RESEARCH ARTICLE

Comparative Plasma Endocrine, Metabolic and Mineral Profile of Cyclic, Acyclic, Endometritic and Pregnant Buffaloes

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Abstract

Circulating concentrations of hormones, metabolites, and minerals reflect the physio-pathological status of reproduction in animals. This study was carried out on infertile (anestrus, endometritic), normal healthy cyclic and pregnant buffaloes to evaluate the comparative plasma progesterone (P_4) and estrogen (E_2) hormones, plasma total protein, total cholesterol, calcium, and phosphorus profile. The study showed higher mean plasma E_2 and lower P_4 levels in the follicular phase of estrous cycle in buffaloes. Significantly ($p < 0.05$) higher mean plasma P_4 level and lower E_2 levels were recorded during the luteal phase and in endometritic and pregnant buffaloes. Total plasma protein concentration was non-significantly higher in normal cyclic than acyclic and endometritic buffaloes. It was also comparatively lower in buffalo with 9 months of pregnancy than 3 and 6 months of pregnancy. The mean plasma total cholesterol level was significantly ($p<0.05$) higher in pregnant than acyclic and endometritic buffaloes. Cyclic buffaloes had significantly ($p <0.05$) higher mean plasma calcium levels than acyclic buffaloes. Plasma phosphorus concentration, however, did not show any significant difference between different stages of the reproductive cycle.

Keywords: Acyclic, Buffalo, Cyclic, Endometritis and Pregnancy, Endocrine metabolic and minerals profile.

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Introduction

Reproductive efficiency is the primary factor affecting the productivity of a dairy buffalo and is greatly influenced by late attainment of puberty, seasonal breeding, long calving intervals, increased number of services per conception, increased days open, uterine infections and various obstetrical problems (Hedaoo *et al*., 2008). The basic causes of the reproductive problems in a herd are multiple and include managerial, nutritional, and pathological factors. Minerals, both macro and micro, are the essential nutrients bearing a significant role in animal reproduction because their excess or deficiency produces a detrimental effect on the performance of livestock (Underwood, 1981). Infertility is one of the major problems which incur losses for the dairy industry. This study was aimed to evaluate comparative steroid hormones, metabolites and mineral status of buffaloes with different reproductive physio-pathological conditions.

Materials and Methods

The study was carried out on infertile (anestrus; endometritic) buffaloes of villages nearby Anand and healthy cyclic as well as pregnant buffaloes of University Farm, A.A.U., Anand from August 2018 to June 2019. The study covered a total of 35 buffaloes comprising five regular cyclic (each buffalo sampled during proestrus, estrus, metestrus, diestrus) and six each acyclic, endometritic and 3, 6 and 9 months pregnant buffaloes. Blood samples were collected from the jugular vein in heparinized vacutainers from all animals with above physio-pathological reproductive status for the estimation of plasma hormonal and biochemical parameters. The blood samples were centrifuged at 3000 rpm for 10 minutes, and the plasma was stored in deep freeze at -20°C with a drop of sodium merthiolate (0.1%) as a preservative until analyzed.

Plasma progesterone (P4)and estradiol-17β(E2) concentrations were determined by employing standard Radio Immuno Assay (R.I.A.) techniques using assay kits procured from Immunotech-SAS, Marseille Cedex, France. The sensitivity of the assay for progesterone and estradiol-17β was 0.1 ng/mL and 9.58 pg/mL, while the intra-assay

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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 total 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 U.V. method, respectively, using standard procedure and assay kits procured from Crest Bio-systems, Goa, with the help of Chemistry Analyzer (Mindray, BS 120).

The data were analyzed using descriptive statistics to calculate the mean ± S.E.s for different groups/stages and the differences among means were tested by ANOVA and NMRT at $p < 0.05$ using SPSS software version 20.0 (Snedecor and Cochran, 1994).

Results and Discussion

The mean estrogen level was significantly ($p < 0.01$) higher, and progesterone level was lower during follicular phase than the luteal phase of the estrous cycle in buffaloes (Table 1). These findings are in agreement with the reports of Mondal *et al.* (2010), Tiwari *et al.* (2012), Butani *et al.* (2011), Ashmawy (2015), and Hafez (2019) in buffaloes with different reproductive status. They also reported significantly ($p < 0.05$) higher mean plasma progesterone levels and lower mean plasma estrogen levels during the luteal phase, pregnancy, and endometritis in buffaloes. The mean plasma progesterone levels in buffaloes during the diestrus phase and in pregnancy were significantly ($p < 0.05$) higher than those during proestrus, estrus, metestrus, anestrus, and endometritis status. The mean progesterone concentration declined significantly ($p < 0.05$) with advancing gestation, The mean estrogen values at estrus and 9th month of gestation were highest as compared to other stages (Table 1).

The mean plasma total protein (g/dl) levels varied non-significantly between 7.11 ± 0.36 and 7.79 ± 0.23 in buffaloes, irrespective of their reproductive status. The mean plasma total protein levels were found to be higher in normal cyclic buffaloes than that of anestrus buffaloes. These findings are in accordance with the reports of Kumar and Atul (2010), Kapadiya and Siddiquee (2013). However, contrary to the present findings, Hedaoo *et al.* (2008) reported significantly ($p < 0.05$) lower mean serum total protein concentration in normal cyclic as compared to anestrus buffaloes. Further, the lower mean total plasma protein levels found in endometritic buffaloes than those of cyclic buffaloes in the present study is in agreement with the report of Sahadev *et al.* (2007). The lower mean plasma total protein levels found at nine months of pregnancy as compared to those of three and six months of pregnancy in the present study corroborated with the observations reported by Ashmawy (2015) in buffaloes.

The mean plasma total cholesterol concentration (mg/dl) varied significantly ($p < 0.05$) between 83.21 ± 2.74 (endometritis) and 127.72 ± 5.31 (6 month pregnancy) in buffaloes, irrespective of their reproductive status (Table 1). The mean plasma total cholesterol levels at six and nine months of pregnancy were significantly ($p < 0.05$) higher as compared to those of acyclic and endometritic buffaloes. In this study, non-significant differences in the mean cholesterol levels were observed in cyclic and acyclic and endometritic buffaloes. The present findings are contrary to the results of Kabir *et al.* (2001), Akhtar *et al*. (2010), Kapadiya and Siddiquee (2013) and Hafez (2019) in buffaloes, whereas Sarvaiya and Pathak (1991) reported non-significantly higher plasma total cholesterol concentration in cyclic buffaloes compared to acyclic buffaloes. The trend of declining the mean serum total cholesterol levels from 8th to 9/10th months of gestation in buffaloes observed in the present study corroborated with the observations reported by Ashmawy (2015).

The mean plasma calcium (mg/dL) levels varied non-significantly between 8.02 ± 0.17 and 8.72 ± 0.13 in buffaloes, irrespective of their reproductive status. The cyclic buffaloes had significantly ($p < 0.05$) higher mean plasma calcium concentrations than that of anestrus buffaloes (7.25 ± 0.24). The present findings were supported by the earlier reports of Newer *et al.* (1999) and Shahzad *et al*. (2018). However, Hedaoo *et al.* (2008) observed non-significant differences in plasma calcium levels in cyclic and anestrus buffaloes.

Table 1: Mean (±SE) plasma endocrine, biochemical and minerals profile in buffaloes with different reproductive stages

Reproductive Status	*Stages*	*Progesterone (ng/ml) P4*	*Estradiol-17β (pg/ml) E2*	*Total protein (g/dL)*	*Total Cholesterol (mg/dL)*	*Calcium (mg/dl)*	*Inorganic phosphorus (mg/dL)*
Cyclic (5 each)	Proestrus	1.38 ± 0.34^{ab}	61.00 ± 6.51^{bc}	7.57 ± 0.17	96.07 ± 6.43^{ab}	8.17 ± 0.22^{b}	3.69 ± 0.11
	Estrus	0.29 ± 0.13^{a}	88.40 ± 0.51^{d}	7.53 ± 0.16	98.36 ± 7.36^{ab}	8.43 ± 0.31^{b}	3.67 ± 0.23
	Metestrus	0.59 ± 0.12^{a}	47.80 ± 12.19^{ab}	7.79 ± 0.23	90.59 ± 7.60^{ab}	8.54 ± 0.49^{b}	3.81 ± 0.16
	Diestrus	3.02 ± 0.39^{de}	37.40 ± 4.06^{a}	7.28 ± 0.22	94.29 ± 7.21^{ab}	8.55 ± 0.34^{b}	3.65 ± 0.16
Acyclic (6)	Anestrus	0.37 ± 0.17^{a}	36.00 ± 6.13^{a}	7.11 ± 0.36	84.97 ± 4.63^{a}	7.25 ± 0.24^{a}	3.75 ± 0.15
Endometritis (6)	Endomet	1.89 ± 0.56^{bc}	61.83 ± 9.59^{bc}	7.21 ± 0.27	83.21 ± 2.74^{a}	8.02 ± 0.17^{b}	3.81 ± 0.18
Pregnant (6 each)	3 Month	4.07 ± 0.31^{e}	66.50 ± 5.83^{bc}	7.60 ± 0.28	107.37 ± 5.62^{b}	8.72 ± 0.13^{b}	3.65 ± 0.12
	6 Month	3.17 ± 0.53^{de}	65.67 ± 6.09^{bc}	7.73 ± 0.28	127.72 ± 5.31^{c}	8.12 ± 0.10^{b}	3.84 ± 0.20
	9 Month	2.65 ± 0.31^{cd}	73.17 ± 7.01^{cd}	7.25 ± 0.19	110.36 ± 7.78^{bc}	8.35 ± 0.24^{b}	3.81 ± 0.24

Means bearing different superscripts within the column differ significantly ($p < 0.05$).
Figures in parenthesis indicate a number of animals.

Fayaz *et al.* (2007) found significantly higher plasma calcium concentration in metritic than healthy buffaloes.

The mean plasma inorganic phosphorus (mg/dl) levels varied non-significantly between 3.65 ± 0.12 and 3.84 ± 0.20 in buffaloes, irrespective of their reproductive status (Table 1). The plasma inorganic phosphorus concentrations did not show any significant variation between different stages of the reproductive cycle. These observations corroborated well with the reports of Hedaoo *et al.* (2008), and Patel *et al.* (2019) either in buffalo and cattle. However, Tiwari *et al.* (2012) and Shahzad *et al.* (2018) recorded a significantly higher value of phosphorus in cyclic buffaloes as compared to anestrus buffaloes.

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