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E Niyas
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

MO Kurien
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

C Jayakumar
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

RS Abhilash
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

KS Anil
Department of Livestock Production and Management, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

S Reshma
M.V.Sc scholar, Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Correspondence
MO Kurien
Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

Sonographic assessment of follicular and luteal characteristics in repeat breeding cattle with prolonged oestrus

E Niyas, MO Kurien, C Jayakumar, RS Abhilash, KS Anil and S Reshma

Abstract
Follicular dynamics (FD), luteal growth and regression characteristics as well as the progesterone (P4) concentration in repeat breeding (RB) cows with prolonged oestrus was assessed. Ten normally cycling cows with oestrus period of less than 36h formed the control group while ten RB animals with prolonged oestrus (>36h) formed Group II. 95 per cent of the selected animals exhibited either two or three waves per cycle. Duration of persistence of pre-ovulatory follicle was longer in RB animals (>24 hrs in 70% animals). The luteal growth and regression rate differed non-significantly between the normal and RB animals. In RB animals, a significant difference in P4 concentration was observed on the day of oestrus (0.67±0.03ng/ml) in contrast to cows in control group (0.36±0.03 ng/ml). In conclusion, a higher duration of persistence of pre-ovulatory follicle and suboptimal luteal profile were observed in RB animals with prolonged oestrus.

Keywords: Repeat breeder, prolonged oestrus, follicular dynamics.

1. Introduction

The animals which failed to conceive after three or more repeated inseminations in the absence of any detectable abnormalities are classified as repeat breeders (Dadarwal *et al.* 2005) [6]. In view of a profitable dairying, which ideally requires an annual calf crop of one, RB has a significant impact on dairy cattle economics. Due to high incidence (20-39%) of RB, huge economic losses are encountered in dairy sector (Nanda and Singh, 2008) [13]. Repeat breeding in dairy cow has an uncertain and multifactorial etiology. Probable etiology of RB includes unsuccessful fertilization (30 to 40 %), embryonic death (20 to 35 %), inadequate luteal P4 and other hormonal disturbances, heat detection errors, abnormal sperm or egg function and nutritional deficiencies.

Till now numerous works were conducted to reduce the impact of RB on livestock sector. Initial studies were based on slaughter specimen and rectal examinations. Though treatment protocols derived from these studies helped to find a solution to some extent, a revolutionary change in bovine reproduction field happened after the introduction of ultrasonography in 1990's. On perusal of literature, ample studies relating to FD in normally cycling animals are available. However, there is a paucity of literature on the peculiarities of FD and luteal characteristics of repeat breeders with prolonged oestrus.

2. Materials and methods

The study was performed at University Livestock Farm and Fodder Research and Development Scheme (ULF &FRDS), Veterinary and Animal Sciences University, Mannuthy during the period from November 2016 to April 2017. Clinically healthy postpartum crossbred dairy cows with a history of normal calving, maintained under identical conditions of feeding and management were selected for the study. All the animals which failed to conceive after three or more consecutive AI were considered as RB and those animals with oestrous period longer than 36 h were considered as RB with prolonged oestrus (Dadarwal *et al.* 2005) [6]. Cows with regular oestrous cycle and exhibiting oestrous period of less than 36 h were considered as normally cycling animals. An absolute body condition score was derived as described by Edmonson *et al.* (1989) [9] for each cow and the animals with a body condition score of 3 or more were selected for the study. Detailed clinico-gynaecological examination was carried out in all selected animals to rule out any anatomical and pathological conditions. Endometritis was ruled out by performing white side test.

2.1 Design of experiment
Ten cows with normal estrous cycle, exhibiting normal estrous period of less than 36h formed the control group (group I). Ten RB animals with history of prolonged oestrus were classified under Group II. Routine trans- rectal ultrasonography (TRUS) was performed to record the ovarian status in all the selected animals. Trans-rectal scanning was done with a real time colour Doppler ultrasound scanner (Mylab™ Gamma, Esoate Sp A, Italy) equipped with linear array, 5-10 MHz frequency trans-rectal transducer (SV3513, Esaote Europe B.V, Netherland).

Follicular dynamics of one estrous cycle (C-1) in all the animals (n=30) were studied by performing TRUS, starting on the day of oestrus and further at two day interval. During the pre-ovulatory period, diameter of the dominant follicle (DF) was recorded at 12 h interval from the day of oestrus till the time of ovulation. Following ovulation, sonographic assessment of the luteal growth and regression was performed at 24h interval up to next oestrus. The largest distance was measured in the scanned image of a pre-ovulatory follicle (D1), another diameter was measured with an angle of 90° to D1 (D2). The actual follicular size was determined by taking the average of D1 and D2 and expressed in millimeters (mm). Blood samples were collected for serum progesterone estimation on day 0 (day of standing oestrus), 5, 10 (C-1) and day of next oestrus (C-2).

The number of follicular waves, interval from onset of oestrous signs to maximum follicular diameter, period of follicular dominance and interval from follicular dominance to ovulation were recorded and was compared between normal and RB cows. Persistence of the DF was deemed based on observation of same follicular diameter at two consecutive ultrasonography observations 12h apart.

3. Results and Discussions
Follicular growth pattern monitored by Real time B-mode trans-rectal ultrasonography was characterized by wave like growth of follicles starting at different stages of oestrous cycle.

3.1 Number of follicular waves
Three different patterns of follicular waves were noticed during the study and the pattern observed in the selected animals were one wave (*n*=1), two wave (*n*=11) and three wave (*n*=8) per cycle. The most common pattern observed in Group I animals was two waves per cycle (n=7) and remaining animals exhibited three waves per cycle (n=3), whereas in RB animals with prolonged oestrus, three different patterns of follicular growth were observed. Three waves per cycle formed the majority (n=5) and other patterns noticed were two waves per cycle (n=4) and one wave per cycle (n=1). The pattern of follicular growth observed in RB animals with prolonged oestrus in group II is contrary to the findings of Perez *et al*. (2003) [14], who reported from their study in RB cows that the most predominant pattern of follicular growth in RB cow was two waves (72.2 %) compared to three waves (16.6 %). Overall, 95 per cent of the animals selected for the study exhibited either two or three wave pattern of follicular growth or 5 per cent animal exhibited one wave cycle. These findings are in accordance with the findings of Adams (1999) [1] who concluded from his study that in majority of oestrous cycle (>95 %) in cattle predominant pattern of follicular wave was composed of either two or three.

3.2 Dominant and subordinate follicles
The mean maximum diameter of DF in control group and RB animals with two waves per cycle was 14.53± 0.51 and 13.83± 0.60 in first wave and 16.30± 0.63 and 14.78± 0.83 in second wave (ovulatory follicle) respectively. An increased diameter of dominant follicle was noticed during second wave when compared to first wave in both the groups and these findings are in agreement with the study by Sunderland *et al*., (1994) [19]; who reported that DF (ovulatory follicle) in the second wave of two wave cycle was found to be greater than that in the first wave, probably due to the ovulatory status in the second dominant follicle which offered the stimulus for the increased growth.

The mean maximum diameter (mm) of follicle that dominated during the first wave in control group and RB animals with prolonged oestrus with three waves per cycle was 13.32± 0.77 and 13.76± 0.60, it was 12.92± 0.96 and 12.01± 0.75in second wave and 13.57± 0.96 and 14.92± 0.74 in third wave (ovulatory follicle) respectively. Animals with three follicular waves of both the groups, the third DF (ovulatory follicle) exhibited the maximum size and there was no significant difference noticed with dominant follicular size of first wave. These findings are in harmony with the studies of Sirois and Fortune (1990) [18] who observed a higher diameter(mm) for the ovulatory (third wave) and first wave DF than in the second wave and the size observed was 12.3± 0.20, 10.2± 0.50 and 12.8± 0.30 in first, second and third wave respectively, probably due to the FSH wave post ovulation and the maximum diameter of preovulatory follicle in the third wave was due to the ovulatory status in the third DF which offered the stimulus for the increased growth.

The mean maximum diameter of subordinate follicle (SF) in the first wave also exhibited significant difference in size (mm) between the normally cycling animals (group I) and RB animals with prolonged oestrus (group II). Subordinate follicle in the ovulatory wave attained the mean maximum size (mm) when compared to other waves in both the groups (7.03± 0.79 in group I and 8.30± 0.63 in group II). Similar studies were done by Ginther *et al*. (1989) [11] who observed that the DF attained a size of approximately 10–20 mm and the largest SF attained as size of approximately 8 mm. A decline in FSH coinciding with an increase in DF inhibin and estradiol secretion is associated with the regression of SF. Inhibin and other follicular fluid factors such as IGF-bioactivity may be involved in DF selection (Rhodes *et al*., 1997) [16].

3.3 Ovulatory follicle
The mean diameter of the 20 ovulatory follicles studied was 15.15± 0.38mm, showing a wide variation between 12.60 and 17.65mm. The mean maximum diameter of DF prior to ovulation in normally cycling animals was 16.30± 0.63mm in two wave cycle (n=7) and 13.57± 0.958mm in three wave cycle (n=3). Similar ovulatory follicular size was observed by Sirois and Fortune (1990) [18] who recorded a follicular size of 12.80± 0.30mm in cows with three follicular waves.

In RB animals, the mean diameter (mm) of 10 ovulatory follicles studied in total was 14.87± 0.48 and varied from 12.85 to 16.85mm.The mean maximum diameter of DF prior to ovulation was 14.78±0.83mm in two wave cycle (n=4), 14.92±0.74mm in three wave cycle (n=5) and 14.95mm in one wave cycle (n=1). Similar studies were carried out in RB animals by Dovensky *et al*. (2000) [7] who recorded an ovulatory follicular diameter of 16.9 and 15.8mm

respectively. But studies conducted by Perez *et al*. (2003) [14] observed slightly higher average diameter of ovulatory follicle in RB cows (17.8± 0.38mm) and varied greatly from 12.5 to 25mm. He also opined that ovulatory follicular size of RB cows varied substantially and was not associated with this syndrome.

Mean maximum diameter of ovulatory follicle in RB cow with one wave follicular growth pattern was 14.95 mm and the follicle attained its dominance in conjunction with ovulatory size. This observation is in accordance with the study of Perez *et al*. (2003) [14] who opined that the cow with a single follicular wave attained its dominance and ovulatory size at the same time and observed an ovulatory follicular size of 16mm.

3.4 Duration of persistence of graafian follicle

In the control group, the duration of persistence of DF size was between 0-12 h in 40 per cent animals and it was between 12-24 h in 60 per cent animals. In RB animals with prolonged oestrus majority of the animals displayed a higher duration of persistence of preovulatory follicle. The duration of persistence was between 0-12 hrs in 20 per cent, 12-24hrs in 10 per cent, 24-36 in 40 per cent and 36-48 hrs in 30 per cent animals respectively.

RB animals with prolonged oestrus exhibited a prolonged duration of persistence of ovulatory follicle at its maximum size (>24 hrs in 70 per cent animals) when compared to normally cycling animals (<24 hrs in 100 per cent animals) with oestrous period less than 36 hrs in the control group. This could be due to the reduced rate of CL regression and a higher level of serum progesterone level on the day of oestrus, which caused a delay in LH surge thereby, delayed ovulation. These findings are in accordance with Sirois and Fortune (1990) [18] who declared that higher progesterone concentrations at oestrus might lead to prolonged maintenance of ovulatory follicle and hence extended duration of oestrus. They observed that this hormonal asynchrony is due to reduced regression rate of CL of previous cycle.

3.5 Interval from onset of oestrus to ovulation of dominant follicle

In group I animals the interval from the commencement of oestrus to ovulation was less than 36 h in 90 per cent of the animals (n=9) and it was between 36-48 hrs in remaining 10 per cent animals (n=1), whereas in RB animals with prolonged oestrus (group II) it was greater than 48 h in 90 per cent animals (n=9) and 10 per cent (n=1) exhibited anovulatory follicle. These findings are in harmony with the study of Walker *et al*. (1996) [20] who observed that the repeat breeder animals occasionally had longer interval from oestrus to ovulation (96 h) which was far beyond the normal duration of approximately 27 h in normally cycling animals. Similarly Bigelow and Fortune (1998) [4] observed that 70 per cent of the delayed ovulators had higher level of progesterone (0.87± 0.21 ng/ml) on the day of oestrus. Bage *et al.* (2000) [2] opined that constant adrenal stimulation related with managemental, environmental and physical stress leads to suprabasal progesterone concentration during oestrus. This suprabasal progesterone concentration is adequate to block the positive feedback effect of estradiol on GnRH release, resulting in delayed LH peak and ovulation.

3.6 Inter-ovulatory interval

Inter ovulatory interval in control group animals with two and three wave cycles were 19.71±0.48 and 21.67± 0.73d respectively, whereas in RB animals with prolonged oestrus in two and three wave cycles the inter-ovulatory interval was 20.80± 0.87 and 22.40± 0.51d. A prolonged Inter ovulatory interval was observed in RB animals (group II) when compared to normally cycling animals (group I), which could have been due to the increased duration of persistence of preovulatory follicle (> 24 h in 70 per cent of RB animals) and longer duration of onset of oestrus to ovulation (ranged between 48-84h in all RB animals). These findings are in agreement with the observation of Walker *et al*. (1996) [20] who noticed that the repeat breeder animals occasionally had longer interval from oestrus to ovulation (96 h) which was far beyond the normal duration of approximately 27 h in normally cycling animals.

3.7 Growth and regression of corpus luteum

The luteal growth and regression characteristics observed did not significantly vary between the normally cycling animals and RB animals with prolonged oestrus.

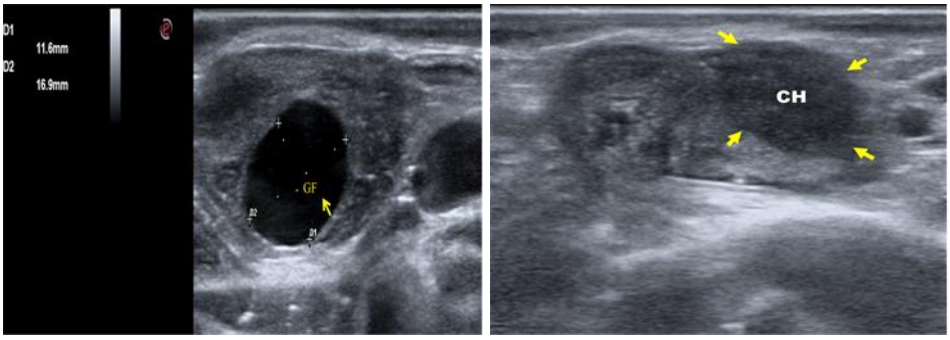
Corpus hemorrhagicum appeared as a more hypo-echoic, more dark area as against the ovarian stroma. Clear appearance of CL by TRUS was identified on third day of ovulation and sonographically imaged as poorly defined, uneven, greyish-black structure with echogenic spots within the ovary. This finding is in accordance with the findings Pierson and Ginther (1984) [11] who reported that the CL was identifiable all over the inter-ovulatory period apart from day 0-2 post ovulation, during that period CL was indistinct.

An increased growth rate was noticed up to day 8 post ovulation. Mean maximum diameter of CL was observed on day10 or 11.From day 8 to 16 of ovulation the luteal growth rate is in a stationary phase and the luteal regression was observed from day 16 or 17 post ovulation. Rate of regression was less in RB animals with prolonged oestrus and was not completed on the day of next oestrus which could be the reason for higher level of progesterone observed on the day of oestrus and prolonged persistence of ovulatory follicle in these animals.

The appearance rate of luteal cavities as well as the appearance and disappearance of cavities in CL in this study agreed with the early reports of Kito *et al.* (1986) [12] who observed an occurrence rate of luteal cavity in 41 per cent animals studied and also reported the appearance of it as early as day 7 of oestrous cycle with a maximum diameter by day 10 and disappearance by day 7 to 17 of AI.

A B C

Fig 1: Follicular emergence on the contra lateral ovary on the day of ovulation: RCL- Regressing Corpus Luteum: FDF- Future Dominant Follicle B) Developing follicles on the day of Deviation: RCL– Regressed Corpus Luteum: F–follicle: OS – Ovarian stroma. C) Dominant follicle**:** SF- Subordinate Follicle: DF- Dominant Follicle; on day 9 post ovulation



A B

Fig 2: Ultrasonography appearance of Graafian follicle: GF- Graafian Follicle. B) Sonographic appearance of Corpus Hemorrhagicum on day after ovulation: CH- Corpus Hemorrhagicum

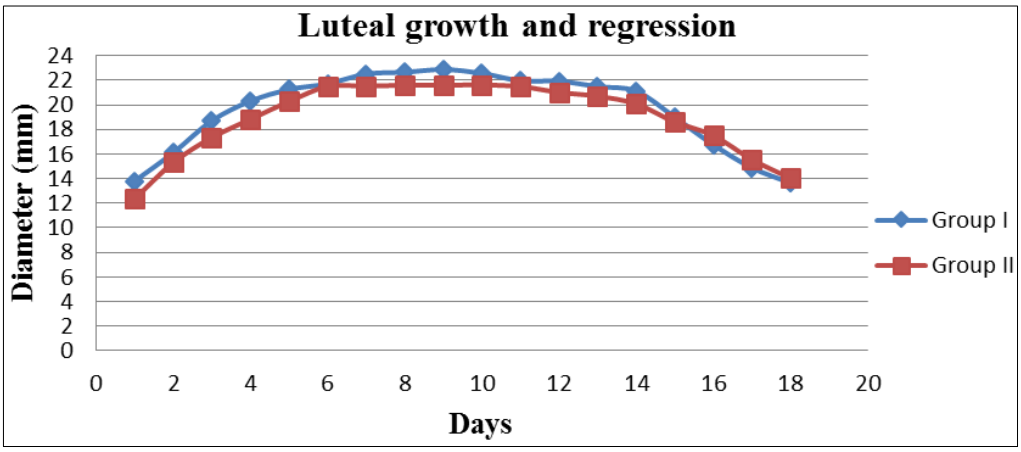


Fig 3: Luteal growth and regression pattern in crossbred cattle

3.7.1 Between Group I and Group II

Even though no significant difference could be observed between the groups during the growth and stationary phase, the mean maximum diameter of CL was smaller in RB animals and a decreased regression rate observed in RB animals as against the animals in control group. The rate of luteal regression observed in RB animals with prolonged oestrus was 1.52± 0.25mm/day between days 17 to 20 where as it was higher (1.77± 0.24mm/day) in control group animals. These findings agreed with reports of Bage *et al.* (2002) [3] who stated that repeat breeder cows with prolonged oestrus showed a suboptimal luteal profile. Ghuman *et al.* (2014) [10] also observed that CL was only partially regressed at the onset of oestrus in RB cows with prolonged oestrus. The faulty luteolysis of CL is attributable to suppressed PGF2α synthesis in RB cows with prolonged oestrus (Duchens *et al*., 1995) [8].

3.8 Serum progesterone profile

Serum progesterone levels (M ± SE) on the day of oestrus of two consecutive cycles (C1-day 0 and C-2 day 0) of normally cycling animals (Group-I) and RB animals with prolonged oestrus (Group II) on C1- day 0 were 0.36±0.03 and 0.67±0.03 ng/mL and on C-2 day 0 were 0.42±0.03 and 0.66±0.03 ng/mL respectively. Statistical analysis of progesterone levels on day 0 (C-1 & C-2) revealed significant difference ($P \leq 0.05$) between the groups. This is in accordance with the reports of Singh *et al.* (2009) [17] who observed a higher serum progesterone concentration of >0.5 ng/ml in repeat breeding cattle exhibiting prolonged oestrus on the day of oestrus as against <0.5 ng/ml in normally cycling cows.

Serum progesterone level on day 10 of oestrous cycle showed a significant difference between group I (4.76± 0.29ng/ml) and group II (3.31± 0.29 ng/ml). Similar observations were

made by Chander *et al*. (2002) [5] who observed that the serum progesterone concentration on day 10 of estrous cycle in normally cycling cows was higher (2.92± 0.71 ng/ml) as against 2.12± 0.16 ng/ml in repeat breeder cows.

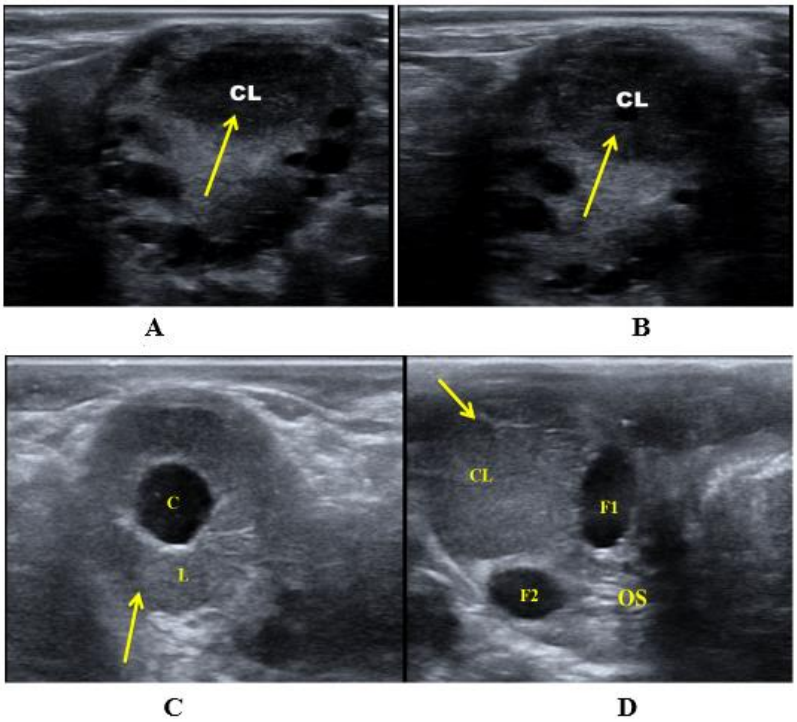


Fig 4: Ultrasonographic appearance of different stages of Corpus Luteum: A) Developing Corpus Luteum: clear visualization by day 3 post ovulation. B) Developing CL on day 5: an increase in luteal size noticed till day 8. Developed Corpus Luteum can be either with (cystic corpora lutea) or without luteal cavity C&D) Developed Compact Corpus Luteum: C- Cavity of cystic corpora lutea, L- Luteal tissue, CL- Compact corpus luteum, OS- Ovarian stroma, F1 & F2 – Follicles

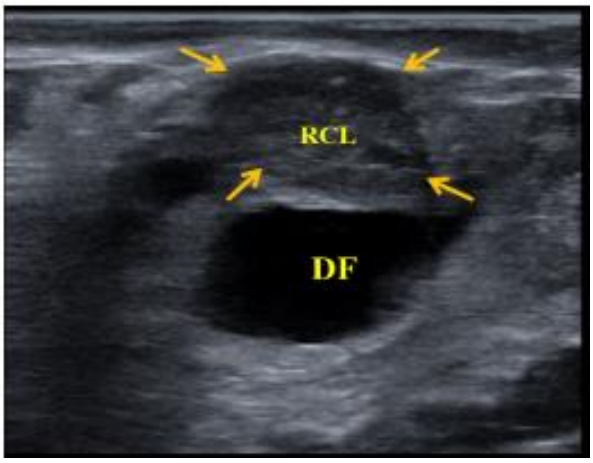


Fig 5: Regressed Corpus Luteum: RCL- Regressed Corpus Luteum on the day of oestrus DF- Dominant/pre ovulatory follicle

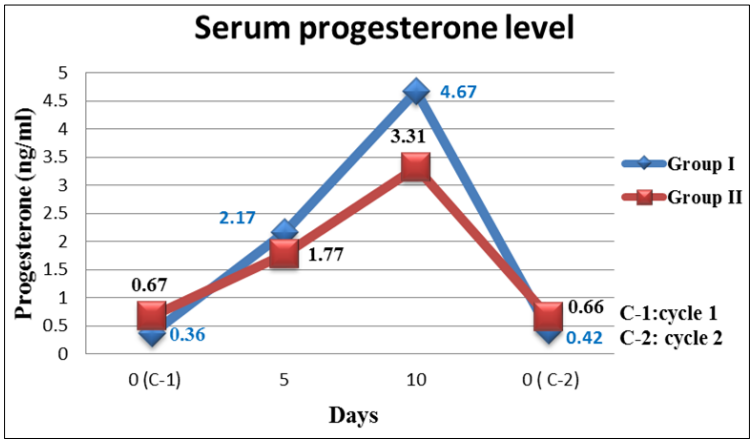


Fig 6: Mean serum progesterone level at different stages of oestrous cycle

4. Conclusion

The predominant follicular growth pattern observed in crossbred cattle of Kerala is either two or three waves per cycle. A prolonged inter oestrous interval, inter ovulatory interval, duration of dominance of graafian follicle, onset of oestrus to ovulation was observed in RB animals with prolonged oestrus when compared to normally cycling animals in the control group. Even though there was no significant difference was observed between the group I and group II in CL growth and regression, the mean maximum diameter attained by the developed CL and regression rate of CL was less in RB animals with prolonged oestrus. A higher progesterone level (>0.5 ng/ml) was observed on day of oestrus in RB animals with prolonged oestrus when compared to normally cycling animals (<0.5 ng/ml). This could be due to incomplete lysis of previous cycle CL during oestrus. This hormonal asynchrony resulted in prolonged persistence of dominant ovulatory follicle, thereby an increased interval from onset of oestrus to ovulation in RB animals.

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