

RESEARCH ARTICLE

Blood Biochemical, Hormonal and Mineral Status of Cyclic, Acyclic, Endometriotic and Pregnant Crossbred Cows

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ABSTRACT

A study was carried out on infertile (acyclic and endometriotic) crossbred cows under field and normal cyclic (all 4 phases of cycle) as well as pregnant (3, 6, 9 month) crossbred cows of University farm to evaluate the plasma progesterone (P4) and estradiol (E2) hormones by RIA, and plasma total protein, cholesterol, calcium and phosphorus concentrations by using assay kits on chemistry analyzer. The mean progesterone levels in cows during the diestrus phase and in pregnancy were significantly ($p < 0.05$) higher than those during proestrus, estrus, metestrus, anestrus, and endometritis status. At six month of gestation, the mean P4 level was significantly ($p < 0.05$) higher than at early or late gestation. The mean E2 values at estrus and 9th month of gestation were highest ($p < 0.01$) as compared to another status. The mean plasma total protein and cholesterol levels were significantly ($p < 0.05$) lower during six and nine months of pregnancy than during cyclic and acyclic stages. The cholesterol profile of all three stages of pregnancy and endometriotic cows were statistically similar, though distinctly low at 9 month of pregnancy. Plasma levels of P4 and E2 thus correlated with the physiological and clinical status of cows, while cholesterol levels reflected steroidogenic status. The mean plasma calcium and inorganic phosphorus concentrations of cyclic, acyclic, pregnant and endometriotic cows, however, did not differ significantly.

Keywords: Blood biochemistry, Crossbred cattle, Cyclic, Acyclic, Pregnant, Endometritis.

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INTRODUCTION

Fertility is one of the key determinants of the cow's performance warranting one calf every year for optimum economic return. Infertility is one of the major problems which incur losses for the dairy industry. Infections of the reproductive tract like endometritis, metritis, pyometra, etc. caused by groups of known and unknown culturable and unculturable micro-organisms play a significant role in infertility, but our knowledge of unculturable microbiota of genital tract of animals is meager (Suthar *et al.*, 2018). The circulatory levels of gonadal steroids, blood metabolites, and minerals are known to vary in different reproductive physio-pathology and are reported in pieces (Habeeb *et al.*, 1999; Ahmad *et al.*, 2004; Ashmawy, 2015; Modi *et al.*, 2017), but comprehensive comparative studies are meager reporting all aspects in the same document from the same lab. Hence, this study was aimed to compare the blood biochemical, steroid hormonal and macro-mineral status of cyclic, acyclic, endometriotic and pregnant crossbred cows from middle Gujarat.

MATERIALS AND METHODS

The present study was carried out on infertile crossbred cows under field condition of village Chikhodra of Anand taluka and healthy cyclic as well as pregnant crossbred cows of Livestock Research Station, AAU, Anand from August 2017 to June 2018. The work was carried out on total 36 crossbred cows covering six each regular cyclic (proestrus, estrus, metestrus, diestrus), acyclic, endometriotic and 3, 6 and 9

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months pregnant animals. Blood samples were collected from a jugular vein in heparinized vacutainers, i.e., during all four phases of estrus cycle of same animals, three stages of pregnancy as well as from acyclic and endometritic cows. The samples were centrifuged at 4500 rpm for 15 minutes and plasma separated was stored in deep freeze at -20°C with a drop of sodium merthiolate (0.1%) until analyzed.

Simultaneously vaginal aspirates were also obtained aseptically from all the cows for bacterial metagenomics and were processed for DNA extraction, amplification using 16S rRNA gene on next generation sequencer and MG RAST libraries were formed to find out the abundance of bacterial phyla, genera and species in each specimen and its association with plasma progesterone and estradiol was derived, which has been reported separately (Patel, 2018).

The plasma progesterone and estradiol-17 β concentrations were determined by employing standard Radio Immuno Assay (RIA) techniques of Kubasic *et al.* (1984) and Robertson and King (1979), respectively, at RIA Laboratory of the College. Labelled antigen (I^{125}), antibody-coated tubes and standards were procured from Immunotech-SAS, Marseille Cedex, France. The sensitivity of the assay for progesterone and estradiol-17 β was 0.1 ng/mL and 9.58 pg/mL, while the intra-assay coefficient of variation was 5.4 and 14.4 percent, and inter-assay variation 9.1 and 14.5 percent, respectively. Plasma total protein and cholesterol contents were determined by Biuret method and CHOD/PAP (Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase) method, respectively, while plasma calcium and inorganic phosphorus concentrations were estimated by arsenazol-III and Molybdate UV method, respectively, using standard procedures and assay kits procured from Crest Bio-systems, Goa, with the help of Chemistry Analyzer (Mindray, BS 120). The data were analyzed to work out the mean \pm SEs for different groups/stages using ANOVA and differences among means were tested by NMRT at $p < 0.05$ using SPSS software version 20.0 (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Plasma Profile of Progesterone and Estradiol

The plasma progesterone (P4) and estradiol (E2) concentrations in cyclic crossbred cows during proestrus, estrus, metestrus, and diestrus phase, as well as those in anestrus cows; three, six, and nine months of gestation and endometriotic crossbred cows, are shown in Table 1. The mean plasma P4 levels in cows under diestrus phase and three, six and nine months of pregnancy were significantly ($p < 0.05$) higher than those in cows under proestrus, estrus, metestrus, anestrus, and endometritis status. Even at six months of gestation, the mean P4 level was significantly ($p < 0.05$) higher than at early or late pregnancy (Table 1). Similarly, the mean plasma estradiol levels in cows under

anestrus, metestrus, and diestrus phase were significantly ($p < 0.05$) lower followed by endometritis than those in proestrus and estrus phase, and three, six and nine months of pregnancy. Further, the mean E2 values at estrus and 9th month of gestation were highest ($p < 0.01$), suggesting its follicular and placental source at the respective stage.

The results of the present study were in agreement with the reports of Shukla *et al.* (2000), Kavani *et al.* (2005), Butani *et al.* (2011) and Ashmawy (2015) in cows with different reproductive status. They all recorded significantly higher plasma progesterone and low estradiol during the luteal phase of cycle, pregnancy, and endometritis as compared to the follicular phase of the cycle in either cattle or buffaloes. Habeeb *et al.* (1999) reported that during the third trimester of gestation, plasma progesterone level decreased to reach the average of 2.5 ng/ml by 9th month in Friesian cows, which is well in line with present findings in crossbred cows. There were positive associations of either plasma progesterone or estradiol levels with the abundance of specific phyla or genera (Patel, 2018), proving the role of local defense mechanism in controlling microbial population and its type in the vagina of cattle during different physio-pathological status.

Plasma Profile of Total Protein and Total Cholesterol

The mean values of plasma total protein and total cholesterol during different stages of the reproductive cycle are represented in Table 2. The mean plasma total protein levels in anestrus and endometriotic cows were non-significantly lower than those of cyclic cows in all 4 phases. Further, the levels declined with advancing pregnancy and were significantly ($p < 0.05$) lower during six and nine months of pregnancy, probably because of its greater utilization for rapid fetal growth *in utero* during last trimester of gestation. These findings of higher mean total plasma protein levels in normal cyclic than the anestrus crossbred cows were in accordance with the report of Vohra *et al.* (1995), Tandle *et al.* (1997), Joe *et al.* (1998), Kavani *et al.*

Table 1: Mean plasma progesterone and estradiol-17 β levels and vaginal microbiota during different stages of the normal and abnormal reproductive cycle in crossbred cows

Reproductive status	Reproductive stage	Steroid hormones		No. of bacterial	
		P4 (ng/mL)	E2 (pg/mL)	Phyla	Genus
Cyclic	Proestrus	0.16 \pm 0.04 ^a	66.00 \pm 8.16 ^{de}	15	273
	Estrus	0.30 \pm 0.16 ^a	78.17 \pm 7.59 ^e	14	203
	Metestrus	1.36 \pm 0.62 ^a	32.67 \pm 2.89 ^{ab}	17	316
	Diestrus	3.15 \pm 0.77 ^{bc}	27.67 \pm 4.56 ^{ab}	17	256
Acyclic	Anestrus	1.26 \pm 0.69 ^a	25.83 \pm 4.56 ^a	17	302
Endometritis	Endometritic	1.67 \pm 1.074 ^{ab}	42.83 \pm 4.41 ^{bc}	16	283
Pregnant	3 Months	4.98 \pm 0.45 ^d	50.17 \pm 4.18 ^{cd}	16	298
	6 Months	7.80 \pm 0.63 ^e	64.50 \pm 2.36 ^{de}	14	293
	9 Months	4.65 \pm 0.58 ^{cd}	94.00 \pm 8.85 ^f	14	305

Mean bears different superscripts within the column differ significantly ($p < 0.01$).



Table 2: Mean plasma biochemical and mineral profile during different stages of the reproductive cycle in crossbred cows

Status	Stage	Total protein (g/dL)	Total cholesterol (mg/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
Cyclic	Proestrus	9.64 ± 0.31 ^d	183.19 ± 36.12 ^b	8.71 ± 0.42	4.03 ± 0.17
	Estrus	9.26 ± 0.28 ^{cd}	157.70 ± 11.92 ^b	8.46 ± 0.40	4.15 ± 0.17
	Metestrus	9.21 ± 0.22 ^{cd}	155.96 ± 12.61 ^b	8.59 ± 0.26	4.26 ± 0.07
	Diestrus	9.14 ± 0.34 ^{cd}	143.89 ± 10.55 ^{ab}	8.35 ± 0.34	4.24 ± 0.26
Acyclic	Anestrus	8.68 ± 0.41 ^{cd}	181.08 ± 21.04 ^b	8.99 ± 0.47	4.27 ± 0.30
Endometritis	Endometriotic	8.27 ± 0.46 ^{bc}	133.54 ± 13.20 ^{ab}	8.81 ± 0.43	4.21 ± 0.36
Pregnant	3 Months	8.18 ± 0.51 ^{bc}	127.21 ± 12.35 ^{ab}	8.17 ± 0.11	4.11 ± 0.06
	6 Months	7.93 ± 0.41 ^{ab}	140.68 ± 15.64 ^{ab}	8.34 ± 0.29	4.54 ± 0.14
	9 Months	7.50 ± 0.44 ^a	99.89 ± 7.64 ^a	8.48 ± 0.42	4.25 ± 0.36

Means bearing common superscripts within the column do not differ significantly ($p < 0.01$).

(2005), Butani *et al.* (2011), Modi *et al.* (2017), whereas Singh *et al.* (2007) and Kumar *et al.* (2009) found contradictory results that the normal cyclic animals had a significantly lower concentration of serum total protein as compared to anestrus cows. Further, the lower mean total plasma protein levels found in endometriotic than the cyclic crossbred cows was in agreement with the reports of Ahmad *et al.* (2004) and Sahadev *et al.* (2007).

The mean plasma total cholesterol level at nine months of pregnancy was significantly ($p < 0.05$) lower as compared to anestrus and different phases of cyclic cows, however the levels in endometriotic and 3 and 6-month pregnant cows, though higher were statistically not different from 9-month stage. The values of cows in proestrus and anestrus phase were the highest though did not differ statistically from other cyclical phases or endometriotic cows (Table 2). The present non-significant difference in total cholesterol levels of cyclic and acyclic cows concurred well with Vahora *et al.* (1995). Similarly, Sahukar *et al.* (1985) recorded the highest total cholesterol at estrus which declined subsequently till 3 months, rose around 6 months and again declined at 9 months of pregnancy in crossbred cows. Ashmawy (2015) also recorded a decline in serum cholesterol from 8th month to 10th month of gestation in buffaloes. Ahmad *et al.* (2004) found significantly higher cholesterol in endometriotic than cyclic and anestrus cows, while in present study inverse trend with non-significant differences were noted for these stages. In contrast, to the present findings, Joe *et al.* (1998) and Jayachandran *et al.* (2013) reported significantly higher plasma cholesterol in cyclic than anestrus and repeat breeding bovines. Thus, the changes in plasma cholesterol levels were negatively associated with gonadal steroidogenesis in cattle.

Plasma Profile of Calcium and Phosphorus

The plasma calcium concentrations did not show any significant variation between different stages of the reproductive cycle (Table 2). Similar observations have also been reported earlier by Sivaiah *et al.* (1986), Singh *et al.* (2007) and Jayachandran *et al.* (2013). The plasma inorganic phosphorus concentrations also did not show any significant variation between different stages of the

reproductive cycle. These observations corroborated with Sivaiah *et al.* (1986). However, Joe *et al.* (1998) and Kumar *et al.* (2009) recorded significantly higher value of phosphorus in cyclic crossbred cows as compared to anestrus cows. Ali *et al.* (2014) reported significantly lower mean serum calcium and phosphorus levels in anestrus and repeat breeder cattle as compared to cows in estrus.

It is thus concluded that the plasma levels of P4 and E2 correlate with physiological and clinical status of cows and their vaginal microbiota, while cholesterol levels reflect steroidogenic status. The mean plasma calcium and inorganic phosphorus concentrations of cyclic, acyclic, pregnant and endometriotic cows, however, did not show significant variation between various reproductive physio-pathological conditions of crossbred cows.

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