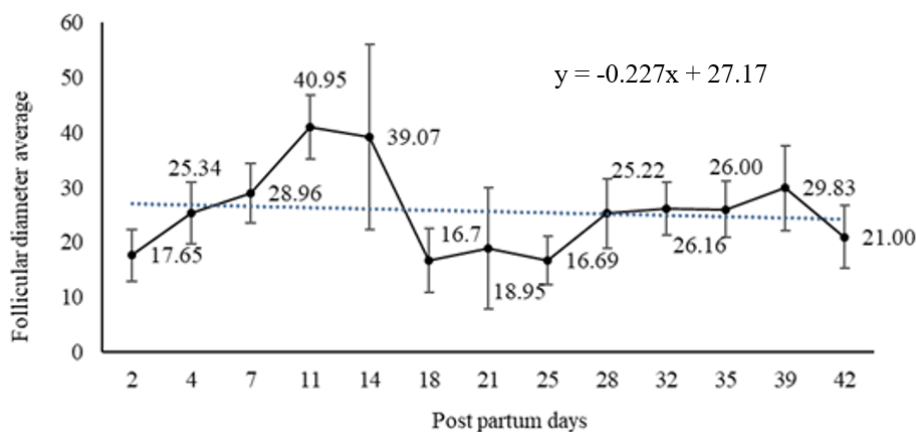


Figure 2. Dynamics and trend line of follicular development in Mangalarga Marchador mares evaluated every three days for 42 consecutive days post parturition.

In Central Ethiopia, with mean annual temperatures ranging from 10.7–23°C, mean follicular diameter ($\pm$ s.d.) was 28.6 ± 0.8 mm, and ovulation occurred between days 7 and 9 postpartum in Anglo-Arabian crossbred mares (Duguma, 2020). Accordingly, hereditary, breed, and environmental factors may influence the pattern of follicular development in mares.

The variation in average follicular diameter (Figure 2) aligns with the oscillatory profile observed in Figure 1. Despite this variation, regression analysis indicated a non-significant linear trend in follicular diameter over assessment days (slope -0.094 ; $p = 0.2775$), and the quadratic component was also non-significant (0.0002 ; $p = 0.9097$). This pattern of follicular development is expected, reflecting the continuous growth of dominant follicles while subordinate follicles progress until selection, followed by regression (Rua et al., 2019).

The follicular wave in this study had a mean duration ($\pm$ s.d.) of 11.75 ± 2.5 days, with a daily growth rate of 3.6 ± 0.6 mm. Lemes et al. (2017b) found no differences in follicular dynamics between early- and late-ovulating mares. Regardless of the timing of postpartum estrus onset, follicles grew at similar rates, reached equivalent preovulatory sizes, and exhibited comparable corpus luteum vascularization across subsequent estrous cycles.

However, follicular recruitment, ovulation, and corpus luteum development occur concurrently with uterine involution only in the absence of endometritis (Lemes et al., 2017a). The uterine mucosal inflammatory process, common in the puerperium, is involved in tissue debris removal and microbial defense (Valacchi et al., 2018). Incomplete endometrial involution disrupts follicular growth and corpus luteum formation, potentially impairing embryonic development and leading to embryonic loss (Falomo et al., 2020).

Corpus luteum development

A significant negative correlation ($p < 0.001$; $r = -0.287$) was observed between corpus luteum transverse diameter and mean follicular diameter across both ovaries, indicating that corpus luteum development coincides with prevalence of small and medium follicles after conception (Abdelnaby & El-Maaty, 2017). Two of the eight mares did not conceive during foal estrus and exhibited corpus luteum regression with estrus repetition. The regression rate in these mares was 1.12%, with a mean regression time of 17 days, consistent with luteolysis occurring between days 14 and 16 (Satué & Gardón, 2013). The mean maximum and minimum diameters of the corpus luteum ($\pm$ s.d.) were 38.57 ± 3.6 mm and 21.00 ± 3.1 mm, respectively. Initially hemorrhagic, the corpus luteum presents larger diameters associated with elevated progesterone, subsequently regressing as fibrous tissue replaces luteal cells (Abdelnaby & El-Maaty, 2017).

Uterine involution and uterine status

Trend lines in Figures 3 and 4 revealed a positive quadratic effect of postpartum days on uterine body mean diameter (quadratic term $+0.0147$; $p = 0.0021$) and mucosal thickness ($+0.0085$; $p = 0.0097$). On day 2 postpartum, uterine body diameter was 47.50 ± 6.70 mm (Figure 4) and mucosal thickness was 18.3 ± 6.2 mm (Figure 3). By day 42, the respective mean diameters were 26.94 ± 5.30 mm and 9.6 ± 1.5 mm. This reduction characterizes uterine involution, encompassing fluid absorption, lochial elimination, apoptosis, myometrial regression, and restoration of non-pregnant status—processes essential for recovery from gestational alterations (Angrimani et al., 2011).

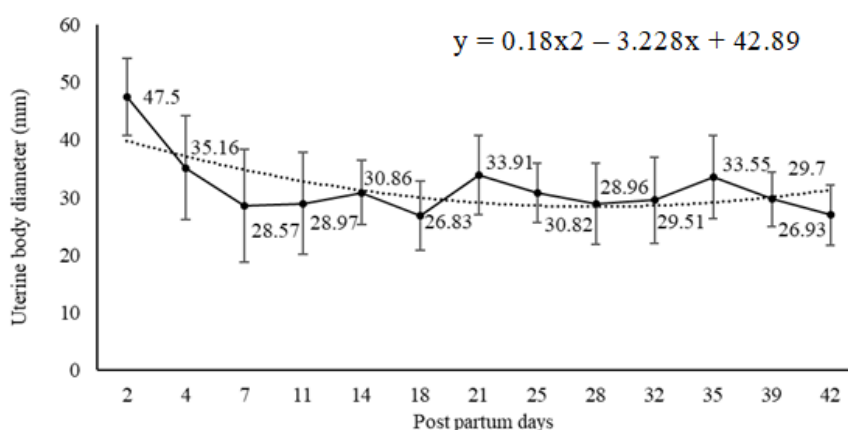


Figure 3. Uterine body mean diameter (mm) and trend line in Mangalarga Marchador mares evaluated every three days for 42 consecutive days post parturition.

Marked reduction in the uterine diameter and mucosal thickness were observed between the 2nd and 18th postpartum days (Figure 3 and 4) with a small increase in mean uterine diameter on days 14th, 21st, and 35th postpartum, corresponding to foal estrus manifestation at 14th day, to maternal-fetal recognition at 21st day, in case of pregnancy (Allen, 2005), and to estrus final period in foal estrous non pregnant mares (Figure 2).

Evidence shows that the uterus is approximately 1.5 times larger than its non-pregnant state 12 hours after parturition. By the 14th postpartum day, it exhibits histological features similar to those observed prior to conception (Davies Morel et al., 2009). The initial rapid reduction in uterine diameter during the early postpartum days is followed by a slower involution rate after day 11. The endometrium returns to its pre-pregnancy condition between 23 and 24 days postpartum. This prompt uterine size reduction, along with the

absence of intrauterine fluid and reduced vascular perfusion during the early postpartum weeks, contributes to the establishment of a favorable uterine environment for embryonic development following fertilization (Lemes et al., 2017a).

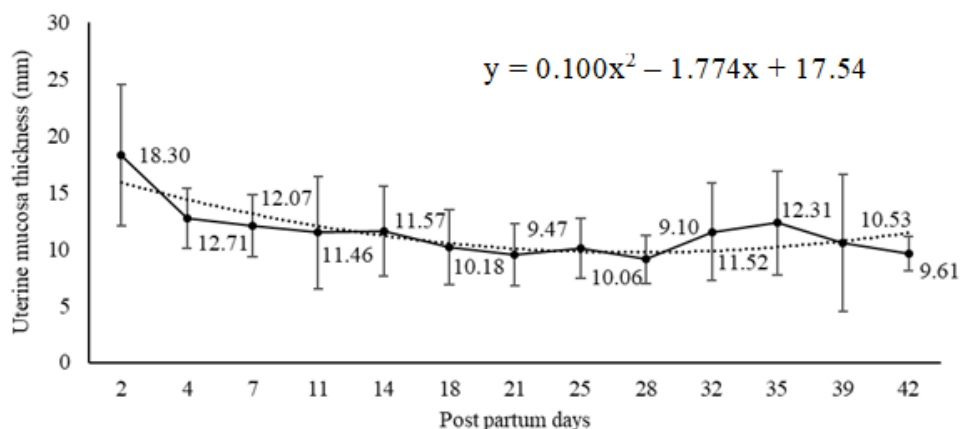


Figure 4. Uterine mucosa thickness (mm) and trend line in Mangalarga Marchador mares evaluated every three days for 42 consecutive days post parturition

A hypoechoic uterine image, classified as grade 2, was identified in 93.69% of mares during their first postpartum estrus ($\chi^2 = 38.44$; $p < 0.0001$). At this stage, the endometrium was preparing for embryo implantation. The reproductive tract tissues exhibited low echogenicity due to the accumulation of fluid associated with follicular growth and elevated estrogen levels, often following prior exposure to progesterone (Satué & Gardón, 2013).

Hypoechoic images may also be associated with the inflammatory process inherent to uterine involution. In mares, this physiological inflammation typically resolves within 24 to 72 hours following mating. If it persists beyond three or four days, the uterine environment becomes unsuitable for embryo survival due to the secretion of prostaglandin F₂-alpha, a decline in progesterone levels, and a rapid return to estrus (Valacchi et al., 2018).

An intense degree of uterine hypertrophy (Grade 3) and mucosal folding was also observed in 91.89% of the mares evaluated during estrus manifestation ($\chi^2 = 260.75$; $p < 0.0001$). Uterine echogenicity changes after follicular dominance due to estrogenic exposure, resulting in a heterogeneous structure that allows visualization of endometrial folds as star-like streaks (Lemes et al., 2017a), a common feature during the estrus period (Satué & Gardón, 2013). In contrast, the diestrus echotexture is homogeneous due to the action of progesterone (Lemes et al., 2017b).

During the first postpartum estrus, 100% of the mares exhibited uterine tone classified as grade 1, indicating flaccidity upon palpation ($\chi^2 = 181.06$; $p < 0.0001$). In mares, the uterus is flaccid during estrus and turgid during diestrus, which contrasts with bovine characteristics. Estradiol levels increase during the ovulatory phase of estrus, inducing sexual receptivity and promoting relaxation of the uterus, cervix, and vulva (Satué & Gardón, 2013).

Reproductive efficiency in foal estrus and body condition

The high conception rate during postpartum estrus (75%) indicates that puerperal physiological processes—uterine involution and subsequent reproductive-tract recovery—did not impair fertility in Mangalarga Marchador mares. Comparable pregnancy rates (75.5%) during first postpartum estrus have been reported (Zúccari et al., 2013). A 10–20% reduction in fertility during first postpartum estrus, relative to subsequent estrous cycles with conception rates $\geq 80\%$, has been documented (Davies Morel et al., 2009). Reduced pregnancy rates during foal estrus may be attributable to incomplete endometrial involution (Lemes et al., 2017b).

Adequate body condition and sanitary status in mares likely contribute to high pregnancy rates. These factors are also associated with daily visual monitoring of estrous signs and ultrasound examinations every three days. Both body weight and body condition score (BCS) are directly linked to reproductive efficiency and influence estrous cycle length, number of cycles, follicular development, conception rates, inter-foaling interval, and pregnancy duration (Rodrigues et al., 2011; Zúccari et al., 2013).

Mean ($\pm$ standard deviation) BCS and body weight declined after parturition from 4.75 ± 0.24 to 4.0 ± 0.21 , and from 417.64 ± 18.73 to 360.00 ± 22.2 Kg, respectively. These physiological losses are attributed to expulsion of the fetus, placenta, and fetal membranes (Gibson et al., 2018). However, this decline did not affect reproductive cyclicity, as all mares exhibited estrus between days 7 and 14 postpartum, with ovulation occurring at 14.5 ± 2.25 days.

Several physiological factors accelerate resumption of fertility: efficient expulsion of fetal membranes, a diffuse epitheliochorial placenta, absence of reproductive-tract trauma during parturition, lochial elimination, and rapid uterine involution all favor prompt recovery of the reproductive system (Blanchard & Macpherson, 2011).

Correlations were observed between body weight change at parturition and mean follicular diameter ($r = -0.124$; $p = 0.0097$), and between BCS change at parturition and mean follicular diameter ($r = -0.103$; $p = 0.0322$). Additionally, body weight change at parturition correlated with mean corpus luteum diameter ($r = -0.1489$; $p = 0.023$). These findings suggest that the magnitude of weight and condition loss may influence follicular development and corpus luteum formation.

Mares with a BCS ≤ 4 (scale 1–9) tend to ovulate 3–4 weeks later in spring than those with a BCS ≥ 5 (Dini et al., 2019). Among embryo donor mares, BCS influences follicular diameter: individuals with BCS 1–4 had smaller follicles than those with BCS 6.5–7.5 (Rodrigues et al., 2011). Mares with higher BCS (> 5) exhibit elevated insulin-like growth factor-1 (IGF-1) levels, a protein involved in dominant follicle selection and ovulation (Peugnet et al., 2010).

A correlation between BCS change and age ($r = 0.521$; $p < 0.0001$) indicates that body condition deficiencies become more pronounced with advancing age in Mangalarga Marchador mares. Senility and low BCS are associated with decreased conception rates during foal estrus due to slower uterine involution (Davies Morel et al., 2009). In this study, only one older mare (11 years) failed to conceive during foal heat (Table 1).

Table 1. Reproductive parameters in Mangalarga Marchador mares evaluated for 42 consecutive days post parturition.

Mares	Age (years)	Parturition date	Beginning day of postpartum estrus and breeding	Ovulation day	Ovulation ovary	Postpartum pregnancy confirmation
1	11	Sep. 25 th	11	14	Right	Yes
2	10	Sep. 26 th	11	21	Right	Yes
3	8	Oct. 2 nd	7	14	Right	Yes
4	10	Oct. 05 th	9	12	Right	Yes
5	11	Oct. 07 th	7	14	Right	No
6	6	Oct. 07 th	10	14	Left	No
7	7	Oct. 11 th	13	17	Right	Yes
8	6	Oct. 21 st	7	10	Right	Yes

By day 30 postpartum, despite parturition demands and high lactational energy requirements, mean body weight (369.3 ± 24.7 Kg) and BCS (4.5 ± 0.24) increased slightly. These values remained stable by day 60 (370.14 ± 19.30 Kg; BCS 4.5 ± 0.28). Milk production may account for 2–3% of mare body weight and body reserves can contribute ~33 % of milk output in the first lactation month. Thus, adequate body condition before and after parturition supports both reproductive performance and lactational capacity (Mazhitova et al., 2015).

Conclusion

Follicular dynamics, uterine involution, body condition score (BCS), and body weight were consistent with the expression of foal estrus and did not affect conception rates in Mangalarga Marchador mares.

Data availability

Does not apply.

Acknowledgments

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