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Progesterone and Prostaglandin F metabolite levels in relation to modes of whelping

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Abstract

Parturition involves a series of changes in the blood levels of various hormones. The present study aimed to examine the concentrations of progesterone and 15-ketodihydroprostaglandin $F_{2\alpha}$ or PGFM (prostaglandin F metabolite) hormones on the day of delivery across different modes of whelping. Bitches that underwent vaginal whelping (VW) (Group I), elective caesarean section (CS) (Group II), emergency CS (Group III) and mifepristone primed whelping (Group IV) presented to University Veterinary Hospitals, Kokkalai and Mannuthy constituted the material for the present study. The study enrolled a total of 28 bitches from different breeds. Serum progesterone was estimated using a chemiluminescent immunoassay (CLIA) and serum PGFM was measured using an enzyme-linked immunosorbent assay (ELISA). Progesterone levels were below 2.25 ng/mL in all groups, with Group I showing the lowest levels on the day of whelping, significantly different from the other groups. Groups II, III and IV had similar progesterone levels on the day of parturition/caesarean. Group I also had the highest PGFM level (89.10 ± 6.12 nM/L) on the day of whelping. Group II had higher PGFM levels (33.00 ± 2.78 nM/L) compared to Group IV (20.94 ± 2.61 nM/L), while Groups III and IV had similar PGFM levels. The significant hormonal variations observed in different groups signify the role of different modes of whelping in modulating the endocrine axis and pregnancy outcome.

Keywords: *Progesterone, PGFM, whelping mode*

In canine pregnancy, progesterone (P_4) is exclusively produced by the corpus luteum. While the average P4 levels in both pregnant and non-pregnant cycles show little difference across various studies, they are generally slightly higher during pregnancy around 25–30 days post-ovulation in most cases (Concannon, 2011). A sharp decline in P_4 levels happens just before parturition. During the prepartum period, there was a sudden decline in the level of serum P_4, dropping to less than 2 ng/mL by (approximately 6.4 nmol/L) within the final 24 h of whelping (Kutzler *et al*., 2003; Kim *et al*., 2007).

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A negative relationship between prostaglandin F_2 alpha ($PGF_{2\alpha}$) and P_4 concentration was observed during the prepartum period in female dogs (Veronesi *et al*., 2002). Verstegen-Onclin and Verstegen (2008) reported that the rapid decrease in P_4 concentration was attributed to the release of $PGF_{2\alpha}$ and the resulting luteolysis, which was initiated 36 hours before whelping. According to recent *in vitro* and gene expression studies, the foetal trophoblast of the placenta was found to be a major source of $PGF_{2\alpha}$ in dogs (Luz *et al*., 2006; Kowalewski *et al*., 2015). In their investigation, Princy *et al*. (2023) used the chemiluminescent immunoassay (CLIA) method and observed a notable decline in serum P_4 concentration as parturition approached.

According to Concannon *et al*. (1988) and Veronesi *et al*. (2002), levels of circulating PGFM start to increase one or two days before whelping, reach their peak during parturition (200-400 pg/mL) and then begin to decrease. They also suggested that an early rise in PGFM could be used as a timely predictor of cervical dilatation, with PGFM levels ideally determined 48–60 hours before parturition. The prepartum luteolysis phase was marked by low circulating progesterone levels and elevated PGFM levels (Kowalewski *et al*., 2010). In dogs which neared parturition, the luteolytic effects of $PGF_2\alpha$ hastened the breakdown of the corpora lutea Kowalewski (2014). In a study conducted by Tamminen *et al*. (2019), found that dogs having normal vaginal deliveries exhibited higher PGFM levels compared to those with dystocia or undergoing elective C-sections. Similarly, Nothling *et al*. (2022) reported that cervical dilation occurred within 36 hours in nearly 90 per cent of the bitches following a 10 per cent rise in PGFM levels (1019 to 52,699 pg/mL). The present study aims to explore the potential relationship between P_4 and PGFM levels across different modes of whelping in canines.

Materials and methods

Bitches (large/ medium/ small breeds) that underwent vaginal whelping, elective CS, emergency CS and whelping induction protocols presented to University Veterinary Hospitals, Kokkalai and Mannuthy constituted the subjects for the present study. Based on the mode of whelping, bitches were grouped into four. Group I included bitches that underwent normal vaginal whelping (VW) (n=7), Groups II and III consisted of those bitches that underwent elective CS (n=8) and emergency CS (n=7) and those bitches that underwent induced whelping/ CS after mifepristone priming formed Group IV (n=6). Bitches in Groups I, II and III, were not presented for early pregnancy ultrasonography and the pet parents presented them shortly before or after the expected whelping day calculated based on breeding dates or since the bitches were exhibiting straining due to difficulty in whelping or visible greenish discharge from the vulva. Such animals underwent a detailed ultrasonographic examination on the day of whelping/ CS. Bitches in Group IV were subjected to early pregnancy ultrasonography on or before 24 days from the date of last breeding and those animals with gestational age (GA) less than or equal to 30 days from LH surge, including different-sized breeds were selected for the study. Animals that did not deliver on the expected date of whelping (EDW) were subjected to B-mode and Doppler ultrasonography to assess the viability of the conceptuses, foetal cardiac oscillations and foetal growth rate. Whelping was induced by administration of mifepristone @ 3.5-5 mg/kg orally at eight-hour intervals three times and the bitches were reviewed 24 h after the commencement of treatment (Simon *et al*., 2017). Misoprostol @ 10 µg/kg twice daily was administered orally in those bitches showing signs of cervical relaxation on vaginoscopic examination. A CS was performed in non-responsive animals 36 hours after the commencement of treatment.

For harvesting sera, a volume of 5 mL peripheral blood samples was collected on the day of whelping/ CS from the cephalic vein into vacutainers with a clot activator from all the dogs. Sera samples were separated, centrifuged and a minimum of 1.5 mL sera samples were aliquoted into two. Aliquoted samples were stored at -20°C for serum progesterone assay and PGFM estimation.

Serum P_4 levels were measured in all dogs before whelping using the Chemiluminescent Immunoassay (CLIA) method (CL-900i3). A commercial CLIA kit (PROG CLIA) for canines (MINDRAY, Shenzen, China) was used as per the manufacturer's instructions, to obtain the results, with progesterone levels expressed in ng/mL. The progesterone concentration was determined using a calibration curve.

The serum PGFM concentration was estimated in all the bitches on the day of whelping/CS using a canine PGFM ELISA kit (ORIGIN, Karunagappalli, Kerala) according to the manufacturer's instructions. The concentration of PGFM was expressed in nM/L. The test principle applied was sandwich enzyme immunoassay. The concentration of PGFM in the samples was determined by comparing the optical density (OD) of the samples to the standard curve.

Statistical analysis of the collected data was performed on SPSS version 24.0. One-way ANOVA followed by Duncan's multiple range test was done to compare parameters between the four groups.

Results and discussion

The mean progesterone levels on the day of whelping were 1.14 ± 0.14, 1.70 ± 0.17, 2.05 ± 0.27 and 2.20 ± 0.10 (ng/mL) for Groups I (Vaginal whelping), II (elective- CS), III (emergency- CS) and IV (mifepristone primed), respectively (Table 1). The mean progesterone concentration was less than 2.25 ng/mL in all the groups. In a study conducted by Niharika (2012), to assess the efficacy of different whelping induction protocols *viz.,* mifepristone protocol and mifepristone-oxytocin combined protocol, mean serum P_4 values of 1.206 ng/mL and 2.43 ng/mL, respectively were observed after 24 h of initiation of treatment.

Similarly, a reducing trend in serum P_4 was documented by Princy *et al*. (2023). The average P_4 levels ranged from 6.16 to 7.51 ng/mL in animals during one to five days before parturition. However, a sharp reduction in P_4 to less than 1ng/mL was evident in animals just before the onset of labour. The researchers also found that analysing P_4 levels alone could serve as a reliable indicator for estimating the approximate time of whelping in canines, as a value below 2 ng/mL indicated impending labour within 12 to 24 hours (Concannon *et al*., 1977).

A highly significant difference (P<0.01) could be observed in the P_4 levels between Group I and others. Group I showed the lowest mean P_4 level on the day of whelping. The obvious reason behind this could be the physiological removal of progesterone block in the normal vaginal whelping process. This finding was in strong alignment with Verstegen-Onclin and Verstegen (2008) who reported that the rapid decrease in P_4 concentration was attributed to the release of $PGF_{2\alpha}$ and the resulting luteolysis, which was initiated 36 hours before whelping.

Similarly, Concannon *et al*. (1988), Kutzler *et al*. (2003), Kim *et al*. (2007) and Johnson (2008) also suggested that in pregnant dogs, progesterone was solely produced by the corpus luteum. A sudden drop in progesterone levels occurs just before delivery. During the prepartum period, there was a sudden decline in the level of serum P_4, dropping to less than 2 ng/mL (approximately 6.4 nmol/L) within the final 24 hours of whelping.

No significant difference (p>0.05) could be noticed in mean progesterone levels between Groups II, III and IV on the day of parturition. This was in accordance with Rota *et al*. (2015), who suggested a cut-off value of 3.4 ng/mL for progesterone estimated by CLIA could be used to safely perform a CS instead of 2 ng/mL. The authors opined that using 3.4 ng/mL as the cut-off ensured that the foetus was sufficiently mature, had reduced neonatal complications and optimised outcomes for both the dam and offspring.

The mean PGFM levels on the day of whelping were 89.10 ± 6.12, 33.00 ± 2.78, 25.06 ± 2.24 and 20.94± 2.61 (nM/L) for Groups I, II, III and IV, respectively (Table 2). The mean PGFM concentration was less than 100 nM/L in all the groups. According to Concannon *et al*. (1988) and Veronesi *et al*. (2002), levels of circulating PGFM started to increase one or two days before whelping, reaching the peak during parturition and then start to decrease. They also suggested that an early rise in PGFM could be used as a timely predictor of cervical dilatation, with PGFM levels ideally determined 48–60 hours before parturition. They reported PGFM levels of 35.2 ± 25.2 nmol/L and 74.9 ± 31.9 nmol/L were observed 24 hours before and on the day of whelping, respectively.

A highly significant difference (P<0.01) could be observed in the mean PGFM levels between Group I and other groups. Group I showed the highest mean PGFM level on the day of whelping. This was in alignment with the findings of Bergstrom *et al*. (2010), who showed that dogs experiencing dystocia had lower plasma PGFM concentrations (23 ± 13 nmol/L) compared to dogs undergoing normal whelping (76 ± 23 nmol/L). Tamminen *et al*. (2019) detected higher levels of PGFM in dogs undergoing normal vaginal delivery compared to those experiencing dystocia or undergoing elective caesarean section. The authors suggested that, during normal vaginal delivery, PGFM levels had raised significantly as prostaglandins played a crucial role in uterine contractions, cervical dilation and expulsion of the foetus. However, in cases of dystocia or elective caesarean section, the normal labour process was either delayed or disrupted leading to reduced PGFM levels.

There was a highly significant difference (p<0.01) between Groups II and IV in mean PGFM concentration. Group II had higher levels of PGFM when compared to Group IV. No significant difference (p>0.05) could be noticed in mean PGFM levels between Groups III and IV on the day of parturition. This might be because most of the dystocia would culminate in emergency CS and dogs suffering from dystocia were observed to have lower levels of PGFM (Bergstrom *et al*., 2010). Although statistical differences were not significant, the mean PGFM concentration was lower in the mifepristone primed group (Group IV). This may be due to the fact that many of the animals underwent C-sections after 36 hours because they did not respond to the induction protocol. The findings were in agreement with Ayana (2024), who observed lower PGFM levels among the induced bitches compared to the spontaneous whelping bitches. Baan *et al*. (2008)

Table 1. Serum progesterone concentration (Mean±SE) on the day of parturition in Groups I, II, III and IV (n=28), (ng/mL)

Variable	Group I	Group II	Group III	Group IV	F-value (P-value)
Progesterone	$1.14^b \pm 0.14$	$1.70^a \pm 0.17$	$2.05^a \pm 0.27$	$2.20^a \pm 0.10$	6.181** (0.003)

** Significant at 0.01 level. Means having different letters as superscripts differ significantly

Table 2. Serum PGFM concentration (Mean±SE) on the day of parturition in Groups I, II, III and IV, (nM/L)

Variable	Group I (n=7)	Group II (n=8)	Group III (n=7)	Group IV (n=6)	F-value (P-value)
PGFM	$89.10^{a} \pm 6.12$	$33.00^{b} \pm 2.78$	$25.06^{bc} \pm 2.24$	$20.94^{c} \pm 2.61$	68.862** (<0.001)

** Significant at 0.01 level. Means having different letters as superscripts differ significantly

also observed that PGFM concentrations increased before parturition in both naturally delivering bitches and those induced to deliver. They also observed that although the PGFM levels were lower in the induced group, before parturition, PGFM levels peaked in both groups during whelping but rapid decline was observed after delivery in the spontaneously whelping group and conversely, PGFM levels remained elevated in the induced group.

The correlation between a reduction in P_4 and a steep rise in PGFM suggested that gradual regression of the corpus luteum, followed by a sharp prepartum drop in P_4 levels initiated the prepartum luteolytic cascade and the initiation of whelping (Kowalewski *et al*., 2020). Changes in foeto-maternal communication, driven by the reduction in progesterone receptors and the effects of P_4, activated this cascade. The sudden increase in $PGF_{2\alpha}$ concentrations is believed to trigger the rapid decline in P_4, a process that does not take place during a non-pregnant cycle where only gradual and consistent reduction could be expected by the natural withdrawal of luteotrophic support.

Conclusion

In conclusion, a sudden drop in progesterone and rising levels of PGFM are essential hormonal factors behind the whelping process. The level of P_4 tended to be lower than the threshold value (2ng/mL) in all the groups, however, a significant reduction could be noticed in the VW group (1.14 ± 0.14 ng/mL). However, no significant difference could be noticed in the mean progesterone levels between elective- CS, emergency- CS and mifepristone-primed groups on the day of parturition. As far as PGFM is concerned, a simultaneous rise in PGFM concentration could be appreciated in all the groups as parturition approached. A highly significant rise was noted in the VW group (89.10 ± 6.12 nM/L) and a lower level in the mifepristone-primed group (20.94 ± 2.61 nM/L). Elective-CS group had higher levels of PGFM when compared to the mifepristone-primed group and emergency-CS group. This might be because, in the emergency- CS group, most of the cases would result from dystocias and dogs suffering from dystocia were observed to have lower levels of PGFM (Bergstrom *et al*., 2010). This might be due to a delayed labour process. Similarly to emergency- CS, mifepristone-primed group also showed lower levels of PGFM. This may be attributed to the fact that the majority of these animals underwent C-sections after 36 hours, likely due to a lack of response to the induction protocol. The different levels of PGFM in different groups reflect a relation between various modes of whelping and progesterone-PGFM ratio. Estimating additional hormones like oestradiol, oxytocin, vasopressin, and cortisol will also be rewarding in future research. The results of the present study suggested an obvious role of both P_4 and PGFM in the pathologic development of total primary uterine inertia and dystocia in bitches.

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Conflict of interest

The authors don't have any conflict of interest.

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