



## Effect of mid luteal administration of PGF<sub>2α</sub> on oestrus duration, serum progesterone profile and ovarian changes in crossbred cows exhibiting prolonged oestrus<sup>#</sup>

Bini Mol Sebastian<sup>1</sup>, M.P. Unnikrishnan<sup>2\*</sup>, C. Jayakumar<sup>1</sup>,  
 B. Bibin Becha<sup>3</sup>, K. Raji<sup>4</sup> and Surej Joseph Bunglavan<sup>5</sup>

<sup>1</sup>Department of Animal Reproduction, Gynaecology and Obstetrics, <sup>4</sup>Department of Veterinary Physiology, <sup>5</sup>Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, <sup>2</sup>Centre for Pig Production and Research, Mannuthy, <sup>3</sup>Base Farm, Kolahalamedu, Idukki, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

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### Abstract

The study compared oestrus duration, duration of follicular persistence, dominant follicle (DF) size, mid-cycle corpus luteum (CL) size and serum progesterone (P<sub>4</sub>) profiles between spontaneous and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>)-induced oestrous cycles in crossbred cows exhibiting prolonged oestrus. Six apparently healthy crossbred cows under six years of age, within six months postpartum and exhibiting prolonged oestrus (>24 h), were selected for the study. Transrectal ultrasonography (TRUS) was performed at 24 hr intervals from the onset of spontaneous oestrus until ovulation to assess DF size and follicular persistence, and again on day 10 post-oestrus to evaluate CL size, also all cows received 500 µg of cloprostenol intramuscularly followed by TRUS during the induced oestrous cycle. Blood samples were collected on each day when TRUS was performed to determine serum P<sub>4</sub>. Data recorded before and after treatment were analysed using repeated measures ANOVA. No significant differences (*p* > 0.05) were observed between spontaneous and induced cycles in oestrus duration (48.00 ± 5.37 h vs. 44.00 ± 5.06 h), duration of follicular persistence (60.00 ± 5.37 h vs. 56.00 ± 5.06 h), DF size (12.72 ± 0.76 mm vs 13.37 ± 0.39 mm), CL size (18.42 ± 1.86 mm vs 18.40 ± 1.56 mm) and serum P<sub>4</sub> (0.51 ± 0.08 ng/mL vs 0.48 ± 0.04 ng/mL) concentrations. These findings suggest that PGF<sub>2α</sub> administration during the mid-luteal phase did not significantly influence morphology and function of ovarian structures in the induced oestrus in cows with prolonged oestrous. Non-significant serum P<sub>4</sub> milieu after the treatment suggests incomplete luteolysis or presence of extra-gonadal sources of P<sub>4</sub>. Further, studies with a larger sample size are required to validate these results and explore more effective strategies to manage the prolonged oestrus in dairy cattle.

**Keywords:** Prolonged oestrus, PGF<sub>2α</sub> luteolysis, Suprabasal- P<sub>4</sub>, transrectal ultrasonography

Prolonged oestrus, defined as an unusually extended duration of behavioural oestrus, is a major cause of reproductive inefficiency in cattle, occurring in up to 50 per cent of repeat breeder cows (Ghuman et al., 2014). Arun et

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\*Corresponding author: [unnikrishnan@kvasu.ac.in](mailto:unnikrishnan@kvasu.ac.in), Ph. 9447027454

al. (2020) reported an overall occurrence of 25.86 per cent under farm conditions in Kerala. This condition complicates accurate determination of the optimal insemination time, resulting in reduced conception rates, increased labour and economic losses.

The most widely accepted causes include suprabasal serum progesterone ( $P_4$ ) concentrations during oestrus or delayed ovulation (Singh et al., 2009). Elevated serum  $P_4$  has been linked to incomplete luteal regression or extra-gonadal secretion, such as from the adrenal glands under stress (Honparkhe et al., 2010). Reduced CL regression may result from the down-regulation of oxytocin and prostaglandin receptors around day 16 of the cycle (Athira et al., 2024). Suprabasal  $P_4$  interferes with the ovulatory cascade, prolonging the interval from oestrus onset to ovulation (Niyas et al., 2019).

Being a potent luteolytic agent,  $PGF_{2\alpha}$  promotes timely CL regression, restores normal follicular growth and facilitates ovulation, when administered during the mid-luteal phase (Ghuman et al., 2014). The present study was aimed to compare the oestrus duration, duration of follicle persistence, follicular and luteal dimensions as well as serum  $P_4$  profiles between spontaneous and  $PGF_{2\alpha}$ -induced oestrous cycles in crossbred cows exhibiting prolonged oestrus.

## Materials and methods

Six apparently healthy, normally calved crossbred dairy cows within six months postpartum, below six years of age and parity ranging from 1 to 3, exhibiting prolonged oestrus were selected for the study from the University Livestock Farm and Fodder Research and Development Scheme, Mannuthy. Cows with an oestrus duration longer than 24 h were considered to have prolonged oestrus (Arun et al., 2020). All animals were maintained under uniform feeding and management conditions throughout the study period. The selected cows were closely monitored for signs of oestrus. Comprehensive clinico-gynaecological examination was conducted during oestrus to confirm prolonged oestrus and to rule out any overt reproductive abnormalities.

Per rectal examination was performed on the 10<sup>th</sup> day after the end of oestrus to detect the presence of corpus luteum (CL), further to confirm ovulation. These animals were monitored daily at 12 hr intervals for the

onset of the subsequent oestrous cycle. Duration of oestrus was recorded and transrectal ultrasonography (TRUS) was carried out using real time colour Doppler ultrasound scanner (Mylab Sigma, EsaoteSpA, Italy), equipped with linear array, 5-10 MHz frequency trans-rectal transducer (SV3513, Esaote Europe B.V, Netherland), at 24 hr intervals from the onset of oestrus until ovulation, to assess dominant follicle size, duration of follicular persistence and ovulation status. The TRUS was repeated on day 10 post-oestrus to assess the size of CL and all the cows were treated with an intramuscular injection of 500  $\mu$ g cloprostenol (Inj. Clostenol-2mL, Zenex Animal Health India Pvt. Ltd). The TRUS was performed during the induced oestrus at 24 hr intervals, starting from the onset of oestrus until ovulation and on day 10 of induced oestrus. Blood samples were collected from jugular vein on each day of TRUS and serum was separated and stored at  $-80^{\circ}\text{C}$  for  $P_4$  assay which was measured using chemiluminescence immunoassay (CLIA) kit ( $P_4$  CLIA, Shenzhen Mindray Biomedical Electronics Co., Ltd., Shenzhen, China) with the CL-900i analyser (Mindray, Shenzhen, China).

Data collected before and after treatment were tabulated and analysed using repeated measures analysis of variance (ANOVA) with SPSS software (version 24.0).

## Results and discussion

### *Duration of oestrus and duration of persistence of dominant follicle*

Before treatment, the mean oestrus duration and duration of follicular persistence was  $48.00 \pm 5.37$  and  $60.00 \pm 5.37$  h, respectively (Table 1). Suprabasal serum  $P_4$  levels during oestrus results in prolonged expression of oestrous signs (Arun et al., 2020; Omari et al., 2020). In the present study, higher serum  $P_4$  concentrations were recorded during oestrus. The suprabasal  $P_4$  inhibits IGF-1 secretion and reduces the responsiveness of follicular cells to luteinising hormone (LH) (Omari et al., 2020).

Ghuman et al. (2014) reported that administration of  $PGF_{2\alpha}$  during the mid-luteal phase regulated oestrus duration by ensuring complete luteolysis. However, in the present study, no significant difference ( $p > 0.05$ ) was observed in the duration of either oestrus or follicular persistence, following treatment. Similar findings were reported in earlier studies involving cattle with prolonged oestrus, treated with  $PGF_{2\alpha}$  (Rajeswari, 2008; Shakir, 2018). This could probably be due to incomplete luteolysis and

**Table 1.** Mean duration of oestrus and duration of persistence of dominant follicle (Mean  $\pm$  SE) among prolonged oestrus cows

Parameter	Duration (h)	
	BT	AT
Duration of oestrus	$48.00 \pm 5.37$	$44.00 \pm 5.06$
Duration of persistence of DF	$60.00 \pm 5.37$	$56.00 \pm 5.06$

Values within a row did not differ significantly at 5% level

**Table 2.** Pre-ovulatory dominant follicle diameter and corpus luteum diameter (Mean  $\pm$  SE) among prolonged oestrus cows before and after treatment with PGF<sub>2 $\alpha$</sub> 

Parameter	Diameter (mm)	
	BT	AT
DF diameter (day 0 oestrus)	12.72 $\pm$ 0.76	13.37 $\pm$ 0.39
DF diameter (day 1 oestrus)	13.38 $\pm$ 0.91	14.45 $\pm$ 0.53
DF diameter (day 2 oestrus)	13.83 $\pm$ 0.81	13.47 $\pm$ 0.85
CL diameter on day 10 post oestrus	18.42 $\pm$ 1.86	18.40 $\pm$ 1.56

Values within a row did not differ significantly at 5% level

consequent suprabasal serum P<sub>4</sub> during oestrus, caused by down-regulation of PGF<sub>2 $\alpha$</sub>  and oxytocin receptors (Athira et al., 2024) or due to existence of extra-gonadal P<sub>4</sub> source (Honparkhe et al., 2010).

### **Diameter of pre-ovulatory dominant follicle and corpus luteum**

The pre-ovulatory DF diameter observed in the present study was 12.72  $\pm$  0.76 mm on day 0 (Table 2). Sartori et al. (2001) reported that ovulation occurred only when DF reached a threshold diameter, accompanied by sufficient oestradiol (E<sub>2</sub>) secretion and LH receptor expression. Incomplete luteolysis was a key factor limiting ovulation, as elevated P<sub>4</sub> levels suppressed the spontaneous preovulatory LH surge and reduced the LH response to exogenous GnRH (Umaña Sedó et al., 2022). The present study demonstrated that the diameter of DF at oestrus did not differ significantly ( $p > 0.05$ ) between spontaneous and PGF<sub>2 $\alpha$</sub> -induced cycles in cows exhibiting prolonged oestrus. Since suprabasal serum P<sub>4</sub> concentrations couldn't be curtailed by PGF<sub>2 $\alpha$</sub>  administration, no significant difference ( $p > 0.05$ ) in DF size could be attained after treatment.

In the present study, the observed CL diameter was 18.42  $\pm$  1.86 mm. Suresh (2024) recorded a larger CL diameter of 22.35  $\pm$  0.53 mm in normal oestrous cows, which is greater than the present value in prolonged oestrous cows. In prolonged oestrous cows, follicular development take place under a subluteal P<sub>4</sub> environment, leading to incomplete maturation of follicular cells and, consequently, suboptimal CL formation post-ovulation (Niribili et al., 2024). Well-established positive correlation is reported between the diameter of the ovulatory follicle and subsequent CL development (Omar et al., 2025). No significant difference ( $p > 0.05$ ) in CL diameter was noticed before and after treatment. The results of present study aligns with those of Quezada-Casasola et al. (2015), who also reported no significant differences ( $p > 0.05$ ) in DF or CL size between synchronised and spontaneous oestrous cycles. Although some previous studies suggested that PGF<sub>2 $\alpha$</sub> -induced shortening of the luteal phase could affect CL morphology and function, potentially reducing its responsiveness to luteotropic factors such as LH and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leading to a lower final CL weight (Skarzynski et al., 2009), the current findings did

not support these reports. Since the ovulatory follicle diameter remained unaffected by PGF<sub>2 $\alpha$</sub>  treatment, CL formation appeared to follow a similar pattern.

### **Serum progesterone concentration**

The overall mean serum P<sub>4</sub> concentration in prolonged oestrous cows on day 0 was 0.51  $\pm$  0.08 ng/mL (Table 3) on the day 0 of oestrus. Seventy per cent of repeat breeders with prolonged oestrus had serum P<sub>4</sub> concentrations above 0.30 ng/mL at oestrus (Singh et al., 2010), resulting in asynchrony between the onset of oestrus and ovulation (Duchens et al., 1995). Athira et al. (2024) reported that reduced expression of oxytocin receptors (OTR) and prostaglandin receptor (PTGS-2R) in endometrial cells on day 16 in cows with prolonged oestrus likely contributes to incomplete luteolysis and hormonal imbalance around the time of oestrus in these animals.

Ghuman et al. (2014) proposed that PGF<sub>2 $\alpha$</sub>  administration during the luteal phase in cows with prolonged oestrus could facilitate complete luteolysis prior to induced oestrus, thereby lowering P<sub>4</sub> levels at the onset of oestrus. However, in the current study, no significant difference ( $p > 0.05$ ) was observed in serum P<sub>4</sub> concentrations on the day of oestrus in the subsequent cycle following PGF<sub>2 $\alpha$</sub>  treatment. This lack of response might be due to a failure to achieve complete luteolysis or due to the presence of extra-gonadal sources of P<sub>4</sub>, such as the adrenal glands or adipose tissue, as suggested by Honparkhe et al. (2010).

Mid-luteal P<sub>4</sub> concentration in prolonged oestrous cows under study was 6.29  $\pm$  0.71 ng/mL. The size of the CL was used as an indirect measure of its function, as both CL diameter and area were previously shown to correlate with circulating P<sub>4</sub> concentrations (Fontes and Oosthuizen, 2022) and CL size was reported to be directly proportional to P<sub>4</sub> output (Omar et al., 2025). However, Pandey et al. (2018) observed that a larger CL size need not necessarily reflect increased P<sub>4</sub> production, as P<sub>4</sub> output is more closely associated with the relative proportions of small and large luteal cells within the CL. Serum P<sub>4</sub> concentrations on day 10 remained unchanged before and after treatment, likely due to the similar CL sizes observed in both cycles. The follicle developed at appropriate hormonal environment led to timely ovulation and formation of a CL having matured

**Table 3:** Serum progesterone concentration P<sub>4</sub> (Mean ± SE) during different days of oestrus and dioestrus among cows exhibiting prolonged oestrus

Days of oestrous cycle	Progesterone concentration (ng/mL)	
	BT	AT
Day 0	0.51 ± 0.08	0.48 ± 0.04
Day 1	0.45 ± 0.07	0.38 ± 0.03
Day 2	0.30 ± 0.05	0.26 ± 0.04
Day 10 after oestrus	6.29 ± 0.71	6.57 ± 0.51

Values within a row did not differ significantly at 5% level

luteal cells and having more capacity to produce P<sub>4</sub> and oxytocin (Abdel et al., 2020).

### Conclusion

This study demonstrated that PGF<sub>2α</sub> administration during the mid-luteal phase in cows with prolonged oestrus did not significantly alter oestrus duration, duration of follicular persistence, DF size, CL dimensions and serum P<sub>4</sub> concentrations. This could be due to failure to achieve complete luteolysis or sustained P<sub>4</sub> secretion from extragonadal sources. Further research with a larger sample size is recommended to validate these observations and explore alternative therapeutic approaches for managing prolonged oestrus.

### Conflict of Interest

The authors declare that no conflicts of interest are related to this study.

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