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## Hormonal dynamics during different phases of oestrous cycle in estrus synchronized indigenous Kangayam cows

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### Abstract

The study was conducted to find out hormonal profile during different stages of estrous cycle in estrus synchronized Kangayam cows. A total of 10 Kangayam cows between 3<sup>rd</sup> and 5<sup>th</sup> calving were selected and serum samples were collected on day 0, 5, 9 and 18<sup>th</sup> day of synchronized estrous cycle. The profiles of progesterone (P<sub>4</sub>), estradiol (E<sub>2</sub>), follicle stimulating hormone (FSH) and luteinizing hormone (LH) during different phases of estrous cycle were estimated. The results revealed that in proestrus phase, the P<sub>4</sub> concentration was 1.45±0.1 ng/mL whereas estradiol and FSH concentration were 20.44±0.2 pg/mL and 1.62±0.07 IU/mL, respectively. The level of LH during proestrus was 1.81±0.04 IU/mL. The P<sub>4</sub> concentration dropped to 0.36±0.2 ng/mL during estrus phase and the estradiol and FSH were increased to 24.29±0.3 pg/mL and 2.05±0.2 IU/mL, respectively. However, estradiol and LH level dropped in metestrus and diestrus phase 12.53±0.1 pg/mL, 1.05±0.01 IU/mL and 14.56±0.1 pg/mL, 1.54±0.02 IU/mL respectively. The results of the study could be useful to various hormonal intervention studies of Kangayam animals to improve the reproductive performance.

**Keywords:** Synchronization, estrous cycle, Hormonal profile and Kangayam cattle

### Introduction

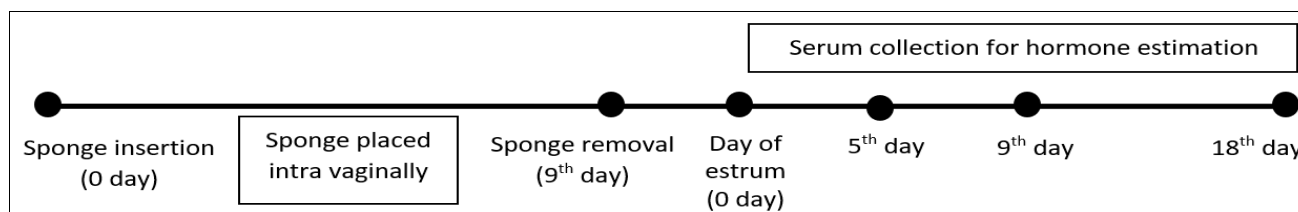
Indigenous breeds are well known for their draft efficiency, heat tolerance and resistance to infectious diseases. Kangayam breed of cattle is considered as one of the best-known superior draught breed in Tamil Nadu and it is known for its superior qualities such as adaptation to poor nutrition and longevity (Kandasamy, 2001) [8]. The indigenous breeds of cattle have poor reproductive performance as compared to crossbreds and European breeds. Though this breed cannot yield more like other indigenous milk purpose breeds, Red Sindhi, Sahiwal, Tharparkar etc., selection of animals can improve the production performance (Akila *et al.*, 2012) [1]. Further the changing climatic conditions definitely have impact on the reproductive efficiency by altering the hormonal physiology. Study on hormonal profile not only enhances basic understanding of reproductive physiology, but also helps to explore opportunities to improve reproductive management in *Bos taurus* cows (Ginther *et al.*, 1996) [3]. However, information on these aspects of reproduction is still fragmented and lacking in *Bos indicus* dairy cows. Studies on serum concentration of reproductive hormones like progesterone, estradiol, FSH and LH is scarce in Kangayam cows. Hence the present study was conducted to estimate the reproductive hormones during different phases of estrous cycle in indigenous Kangayam cattle.

### Materials and Methods

The present study was conducted at the Department of Veterinary Physiology, Veterinary College and Research Institute, Namakkal. A total number of 10 pluriparous, healthy Kangayam cows between 3<sup>rd</sup> and 5<sup>th</sup> lactation were selected at farmer's field in Tiruppur and Erode Districts. In gynaeco-clinical examination, animals with no palpable abnormalities but having smooth ovaries and no palpable structures on both the ovaries were selected for the study. The estrus synchronization was performed in all the animals using intravaginal sponge.

The intravaginal sponge containing 1.5 g of progesterone was inserted intravaginally for induction of estrus in selected cows. The animals were restrained in Travis and the perineum was cleaned with water to remove the dung and other dust particles from the vulval lips. The vaginal speculum was lubricated with liquid paraffin was inserted through vulva into

vagina slowly and gently without causing any damage to the genital tract. The progesterone sponge was passed into vagina through the speculum and with the help of plastic plunger pushed up to anterior vagina. The sponge was removed on 9<sup>th</sup> day of insertion and the animals were observed for estrus signs.



The blood samples were collected in the induced estrus on day 0, 5, 9 and 18<sup>th</sup> day of estrous cycle and the serum was separated by centrifuging at 3000 rpm for 30 minutes. The serum samples were stored at -20 °C until the analysis of hormones. The concentration of hormones progesterone, estrogen, FSH and LH in serum samples were estimated in duplicate using commercial radioimmunoassay Kit (Immunotech, Prague, Czech Republic) as per manufacturer's protocol. The radioactivity in samples was measured in gamma counter (Stratec, Germany) in the Department of

Veterinary Physiology, VCRI, Namakkal.

The collected data were analysed using one-way ANOVA and Independent 't' test using SPSS software version 21.0 and the results are presented as means with standard error of means.

### Results and Discussion

The reproductive hormones *viz.*, Progesterone, Estradiol, Follicle-Stimulating Hormone (FSH) and Luteinizing hormone (LH) were estimated at different stages induced estrous cycle in Kangayam cows and are presented in table 1.

**Table 1:** Mean ( $\pm$ SE) reproductive hormone concentration of Kangayam cows at different phases of estrous cycle

Phases of estrous cycle	Progesterone (ng/ml)	Estradiol (pg/ml)	FSH (IU/L)	LH (IU/L)
Proestrus	1.45 <sup>b</sup> $\pm$ 0.1	20.44 <sup>c</sup> $\pm$ 0.2	1.62 <sup>b</sup> $\pm$ 0.07	1.81 <sup>a</sup> $\pm$ 0.04
Estrus	0.36 <sup>a</sup> $\pm$ 0.2	24.29 <sup>d</sup> $\pm$ 0.3	2.05 <sup>d</sup> $\pm$ 0.2	6.92 <sup>b</sup> $\pm$ 0.3
Metestrus	2.53 <sup>c</sup> $\pm$ 0.2	12.53 <sup>a</sup> $\pm$ 0.1	1.85 <sup>c</sup> $\pm$ 0.1	1.05 <sup>a</sup> $\pm$ 0.01
Diestrus	5.95 <sup>d</sup> $\pm$ 1.2	14.56 <sup>b</sup> $\pm$ 0.1	1.33 <sup>a</sup> $\pm$ 0.1	1.54 <sup>a</sup> $\pm$ 0.02

Each value is mean of ten observations.

Means with different superscripts in a column differ significantly ( $p < 0.05$ )

### Serum progesterone

The mean  $\pm$  SE progesterone concentration during proestrus was 1.45 $\pm$ 0.1 ng/mL, which dropped to 0.36 $\pm$ 0.2 ng/mL during in estrus phase which indicated regression of corpus luteum of previous cycle. The metestrus phase showed progesterone concentration of 2.53 $\pm$ 0.2 ng/mL, which indicated the development of new corpus luteum. The progesterone concentration was increased two fold during diestrus (5.95 $\pm$ 1.2ng/mL), indicating the continuance of life of corpus luteum.

On statistical analysis, the progesterone concentration exhibited a significant difference ( $p < 0.05$ ) in each stage of estrus cycle. Hafez *et al.* (2008) [4] reported that higher progesterone concentration during diestrus could be due to the functional activity of the corpus luteum and decreasing trend during proestrus phase due to the initiation of luteolysis and similar trend was also noticed in the study. The observations of the progesterone concentration in the present study concurred with the observations made in Punganur cattle at different phases of estrus cycle. Naik *et al.* (2013) [11] Furthermore, the change in the progesterone trend in present study is similar to previous report showing increased concentration of progesterone on 17 and 21 day of post insemination in Ongole cattle fed with different levels of crude protein (Hayati *et al.*, 2021) [6]. Contrary to present report, higher progesterone concentration was observed in Sahiwal cows during estrum with overt estrus sign group (1.23  $\pm$  0.99 ng/ml) and silent estrus group (1.08 $\pm$  0.99 ng/ml) by Mondal and Prakash (2003) [9]. The variations in the concentration of progesterone hormone might be due to the

differences in the sampling frequency, seasons and climate at the time of sampling, age and physiological stage (lactating or non-lactating) of the animals (Alvarez *et al.*, 2000) [2]. Howell *et al.* (1991) [7] reported that the HF breed is more susceptible to heat stress than Jersey breed, so that increased level of cortisol may cause reduction in secretion of progesterone by reducing the GnRH release from hypothalamus.

### Serum estradiol

The estradiol concentration in Kangayam cows during proestrus was 20.44 $\pm$ 0.2 pg/mL, which further increased during estrus to 24.29 $\pm$ 0.3 pg/mL. However, estradiol level in metestrus dropped to 12.53 $\pm$ 0.1 pg/mL and the level remained comparable in diestrus (14.56 $\pm$ 0.1pg/mL).

The trend in change of estradiol concentration was similar to findings of Hassan *et al.* (2021) [5] reported in indigenous Sahiwal cattle. The intensity of estrus in each phase of estrus cycle differed significantly ( $p < 0.05$ ) in different breed of cows. Contrary to present study, Naik *et al.* (2013) [11] reported mean estrogen concentration of 20.24 pg/ml in Punganur cows during 0 day of estrous cycle. The higher level (24.29 pg/ml) of estrogen in present study during estrus might be due to the influence of negative feedback effect of progesterone from intra vaginal sponge. Upon removal of sponge from vagina, the release of more amount of FSH and LH hormones might caused the follicle development and more estrogen secretion during estrus. They also stated that significantly higher estrogen concentration one day before estrus might be due to the development of preovulatory follicle. The estrogen concentration decreased from day of

estrus to metestrus stage, which could be due to ovulation and influence of luteinizing hormone to development and growth of luteal cells (Hafezet *et al.*, 2008) [4].

### Serum follicle stimulating hormone

The mean serum follicle stimulating hormone (FSH) concentration during proestrus was  $1.62 \pm 0.07$  IU/mL, which further elevated to  $2.05 \pm 0.2$  IU/mL during estrus. The FSH concentration in metestrus was estimated as  $1.85 \pm 0.1$  IU/mL and it was dropped to  $1.33 \pm 0.1$  IU/mL during diestrus. On statistical analysis, the FSH concentration during estrus and metestrus differed significantly with other three stages of estrus cycle. These findings are in agreement with the earlier reports recorded in Sahiwal cow during proestrus stage of estrous cycle. The report showed peak concentration of FSH during estrus phase ( $2.40 \pm 0.94$ ) and decreased ( $1.06 \pm 0.20$ ) at metestrus phase of estrous cycle with increasing level during luteal phase ( $1.58 \pm 0.18$  -  $1.69 \pm 0.31$ ) which declined to  $1.67 \pm 0.21$  during proestrus phase of estrous cycle (Mondal *et al.*, 2003) [9]. The same trend in hormonal dynamics also reported by Hassan *et al.* (2021) [5] in sahiwal cattle.

### Serum luteinizing hormone

The mean luteinizing hormone (LH) concentration in lactating Kangayam cows during proestrus stage was  $1.81 \pm 0.04$  IU/mL, which significantly elevated in estrus phase ( $6.92 \pm 0.3$  IU/mL). However, the LH level dropped in metestrus and diestrus to  $1.05 \pm 0.01$  and  $1.54 \pm 0.02$  IU/mL, respectively. The concentration of LH during the proestrus observed as in basal level ranged from 0.23 to 1.77 IU/mL. The concentration of LH in the present study elevated from basal level to peak level (6.92) during estrus might be due surge release to ovulate the mature Graafian follicle. The rise in the LH at estrus phase in present study concurs with the earlier reports of Peters (1985) who reported that the LH concentration in beef cows (Hereford X Frisian) during mid-luteal phase was  $1.80 \pm 0.05$  ng/ml and gradually increased to 2.46 ng/ml during early follicular phase (one day before LH surge). He also reported that LH pulse was more during early luteal (36 pulses) phase than mid luteal (7 pulses) phase. Similarly, Rahe *et al.* (1980) observed lesser amplitude and greater frequency of LH pulse in luteal phase whereas greater amplitude and lesser frequency in mid luteal phase.

The reproductive hormone profile in Kangayam cows differ during different stages of estrous cycle. In proestrus phase, the progesterone concentration was  $1.45 \pm 0.1$  ng/mL whereas estradiol and FSH concentration was increased to  $20.44 \pm 0.2$  pg/mL and  $1.62 \pm 0.07$  IU/mL, respectively. The LH concentration was at basal level during proestrus ( $1.81 \pm 0.04$  IU/mL). The serum progesterone dropped to  $0.36 \pm 0.2$  ng/mL during estrus and the estradiol and FSH was increased to  $24.29 \pm 0.3$  pg/mL and  $2.05 \pm 0.2$  IU/mL, respectively and further reduced during other two phases of estrous cycle. However, estradiol and LH level dropped in metestrus and diestrus phase  $12.53 \pm 0.1$  pg/mL,  $1.05 \pm 0.01$  IU/mL and  $14.56 \pm 0.1$  pg/mL,  $1.54 \pm 0.02$  IU/mL, respectively. However the values should be comparable with other Bos taurus breeds to create baseline data.

### Conclusion

The present study concludes that the characterization of oestrus cycle with hormonal changes during different phases seemed to be related to Bos taurus cows and this relevance might be used to develop synchronization and resynchronization strategies, standardize potential protocols

for superovulation, and to carry out commercial embryo transfer programs in indigenous Kangayam cows

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### Conflict of Interest

All the authors declare no conflict of interest.

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