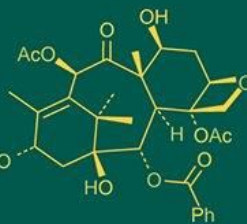


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Studies on ovarian structural, steroidal and biochemical changes in pregnant and non-pregnant Sahiwal heifers

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Abstract

The present study was conducted to study the ovarian structural, steroidal and biochemical changes in pregnant and non-pregnant Sahiwal heifers. A total of forty (40) Sahiwal heifers were selected and artificial insemination (AI) was done with sex sorted semen in all animals at appropriate time during estrus. Ovarian sonography was performed on days 0, 10 and 20 after estrus for the assessment of diameter of the dominant follicle and diameter of corpus luteum. Blood samplings were done on days 0, 10 and 20 after estrus for the estimation of serum estrogen, progesterone, glucose and total protein. Pregnancy diagnosis was done at 60 day after AI using ultrasonography in all animals with an overall conception rate of 47.5%. The average dominant follicle and corpus luteum sizes were 9.48 ± 0.12 mm and 10.38 ± 0.45 mm, respectively. Pregnant heifers had a significantly larger ($p < 0.05$) mean dominant follicle and corpus luteum size compared to non-pregnant heifers. The overall mean serum estrogen and progesterone values were 10.23 ± 0.33 pg/ml and 2.60 ± 0.63 ng/ml, respectively. Pregnant heifers had significantly higher ($p < 0.05$) mean serum estrogen and progesterone levels on day 0 and day 10 compared to non-pregnant heifers. Mean values of serum glucose and total proteins were 47.87 ± 0.56 mg/dl and 6.38 ± 0.45 g/dl and these values were significantly higher ($p < 0.05$) in pregnant heifers compared to non pregnant heifers. Thus, monitoring these parameters might be useful in assessing reproductive status and also helpful in optimizing fertility management in dairy cow.

Keywords: Sahiwal heifers, estrogen, progesterone, glucose, protein and ultrasonography

Introduction

Hormonal and biochemical parameters such as serum estradiol, progesterone, glucose and total proteins etc. are critical indicators of reproductive status in cow. These parameters undergo significant changes during the estrous cycle and early pregnancy. Thus their variations during the estrous cycle and early pregnancy provide valuable insights into physiological changes that support conception and pregnancy maintenance. Pregnant cattle show low progesterone, higher estradiol at estrus and greater glucose and total protein concentrations compared to non-pregnant cattle (Datta *et al.*, 2023; Shukla *et al.*, 2000; Chinnasamy *et al.*, 2024) [6, 39, 41]. These differences highlight the physiological adaptations that support pregnancy establishment and maintenance (Lopes *et al.*, 2007) [16]. Monitoring these parameters can aid in assessing reproductive status and optimizing fertility management in dairy cow.

Materials and Methods

The present study was conducted at Instructional Dairy Farm, Nagla, G.B. Pant University of Agriculture and Technology, Pantnagar during March to December, 2024 to study the ovarian structural (dominant follicle & corpus luteum), steroidal and biochemical changes in pregnant and non-pregnant Sahiwal heifers during estrous cycle. A total of 40 pubertal Sahiwal heifers of 24 to 36 months age having more than 220 kg body weight with regular estrous cycle, were selected for the study. The selected animals were maintained under uniform managemental conditions, totally on stall feeding twice a day with concentrate @ 2.5 kg per head per day and ad lib access to water. Rectal palpation was performed for the assessment of optimum development of genitalia before the final selection of animals.

All the animals were also screened for subclinical endometritis using white side test and animals with negative white side test were included for the study. White side test (WST) on cervical mucus was performed before AI as per the method described by Pateria and Rawal (1990) [29]. Cervical mucus sample was aseptically collected from the animal using sterile AI sheath connected with 20 ml syringe (Raja *et al.* 2012) [32]. AI was done in all animals using sex sorted semen at appropriate time. Real time B mode trans-rectal ultrasonography with linear array transducer having a frequency of 7.5 MHz (B1 PRO Ultrasound Machine, Konika Minolta, Marunouchi, Chiyoda, Tokyo, Japan) was used for ovarian screening in the present study. Ovarian sonography was performed on days 0 (at about 6 hours of estrus initiation), 10 and 20 after estrus for the assessment of diameter of the dominant follicle and diameter of corpus luteum. Blood samples from the jugular vein were collected on day 0, 10, 20 after initiation of estrus. A 18-gauge sterilized needle with a 10 ml sterilized syringe and vacutainer tube were used for the collection of blood samples. The serum was separated within 30 minutes by centrifuging the samples at 3000 rpm for 10 minutes. The serum obtained was marked for identification and stored at -20 °C until analysis for biochemical and hormonal parameters in 2 ml Eppendorf tubes. Serum estrogen, progesterone, glucose and total proteins were estimated using commercial bio-assay kit according to the manufacturer's instructions (catalog number ES380S & PG362S, CALBIOTECH and catalog number 93DP100-74 & 83LS100-60, ARKRAY, respectively).

Statistical Analysis

All the quantitatively data pertaining to the experiment were expressed as mean±SEM. The data were analyzed statistically using one-way ANOVA in SPSS (Snedecor and Cochran, 1994) [40].

Results and Discussion

Ovarian Sonography

Size of dominant follicle (diameter in mm)

Table 1 depicts the size of the dominant follicle during estrus. The dominant follicle size on day of estrus in the present study ranged from 7.26 mm to 11.42 mm, with an overall mean value of 9.48±0.12 mm in all 40 Sahiwal heifers. These findings are slightly less than those reported by Sharma *et al.* (2024) [38] in Sahiwal heifers (10.52±0.13 mm) and Jersey crossbred heifers (10.15±0.51 mm). Dodiya (2016) [7] and Hassan *et al.* (2021) [9] also reported larger mean dominant follicle size (11.42±0.20 mm and 14.70±0.70 mm) in Sahiwal cow, respectively. Similarly, Viana *et al.* (2000) [42] also observed a higher value of mean dominant follicle size of 12.44±1.59 mm in Gir. In crossbred heifers, significantly higher mean dominant follicle size (15.90±0.50 mm) was recorded by Savio *et al.* (1988) [37]. The mean size of dominant follicle in pregnant heifers (9.56±0.19 mm) was significantly higher ($P<0.05$) than non-pregnant heifers (9.40±0.17 mm) in the present study. These findings are in accordance with Perry *et al.* (2007) [31], who reported significantly higher ($p<0.05$) mean dominant follicle size (10.70±0.65mm) in pregnant beef heifers compared to non-pregnant ones. Similarly, Vasconcelos *et al.* (2001) [41] also observed that decrease in the size of the ovulatory follicle leads to a reduction in subsequent luteal size and lowers the pregnancy rate in

lactating dairy cows.

Reproductive success in cattle depends on multiple maternal factors including the ovulation of a competent oocyte, sufficient progesterone production by the corpus luteum (CL) and the establishment of an optimal uterine environment. The size of the ovulatory follicle plays a pivotal role in these processes. Larger ovulatory follicles are positively correlated with higher estradiol concentrations during the preovulatory period, which are essential for optimizing uterine conditions to support embryonic development, implantation and pregnancy maintenance (Miller and Moore, 1976; Miller *et al.*, 1977) [21, 22]. The granulosa cells within preovulatory follicles are key determinants of the steroidogenic capacity of the subsequent CL. Larger follicles contain more granulosa cells, which translate into a CL with enhanced progesterone production potential (Pfeifer *et al.*, 2012) [30]. Higher progesterone levels are critical for maintaining pregnancy. Furthermore, ovulation of larger follicles has been linked to improved oocyte competence, increased conception rates and greater pregnancy success (Perry *et al.*, 2007) [31].

Size of corpus luteum (diameter in mm)

The size of the corpus luteum as determined by ultrasonography is presented in table 1. In the present study the size of the corpus luteum ranged from 6.45 to 15.25 mm with an overall mean value of 10.38±0.45mm. Our findings are almost similar to the findings of Ireland *et al.* (1980) [10], who reported a range of 5.0 to 15.0 mm size of corpus luteum in crossbred heifers. In contrast, Lynch *et al.* (2010) [18], observed a higher overall mean size of corpus luteum (21.61±4.51) with a range of 13.00 to 37.25 mm in beef heifers. Similarly, Hassan *et al.* (2021) [9] also documented a higher average size of corpus luteum as 15.9±2.5 mm in Sahiwal cows. Significant difference ($p<0.05$) was observed between the mean corpus luteum size in pregnant (11.58±0.29) and non-pregnant (9.31±0.33) heifers in the present study. These results corroborate with those reported by Pandey *et al.* (2018) [26], who also observed a significantly higher conception rate in zebu cattle with a larger corpus luteum. Similarly, Parkinson *et al.* (1994) [28], also observed significantly ($p<0.05$) higher mean size of corpus luteum (14.9±0.24) in pregnant dairy cows compared to cyclic cows (13.9±0.22). These findings reinforce the role of the corpus luteum in supporting pregnancy.

The larger CL size observed in pregnant animals suggests a higher progesterone producing potential, which is crucial for sustaining early embryonic development and creating a favorable uterine environment for pregnancy. In contrast, the smaller CL size in non pregnant animals may reflect insufficient luteal function, which could negatively impact reproductive outcomes by failing to produce adequate progesterone. These results support the importance of CL size as a reliable indicator of luteal function and its critical role in determining the success of pregnancy maintenance.

Steroid Status

Serum estrogen (pg/ml)

The serum estrogen values (pg/ml) at different days of estrous cycle along with its range are depicted in table 1. The overall mean estrogen level was recorded as 10.23±0.33 pg/ml with a range of 4.67 to 22.91 pg/ml in all the 40 animals under the study. Our findings are almost similar to the findings of Wettemann and Hafs (1973) [44], who

reported mean value (9.7 pg/ml) of serum estrogen in Holstein heifers. Similarly, Kaneko *et al.* (1995) [14] recorded a mean concentration (12.00 ± 0.70 pg/ml) of blood estrogen in dairy cows. The mean serum estrogen level was significantly ($p < 0.05$) higher (15.30 ± 0.81 and 8.69 ± 0.16) on day 0 and day 10 in pregnant heifers compared to non pregnant (12.65 ± 0.59 and 7.52 ± 0.33), respectively. In contrast to this on day 20 of onset of estrus the mean estrogen level was significantly ($p < 0.05$) higher (11.91 ± 0.55) in non pregnant heifers compared to pregnant heifers (7.11 ± 0.43). Our findings are similar to Lopes *et al.* (2007) [16], who reported significantly ($p < 0.05$) higher plasma estradiol concentrations on the day of AI in pregnant Holstein cows. On the other hand, Naik *et al.* (2013) [23] also observed the estrogen levels were significantly ($p < 0.01$) higher (20.24 ± 0.01 pg/ml) on day 0 in non-pregnant Punganur cattle. Shukla *et al.* (2000) [39] also recorded higher (16.45 ± 0.41 pg/ml) estrogen level in non-pregnant crossbred cows on day 0 of estrous cycle.

Variations in estrogen level may be attributes due to differences in sampling frequency, seasonal and climatic conditions at the time of sample collection as well as the age and physiological status of the animals (Alvarez *et al.*, 2000) [1]. Plasma estrogen concentrations on the day of AI were higher in cows confirmed pregnant compared to non-pregnant cows, suggesting that pre-ovulatory follicular steroid biosynthesis influences pregnancy success (Lopes *et al.*, 2007) [16]. On the day of estrus in cattle, estrogen plays a critical role in regulating reproductive processes and preparing the cow for ovulation. High estrogen concentrations stimulate the central nervous system, leading to characteristic estrous behaviour (Lyimo *et al.*, 2000) [17]. As estrogen reaches peak levels, it stimulates the hypothalamus and anterior pituitary gland, leading to the preovulatory luteinizing hormone surge. This luteinizing hormone surge is essential for final follicular maturation and ovulation (Julie E. Holesh, 2025) [12].

Serum progesterone (ng/ml)

The findings of serum progesterone level are presented in table 1. Serum progesterone values varied between 0.26 and 8.02 ng/ml, with an overall mean value of 2.60 ± 0.63 ng/ml in all 40 animals under the study. Our findings closely resemble to those of Datta *et al.* (2023) [6], who reported a higher mean serum progesterone value (5.74 ± 0.56) in Lakhimi cow. Further, Sartori *et al.* (2004) also recorded a higher mean serum progesterone value (7.3 ± 0.4 ng/mL) in Holstein heifers. A non-significant ($p > 0.05$) difference in the mean serum progesterone value was recorded between pregnant (0.38 ± 0.07) and non-pregnant (0.39 ± 0.01) heifers on day 0 of onset of estrus. The mean progesterone levels were significantly higher ($p < 0.05$) in pregnant heifers (4.51 ± 0.27 and 5.22 ± 0.35) compared to non-pregnant heifers (3.62 ± 0.30 and 1.75 ± 0.35) on days 10 and 20 after the onset of estrus, respectively. In contrast to this Ayam (2006) [2], reported a higher (6.38 ± 0.63 ng/ml) mean progesterone value in pregnant sahiwal cows on day 10 post insemination. The mean values of progesterone on day 0 of estrous cycle observed in the present study is similar to the findings of Kumaresan *et al.* (2001) [15] and Rekwot *et al.* (2000) [33], who also reported less than 1 ng/ml mean serum progesterone during the estrus in Zebu cattle. In another study on Holstein heifers conducted by Wettemann and Hafz (1973) [44] it was reported that progesterone levels increased markedly from 0.4 ± 0.1 ng/ml at estrus to 6.8 ± 0.4 ng/ml by day 11 after estrus.

For optimal fertility, low circulating P4 concentrations near the time of artificial insemination (AI) are indicative of effective CL regression of previous cycle, which is essential for further successful conception. Conversely, elevated P4 concentrations post-AI can impact embryonic development and in certain cases enhance fertility outcomes (Wiltbank *et al.*, 2014; Yan *et al.*, 2016) [45, 46]. Progesterone production is primarily regulated by the corpus luteum (CL), which develops following the ovulation. The number of granulosa cells that luteinize into large luteal cells along with the constitutive production of progesterone by these cells significantly influences circulating progesterone levels (Niswender, 2002) [25]. Progesterone plays a crucial role in stimulating the production of various endometrial secretions necessary for successful embryonic development (Nephew *et al.*, 1991; Giesert *et al.*, 1992) [24, 8]. Mann and Lamming (2001) [20] reported that inadequate embryonic development is associated with a delayed post-ovulatory rise in progesterone and low luteal phase P4 concentrations. Insufficient circulating P4 concentrations have been linked to early embryonic loss in cattle (Mann and Lamming, 1999) [19].

Biochemical Status

Serum glucose (mg/dl)

Results related to serum glucose level (mg/dl) are shown in table 1. The values of serum glucose ranged from 38.18 to 59.86 mg/dl with an overall mean value of 47.87 ± 0.56 in all 40 animals under the study. Our findings corroborate with the findings of Kadhim and Ali (2019) [13], who reported a range (45.00 to 75.00 mg/dl) of serum glucose level with a mean value (54.6 ± 2.69) in lactating dairy cows. Similarly, In Jersey crossbred cows the mean value of serum glucose was found 53.46 ± 0.69 mg/dl (Chinnasamy *et al.*, 2024) [4]. The mean values were found significantly ($p < 0.05$) higher (52.33 ± 0.98 , 53.52 ± 1.01 and 52.83 ± 0.88) in pregnant heifers compared to non-pregnant heifers (44.79 ± 0.81 , 43.33 ± 0.32 and 41.87 ± 0.56) on days 0, 10 and 20 of onset of estrus, respectively. However, the mean serum glucose levels between pregnant heifers showed non-significant ($p > 0.05$) difference on days 0, 10 and 20 of onset of estrus, respectively. Our results are in accordance with Pandey *et al.* (2016) [27], who also noted the significantly ($p < 0.05$) higher glucose concentrations (51.53 ± 2.26 , 55.92 ± 1.61 and 52.52 ± 1.34) in pregnant cross breed cows compared to non-pregnant (38.44 ± 1.44 , 42.59 ± 0.90 and 39.35 ± 1.06) on days 0, 10 and 20 of estrous cycle, respectively. Similar results were also reported by (Chinnasamy *et al.*, 2024) [4] with significantly ($p < 0.01$) higher (58.48 ± 0.64 mg/dl) blood glucose concentration in pregnant Jersey crossbred cows. In contrast to our findings (Kadhim and Ali, 2019) [13] reported non-significant ($p > 0.05$) difference in the mean values of serum glucose between pregnant (54.22 ± 0.72 mg/dl) and non-pregnant dairy cows (53.87 ± 0.67 mg/dl).

The concentration of blood glucose is closely linked to the animal's overall energy balance and reproductive effectiveness. Parmar *et al.* (1986) observed that glucose levels tend to rise during the luteal phase of the estrous cycle. The increase in blood glucose can either directly boosts the production of progesterone by enhancing both the frequency and the average concentration of luteinizing hormone (LH) or indirectly stimulates progesterone release during early luteal phase by increasing insulin level, which supports pregnancy (Richards *et al.*, 1989) [34]. Elevated blood glucose levels also provide the necessary energy to support optimal ovarian function and thus enhancing

follicular development and ovulation. Additionally, increased glucose availability may improve the uterine environment, promoting better sperm survival and embryo implantation. This highlights the critical role of metabolic status in maximizing reproductive performance and AI outcomes in dairy cows.

Serum total protein (g/dl)

Table 1 represents the serum total protein levels in animals under study. Serum total protein value ranged from 4.36 to 7.95 g/dl and with an overall mean value of 6.38 ± 0.45 g/dl. Our results are in accordance with Chinnasamy *et al.* (2024) [4], who observed an overall serum total protein value as 6.62 ± 0.44 g/dl in Jersey crossbred cows. In contrast to this, Kadhim and Ali (2019) [13], reported higher (8.23 ± 0.42 g/dl) mean serum total protein value with a range of 5.7 to 8.1 g/dl in lactating dairy cows. The mean values of serum total protein were (6.57 ± 0.11 , 6.69 ± 0.10 and 6.76 ± 0.11) in pregnant heifers and (6.12 ± 0.20 , 6.11 ± 0.19 and 6.12 ± 0.19) in non-pregnant heifers on days 0, 10 and 20 of onset of estrus, respectively. A non-significant ($p > 0.05$) difference was observed in the mean total serum protein value on day 0 between pregnant and non-pregnant heifers, whereas this value increased significantly ($p < 0.05$) in pregnant heifers on days 10 and 20 compared to non-pregnant heifers. Our results are similar to the findings of (Chinnasamy *et al.*, 2024) [4], who observed significantly ($p < 0.01$) higher

(7.46 ± 0.56 g/dl) total serum protein concentration in pregnant Jersey crossbred cows. Similarly (Pandey *et al.*, 2016) [27] reported a significant ($p < 0.05$) increase in the total protein concentrations in pregnant (6.74 ± 0.27 , 6.31 ± 0.14 and 6.24 ± 0.20) crossbred cows compared to non-pregnant (6.30 ± 0.14 , 6.26 ± 0.15 and 6.03 ± 0.21) on days 0, 10 and 20 of onset of estrus, respectively. (Kadhim and Ali, 2019) [13] further reported significantly higher ($p < 0.05$) total serum protein in pregnant dairy cows (8.54 ± 0.17 g/dl) compared to non-pregnant ones (7.92 ± 0.68 g/dl). However, contrary to our results, Chandrarahar *et al.* (2003) [3] and Das and Bisnoi (2005) [5] found no significant difference in the plasma total protein levels between pregnant and non-pregnant cows.

Total protein levels are commonly assessed to determine an animal's nutritional status, considering both its dietary intake and metabolic processes (Chinnasamy *et al.*, 2024) [4]. In our study, elevated serum total protein levels in pregnant animals may be linked to their metabolic demands. A low concentration of plasma total protein can lead to a deficiency of specific amino acids essential for the biosynthesis of gonadotropins and gonadal hormones (Vohra *et al.*, 1995; Joe Arosh *et al.*, 1998) [43, 11], potentially resulting in reproductive hormonal imbalances that cause ovarian inactivity (Roberts, 1971) [35]. Studies indicate that cows with elevated serum protein levels tend to have higher conception rates, possibly due to enhanced ovarian activity and more favorable uterine environment.

Table 1: Size of dominant follicle and corpus luteum & values of serum estrogen, progesterone, glucose and total protein (Mean $\pm$ SE) in pregnant and non-pregnant Sahiwal heifers (n = 40)

Size of Dominant follicle (mm)				
Type of animal	Number of animals	Size of dominant follicle in mm at about 6 hours of onset of estrus		
		Minimum	Maximum	Mean
Pregnant	19	7.75	11.42	9.56 ± 0.19^A
Non pregnant	21	7.26	10.43	9.40 ± 0.17^B
Total	40	7.26	11.42	9.48 ± 0.12^A
Size of corpus luteum (mm)				
Type of animal	Number of animals	Size of corpus luteum		
		Minimum	Maximum	Mean
Pregnant	19	8.50	15.10	11.58 ± 0.29^A
Non pregnant	21	6.45	15.25	9.31 ± 0.33^B
Total	40	6.45	15.25	10.38 ± 0.45^C
Mean serum estrogen (pg/ml)				
Type of animal	Number of animals	Mean serum estrogen		
		Day 0	Day 10	Day 20
Pregnant	19	15.30 ± 0.81^{Aa}	8.69 ± 0.16^{Aa}	7.11 ± 0.43^{Cc}
Non pregnant	21	12.65 ± 0.59^{Bb}	7.52 ± 0.33^{Bb}	11.91 ± 0.55^{Aa}
Total	40	13.91 ± 0.53^{ABab}	8.08 ± 0.21^{ABab}	8.71 ± 0.71^{Bb}
Mean serum progesterone (ng/ml)				
Type of animal	Number of animals	Mean serum progesterone		
		Day 0	Day 10	Day 20
Pregnant	19	0.38 ± 0.07^{Aa}	4.51 ± 0.27^{Ab}	5.22 ± 0.35^{Ab}
Non pregnant	21	0.39 ± 0.01^{Aa}	3.62 ± 0.30^{Bb}	1.75 ± 0.35^{Cc}
Total	40	0.38 ± 0.01^{Aa}	4.04 ± 0.21^{ABab}	3.40 ± 0.37^{Bb}
Mean serum glucose (mg/dl)				
Type of animal	Number of animals	Mean serum glucose		
		Day 0	Day 10	Day 20
Pregnant	19	52.33 ± 0.98^{Aa}	53.52 ± 1.01^{Aa}	52.83 ± 0.88^{Aa}
Non pregnant	21	44.79 ± 0.81^{Cc}	43.33 ± 0.32^{Cc}	41.87 ± 0.56^{Cc}
Total	40	48.37 ± 0.86^{Bb}	48.17 ± 0.95^{Bb}	47.07 ± 1.01^{Bb}
Mean serum total protein (g/dl)				
Type of animal	Number of animals	Mean serum total protein		
		Day 0	Day 10	Day 20
Pregnant	19	6.57 ± 0.11^{Aa}	6.69 ± 0.10^{Aa}	6.76 ± 0.11^{Aa}
Non pregnant	21	6.12 ± 0.20^{Aa}	6.11 ± 0.19^{Bb}	6.12 ± 0.19^{Bb}
Total	40	6.33 ± 0.12^{Aa}	6.38 ± 0.12^{ABab}	6.43 ± 0.12^{ABab}

Mean values with at least one common superscript within column (A, B, C) and within row (a, b, c) do not differ significantly ($p \geq 0.05$)

Conclusion

On the basis of the results obtained in the present study on a smaller number of Sahiwal animals it could be concluded that higher values of serum estrogen, progesterone, glucose and total protein were observed in pregnant heifers compared to non pregnant one. These differences highlight the physiological adaptations that support pregnancy establishment and maintenance. Monitoring these parameters might be useful in assessing reproductive status and optimizing fertility management in dairy cow.

Conflict of Interest

The authors declare no conflict of interest.

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References

- Alvarez P, Spicer LJ, Chase CC, Stewart TD, Hammond RE, Olson TA, *et al.* Ovarian and endocrine characteristics during anestrous cycle in Angus, Brahman and Senepol cows in a subtropical environment. *J Anim Sci.* 2000;78:1291-1302.
- Ayam VBS. Metabolic, mineral and progesterone profiles in conceived and non-conceived Sahiwal cows [Master's thesis]. Raipur: Indira Gandhi Agricultural University; 2006.
- Chandrarah D, Tiwari RP, Awasthi MK, Datta GK. Serum biochemical profile of repeat breeder crossbred cows. *Indian J Anim Reprod.* 2003;24(2):125-127.
- Chinnasamy TB, Pandian CS, Sivakumar SA, Sivakumar P, Perumal P. Comparative study on serum biochemical profiles during different states of reproduction in Jersey crossbred cows. *IJABR.* 2004;SP-8(5):5-10.
- Das PB, Bisnoi PC. Clinical importance of some serum constituents in repeat breeder cows. *Indian Vet J.* 2005;82:905-908.
- Datta A, Baruah A, Bora A, Dutta D, Goswami J, Bhuiya D, *et al.* Insight into ovarian follicular dynamics and hormonal interplay during estrus period in Lakhimi cow of Assam. *Indian Vet J.* 2023;78:9-84.
- Dodiyar V. Studies on follicular dynamics and hormonal interventions to enhance fertility in Sahiwal cattle [M.V.Sc. thesis]. Ludhiana: Guru Angad Dev Veterinary and Animal Sciences University; 2016.
- Geisert RD, Morgan L, Short EC, Zavy MT. Endocrine events associated with endometrial function and conceptus development in cattle. *Reproduction.* 1992;4(3):301-305.
- Hassan M, Arfat MY, Arshad U, Ahmad N. Ovarian dynamics, hormone profiles, and characterization of ovarian and uterine blood flow in cycling Sahiwal cows. *S Afr J Anim Sci.* 2021;51(2):195-204.
- Ireland JJ, Murphee RL, Coulson PB. Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *J Dairy Sci.* 1980;63(1):155-160.
- Arosh ADJ, Kathiresan TG, Devanathan RC, Rajasundara J, Rajasekaran. Blood biochemical profile in normal cyclical and anoestrous cows. *Indian J Anim Sci.* 1998;68:1154-1156.
- Holsh JE. Physiology of ovulation. Treasure Island (FL): StatPearls Publishing; 2025. p. 1-122.
- Kadhim M, Ali B. Levels of some biochemical markers in sera of pregnant and non-pregnant lactating dairy cows in Baghdad, Iraq. *Iraqi J Vet Sci.* 2019;33(2):353-358.
- Kaneko H, Kishi H, Watanabe G, Taya K, Sasamoto S, Hasegawa Y. Changes in plasma concentrations of immunoreactive inhibin, estradiol and FSH associated with follicular waves during the estrous cycle of the cow. *J Reprod Dev.* 1995;41(4):311-320.
- Kumaresan A, Prabhakara PP, Bujarbaruah KM, Pathak KA, Chhetri B, Ahmed SK. Reproductive performance of crossbred dairy cows reared under traditional low input production system in the eastern Himalayas. *Trop Anim Health Prod.* 2009;41:71-78.
- Lopes AS, Butler ST, Gilbert RO, Butler WR. Relationship of pre-ovulatory follicle size, estradiol concentrations and season to pregnancy outcome in dairy cows. *Anim Reprod Sci.* 2007;99(1-2):34-43.
- Lyimo ZC, Nielen M, Ouweltjes W, Kruip TM, Van Eerdenburg FJCM. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology.* 2000;53(9):1783-1795.
- Lynch CO, Kenny DA, Childs S, Diskin MG. Relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. *Theriogenology.* 2010;73(2):190-198.
- Mann GE, Lamming GE. The influence of progesterone during early pregnancy in cattle. *Reprod Domest Anim.* 1999;34:9-12.
- Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction.* 2001;121(1):175-180.
- Miller BG, Moore NW. Effects of progesterone and oestradiol on RNA and protein metabolism in the genital tract and on survival of embryos in the ovariectomized ewe. *Aust J Biol Sci.* 1976;29:565-573.
- Miller BG, Moore NW, Murphy L, Stone GM. Early pregnancy in the ewe: Effects of oestradiol and progesterone on uterine metabolism and embryo survival. *Aust J Biol Sci.* 1977;30:379-388.
- Naik BR, Kumar AVNS, Bramhaiah KV, Ravi A, Chakravarthi VP. Estrogen and progesterone hormone levels in Punganur cattle. *IOSR J Agric Vet Sci.* 2013;2:50-53.
- Nephew KP, McClure KE, Ott TL, Dubois DH, Bazer FW, Pope WF. Relationship between variation in conceptus development and differences in estrous cycle duration in ewes. *Biol Reprod.* 1991;44(3):536-539.
- Niswender GD. Molecular control of luteal secretion of progesterone. *Reproduction.* 2002;123(3):333-339.
- Pandey AK, Ghuman SPS, Dhaliwal GS, Honparkhe M, Phogat JB, Kumar S. Effects of preovulatory follicle size on estradiol concentrations, corpus luteum diameter, progesterone concentrations and subsequent pregnancy rate in buffalo cows (*Bubalus bubalis*). *Theriogenology.* 2018;107:57-62.

27. Pandey NKJ, Gupta HP, Prasad S, Sheetal SK. Alteration in blood biochemical profile in pregnant and non-pregnant crossbred cows following exogenous supplementation of GnRH, HCG and progesterone releasing intravaginal devices. *Indian J Anim Reprod.* 2016;36(2):45-50.
28. Parkinson TJ, Turvey A, Jenner LJ. A morphometric analysis of the corpus luteum of the cow during the estrous cycle and early pregnancy. *Theriogenology.* 1994;41(5):1115-1126.
29. Pateria AK, Rawal CVS. White side test for subclinical metritis in buffaloes. *Indian J Anim Reprod.* 1990;11(2):142-144.
30. Pfeifer LFM, Leal SDCBDS, Schneider A, Schmitt E, Corrêa MN. Effect of ovulatory follicle diameter and progesterone concentration on pregnancy rate of fixed-time inseminated lactating beef cows. *Rev Bras Zootec.* 2012;41:1004-1008.
31. Perry GA, Smith MF, Roberts AJ, MacNeil MD, Geary TW. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J Anim Sci.* 2007;85(3):684-689.
32. Raja S, Devanathan TG, Kulasekar K, Pazhanivel N, Balachandran C. Whiteside test and endometrial biopsy for diagnosis of endometritis in repeat breeding cows. *Indian J Anim Reprod.* 2012;33(1):56-58.
33. Rekwot PI, Ogwu D, Oyedipe EO. Influence of bull biostimulation, season and parity on resumption of ovarian activity of zebu cattle following parturition. *Anim Reprod Sci.* 2000;63(1-2):1-11.
34. Richards MW, Wettemann RP, Schenemann MH. Nutritional anestrus in beef cows: Concentrations of glucose and non-esterified fatty acids in plasma and insulin in serum. *J Anim Sci.* 1989;67:2354-2362.
35. Roberts SJ. Veterinary obstetrics and genital diseases. 2nd ed. New Delhi: CBS Publishers and Distributors; 1971. p. 1-105.
36. Sartori R, Rosa GJM, Wiltbank MC. Ovarian structures and circulating steroids in heifers and lactating cows in summer and dry cows in winter. *J Dairy Sci.* 2002;85(11):2813-2822.
37. Savio JD, Keenan L, Boland MP, Roche JF. Pattern of growth of dominant follicles during the estrous cycle of heifers. *J Reprod Fertil.* 1988;83:663-671.
38. Sharma P, Kumar P, Sharma A, Negi V, Kumar H, Sood P. Short estrous cycle post-ovulation in peri-pubertal Sahiwal and Jersey crossbred heifers. *Int J Biol Res Rev.* 2004;33(1):31-38.
39. Shukla SP, Sharma RD, Jindal R. Serum estradiol and progesterone levels during estrous cycle in repeat breeding crossbred cows. *Indian J Anim Reprod.* 2000;21(2):112-114.
40. Snedecor GW, Cochran WG. *Statistical methods.* 8th ed. Ames (IA): Iowa State University Press; 1994. p. 1-524.
41. Vasconcelos JLM, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology.* 2001;56(2):307-314.
42. Viana JHM, Ferreira M, Sá WF, Camargo LSA. Follicular dynamics in Zebu cattle. *Pure Appl Biol.* 2000;35:2501-2509.
43. Vohra SC, Dindorkar CV, Kaikini AS. Studies on blood serum levels of certain biochemical constituents in normal cycling and anestrous crossbred cows. *Indian J Anim Reprod.* 1995;16:85-87.
44. Wettemann RP, Hafs HD. LH, prolactin, estradiol and progesterone in bovine blood serum during early pregnancy. *J Anim Sci.* 1973;36(1):51-56.
45. Wiltbank MC, Souza AH, Carvalho PD, Cunha AP, Giordano JO, Fricke PM, *et al.* Physiological and practical effects of progesterone on reproduction in dairy cattle. *Animal.* 2014;8(Suppl 1):70-81.
46. Yan L, Robinson R, Shi Z, Mann G. Efficacy of progesterone supplementation during early pregnancy in cows: A meta-analysis. *Theriogenology.* 2016;85(8):1390-1398.