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## Can Peripartum Plasma Profiles of Steroid Hormones, Metabolites and Minerals Predict Postpartum Fertility in Buffaloes?

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### Abstract

The plasma profile of various hormonal, biochemical and mineral constituents was studied in 32 buffaloes under nutritional management of transition period for 2 months before to 2 months postpartum. The buffaloes that expressed estrus and conceived within 120 days postpartum, irrespective of nutrient treatment or control groups, were classified as fertile (n=15) and rests as infertile (n=17) buffaloes. The variations in blood glucose and plasma profile of progesterone, estradiol, protein, cholesterol, triglycerides, Ca, P, Mg and trace minerals (Zn, Fe, Cu, Co, Mn), if any, on day 60, 30, 15 prepartum, on the day of calving, and on day 15, 30, 45 and 60 postpartum in postpartum conceiving and non-conceiving buffaloes were examined. There was no significant effect of fertile-infertile groups on plasma P<sub>4</sub> and E<sub>2</sub> profile on any day, except on day 45 postpartum, where E<sub>2</sub> was (p<0.05) higher in fertile than infertile group (31.47±2.37 vs. 24.29±1.76 pg/ml), indicating early onset of follicular activity with ovulatory estrus in postpartum fertile group. The blood glucose and plasma protein profile also did not vary significantly at any of the intervals between two groups. Further, no significant difference in plasma cholesterol or triglyceride concentrations was found between conceived and non-conceived buffaloes at any of the intervals pre- or postpartum, except that the pooled mean cholesterol level was higher (P<0.05) in non-conceived than conceived buffaloes. The plasma concentrations of calcium, inorganic phosphorus and magnesium also did not reveal significant variation between fertile and infertile buffaloes at any of the intervals, though the magnesium value was higher in conceived than non-conceived buffaloes. Similarly, no significant difference was seen in the levels of plasma trace minerals, viz., Zn, Fe, Mn, Cu or Co between fertile and infertile buffaloes at any of the intervals studied. These non-significant variations noted between fertile and infertile buffaloes in blood profile could perhaps be due to masking effect of nutritional supplementation as the control group was also optimally managed by the farmers; hence the animals were pooled irrespective of groups for this comparison.

**Key Words:** Buffalo, Postpartum, Fertile, Infertile, Metabolic profile, Steroid hormone profile.

### Introduction

The blood biochemical, hormonal and mineral profile during transition period has great relevance to future fertility in dairy animals. Cholesterol, a precursor of steroid hormones, is the principal means

of synthesis of sex hormones from the gonads, placenta and adrenals. Similarly, optimum protein level is also necessary for the development of endocrine and sex glands, and for early onset of postpartum estrus. About half of the calcium present in the plasma is bound to protein in a non-diffusible form and the remainder is in ionized (physiologically active,  $\text{Ca}^{++}$ ) form. The inorganic phosphorus is practically entirely found in the plasma. Calcium and phosphorus influence the ability of animals to utilize other trace elements. Their influence on certain enzyme systems may affect reproductive efficiency. Trace elements, viz., Zn, Cu, Co, Se and Mn are the integral parts of many metallo-enzymes and carrier proteins, and play an important role in body metabolism, protein synthesis, haemopoiesis, immunocompetence and even in the maintenance of vascular and skeletal integrity and structure and function of the central nervous system. The imbalance or deficiency of trace elements leads to inactive ovaries and repeat breeding in dairy animals (Hidiroglou, 1979). The present study was therefore aimed to relate the blood profile of these components during transitional period with postpartum fertility in buffaloes.

### Materials and Methods

This study covered 32 advanced pregnant pluriparous buffaloes divided into treatment and control groups, and subjected to either nutritional management of transition period for 2 months before to 2 months postpartum or control group managed as per local farmers' feeding practices. The buffaloes of treatment group, in addition to farmers feeding schedule, received 1.5 kg compound concentrate mixture (22 % CP) and 50 g of area specific chelated mineral mixture. The buffaloes that expressed estrus and conceived through AI within 120 days postpartum, irrespective of nutrient treatment or control groups, were classified as fertile (n=15) and rests as infertile (n=17) buffaloes. The blood samples were collected in heparinized vacutainers from all these animals at fortnight intervals, i.e. on days 60, 30 and 15 pre-partum, the day of calving and on days 15, 30, 45 and 60 postpartum. The blood glucose was determined on the spot by direct strip technique using Accu-chek Integra machine. The plasma samples separated out by centrifugation of blood samples were stored in deep freeze at  $-20^{\circ}\text{C}$  with a drop of merthiolate (0.1%) until analyzed. The levels of plasma progesterone and estradiol were estimated by standard RIA techniques. Plasma total protein, cholesterol, triglycerides, Ca, P and Mg were estimated on biochemistry analyzer using standard procedures and kits procured from Crest Biosystem, Goa. The trace minerals (Zn, Fe, Cu, Co, Mn) were analysed in 1:3 diluted plasma samples on atomic absorption spectrophotometer. The data were analysed statistically using ANOVA and 't' test to know the variations in these constituents between days and between postpartum conceiving and non-conceiving buffaloes on different days peripartum.

### Results and Discussion

#### Plasma Progesterone and Estradiol-17 $\beta$ Profile

The mean plasma progesterone ( $\text{P}_4$ ) and estradiol-17 $\beta$  ( $\text{E}_2$ ) concentrations recorded on day 60, 30, 15 pre-partum, on the day of calving, and on day 15, 30, 45 and 60 postpartum in transitional buffaloes revealed that there was sudden and significant drop in plasma  $\text{P}_4$  and rise in plasma  $\text{E}_2$  concentrations on the day of calving as compared to pre-partum values, and very low or basal values were noted around days 3 and 14 postpartum, thereafter the levels of both the hormones gradually increased and showed a fluctuating trend with higher values of  $\text{E}_2$  around day 45 postpartum (Table 1). However, no significant difference was found in values of any of these hormones between fertile and infertile buffaloes, except  $\text{E}_2$  at day 45 was significantly ( $P<0.05$ ) higher in postpartum fertile than infertile group ( $31.47\pm 2.37$  vs.  $24.29\pm 1.76$  pg/ml), indicating early onset of follicular activity with ovulatory estrus postpartum in this group as has been documented by Shah *et al.* (2004) and Butani *et al.* (2010). Hussein *et al.* (2013), however, reported higher plasma  $\text{P}_4$  and  $\text{E}_2$  concentrations during 14<sup>th</sup> to 75<sup>th</sup> days postpartum in pregnant as compared to non-pregnant ( $5.47\pm 0.17$  vs.  $3.18\pm 0.21$  ng/ml) buffaloes. The paradigm shift recorded in both the steroid hormones during transition period concurred well with the observations of Kindahl *et al.* (2004) and

Dhami *et al.* (2015) in crossbred cows.

**Table 1: Mean hormonal and biochemical profile during transition period in nutritionally managed buffaloes**

Days pre- & post partum	Plasma profile					
	Progesterone (ng/ml)	Estradiol-17 $\beta$ (pg/ml)	Blood glucose (mg/dl)	Total protein (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
-60	4.66 $\pm$ 0.08 <sup>c</sup>	45.45 $\pm$ 1.69 <sup>c</sup>	45.48 $\pm$ 0.97 <sup>a</sup>	8.05 $\pm$ 0.10 <sup>c</sup>	62.63 $\pm$ 3.16 <sup>de</sup>	23.71 $\pm$ 1.38 <sup>c</sup>
-30	4.34 $\pm$ 0.10 <sup>de</sup>	55.59 $\pm$ 1.98 <sup>d</sup>	47.07 $\pm$ 1.21 <sup>ab</sup>	7.92 $\pm$ 0.10 <sup>bc</sup>	58.41 $\pm$ 2.81 <sup>cde</sup>	23.39 $\pm$ 1.34 <sup>c</sup>
-15	4.04 $\pm$ 0.10 <sup>d</sup>	74.48 $\pm$ 1.85 <sup>e</sup>	47.72 $\pm$ 1.38 <sup>abc</sup>	7.80 $\pm$ 0.11 <sup>bc</sup>	53.60 $\pm$ 2.88 <sup>bc</sup>	21.45 $\pm$ 1.23 <sup>c</sup>
0	0.50 $\pm$ 0.04 <sup>a</sup>	146.31 $\pm$ 2.59 <sup>f</sup>	58.10 $\pm$ 1.44 <sup>d</sup>	7.30 $\pm$ 0.12 <sup>a</sup>	43.46 $\pm$ 2.03 <sup>a</sup>	13.45 $\pm$ 1.07 <sup>a</sup>
15	0.31 $\pm$ 0.03 <sup>a</sup>	20.83 $\pm$ 1.30 <sup>a</sup>	50.93 $\pm$ 1.15 <sup>c</sup>	7.61 $\pm$ 0.09 <sup>b</sup>	49.93 $\pm$ 2.14 <sup>ab</sup>	16.47 $\pm$ 1.16 <sup>ab</sup>
30	0.66 $\pm$ 0.13 <sup>ab</sup>	25.21 $\pm$ 1.10 <sup>ab</sup>	50.72 $\pm$ 1.04 <sup>c</sup>	7.64 $\pm$ 0.10 <sup>b</sup>	56.53 $\pm$ 2.43 <sup>bcd</sup>	17.80 $\pm$ 1.37 <sup>b</sup>
45	1.01 $\pm$ 0.17 <sup>bc</sup>	28.00 $\pm$ 1.62 <sup>b</sup>	50.48 $\pm$ 1.06 <sup>bc</sup>	7.74 $\pm$ 0.13 <sup>bc</sup>	62.43 $\pm$ 2.39 <sup>de</sup>	16.81 $\pm$ 1.39 <sup>ab</sup>
60	1.35 $\pm$ 0.27 <sup>c</sup>	26.90 $\pm$ 1.82 <sup>b</sup>	51.07 $\pm$ 1.28 <sup>c</sup>	7.70 $\pm$ 0.11 <sup>b</sup>	65.49 $\pm$ 2.23 <sup>e</sup>	17.61 $\pm$ 1.28 <sup>b</sup>
Overall	--	--	50.20 $\pm$ 0.48	7.72 $\pm$ 0.04	56.56 $\pm$ 0.99	18.84 $\pm$ 0.50

Means bearing uncommon superscripts within the column differ significantly (P<0.05).

### Blood Biochemical Profile

The blood glucose levels on the day of calving were significantly higher than before and after calving and the mean plasma total protein concentrations showed inverse trend, but there was no significant variation at any of the intervals between conceived and non-conceived groups for any of these traits. Shah *et al.* (2003) reported similar findings for plasma total protein between fertile and infertile Surti buffaloes; however, Khasatiya *et al.* (2005<sup>a</sup>) found lower level of glucose while Butani *et al.* (2010) found higher level of protein in conceived than the non-conceived buffaloes.

The mean plasma cholesterol levels decreased gradually and significantly (P<0.05) from day 60 pre-partum to day of calving and then again increased till day 60 postpartum (Table 1). The levels were apparently higher in non-conceived than conceived buffaloes throughout the evaluation, but differed significantly in the overall pooled means only (58.69 $\pm$ 1.45 vs. 54.57 $\pm$ 1.34 mg/dl). The mean plasma triglycerides concentrations also followed the trend similar to cholesterol, but there was no significant difference between conceived and non-conceived buffaloes at any of the intervals pre- or postpartum or even in overall mean values. The present findings on cholesterol concurred with the observations of Khasatiya *et al.* (2005<sup>a</sup>) in Surti buffaloes. The present findings on triglycerides appropriated with Guedon *et al.* (1999), who stated that triglycerides levels remained more or less constant without showing relationship with fertility and it was probably more related with lipid metabolism in the mammary gland. On the contrary, Patel and Dhami (2005) documented significantly (P<0.05) higher level of triglycerides in conceived cows as compared to non-conceived cows (63.19 $\pm$ 0.70 vs 60.45 $\pm$ 0.99 mg/dl) and Hussein *et al.* (2013) recorded highest level of plasma total cholesterol and triglycerides in conceiving than the non-conceiving buffaloes between 14<sup>th</sup> and 45<sup>th</sup> day postpartum. Further, the changes found in the biochemical constituents during transition period have also been reported earlier by many researchers Piccione *et al.* (2012) and Theodore (2015) in bovines.

### Plasma Macro-Minerals

The mean plasma calcium concentrations increased slightly from day 60 to day 15 prepartum, dropped on the day of calving, and again showed rising trend till day 45-60 postpartum (Table 2).

No significant difference was found in calcium concentrations between fertile and infertile buffaloes at any of the intervals studied. There might be beneficial effect of nutritional supplementation in raising the plasma calcium level and on postpartum fertility by hastening uterine involution. The present findings on plasma calcium profile of fertile and infertile buffaloes corroborated with the earlier report of Patel and Dhama (2005) in HF cows. Khasatiya *et al.* (2005<sup>b</sup>) and Butani *et al.* (2009) reported significantly higher calcium concentration in conceived than non-conceived buffaloes with relatively higher values than the present ones.

The mean plasma inorganic phosphorus concentrations gradually declined from day 30 prepartum with approaching parturition and was significantly low on the day of calving, and then again increased gradually to prepartum levels by day 45-60 postpartum. No significant difference was observed between fertile/conceived and infertile/non-conceived buffaloes at any of the intervals studied or even in pooled values ( $4.97 \pm 0.04$  vs  $5.05 \pm 0.05$  mg/dl). The present findings corroborated with the observations of Khasatiya *et al.* (2005<sup>b</sup>) and Butani *et al.* (2009). However the present values of phosphorus were quite lower than that reported by Paul *et al.* (2000) in conceived buffaloes ( $7.69 \pm 0.23$  mg/dl) and by Patel and Dhama *et al.* (2005) in cows ( $6.67 \pm 0.50$  mg/dl). The calcium and phosphorus ratios in both the conceived and non-conceived buffaloes under study were the same (1.63:1) and in normal limit reported in the literature.

The mean plasma magnesium concentrations followed the trend of phosphorus, but the differences between periods were non-significant. Moreover, the values were consistently higher in non-conceived than conceived buffaloes. However, Patel and Dhama (2005) reported significantly higher magnesium levels in conceived than non-conceived HF cows. Moreover, the periparturient changes observed in the macro-minerals profile during transition period were in accordance with the previous reports of Piccione *et al.* (2012) and Theodore (2015) in dairy cows.

### Plasma Trace Minerals

In the present study, no significant difference in plasma zinc concentration was recorded between pregnant and non-pregnant buffaloes. The present findings closely corroborated with Khasatiya *et al.* (2005<sup>b</sup>) and Butani *et al.* (2009). Saxena and Gupta (1995), however, recorded significantly higher

**Table 2: Mean plasma minerals profile during transition period in nutritionally managed buffaloes**

Days pre- & postpartum	Plasma profile					
	Calcium (mg/dl)	Inorganic phosphorus (mg/dl)	Magnesium levels (mg/dl)	Zinc levels (ppm)	Iron levels (ppm)	Copper levels (ppm)
-60	$8.27 \pm 0.14^b$	$5.24 \pm 0.08^c$	$2.55 \pm 0.11$	$0.94 \pm 0.04^a$	$2.57 \pm 0.07^{cd}$	$1.04 \pm 0.19^{ab}$
-30	$8.47 \pm 0.15^b$	$5.22 \pm 0.09^c$	$2.50 \pm 0.09$	$1.07 \pm 0.05^{ab}$	$2.63 \pm 0.09^d$	$1.18 \pm 0.22^c$
-15	$8.28 \pm 0.12^b$	$5.05 \pm 0.09^{bc}$	$2.50 \pm 0.09$	$1.09 \pm 0.05^b$	$2.62 \pm 0.07^d$	$1.15 \pm 0.22^{bc}$
0	$7.57 \pm 0.11^a$	$4.51 \pm 0.07^a$	$2.35 \pm 0.09$	$0.98 \pm 0.04^{ab}$	$2.07 \pm 0.06^a$	$1.01 \pm 0.17^a$
15	$8.10 \pm 0.15^b$	$4.84 \pm 0.07^b$	$2.42 \pm 0.10$	$1.09 \pm 0.05^b$	$2.30 \pm 0.07^b$	$1.18 \pm 0.22^c$
30	$8.17 \pm 0.13^b$	$4.99 \pm 0.09^{bc}$	$2.44 \pm 0.10$	$1.09 \pm 0.04^b$	$2.39 \pm 0.07^{bc}$	$1.20 \pm 0.24^c$
45	$8.14 \pm 0.14^b$	$5.08 \pm 0.08^{bc}$	$2.60 \pm 0.09$	$1.07 \pm 0.06^{ab}$	$2.36 \pm 0.07^{bc}$	$1.14 \pm 0.22^{bc}$
60	$8.18 \pm 0.13^b$	$5.14 \pm 0.07^c$	$2.46 \pm 0.10$	$1.01 \pm 0.04^{ab}$	$2.31 \pm 0.09^b$	$1.18 \pm 0.21^c$
Overall	$8.15 \pm 0.05$	$5.01 \pm 0.03$	$2.48 \pm 0.03$	$1.04 \pm 0.02$	$2.41 \pm 0.03$	$1.13 \pm 0.22$

Means bearing uncommon superscripts within the column differ significantly ( $P < 0.05$ ).

plasma zinc concentration in crossbred cows that conceived within 120 days postpartum as compared to those non-conceived ones (164.85 vs 110.19 µg/dl). The mean plasma iron concentrations dropped significantly on the day of calving when compared with prepartum levels, and then again gradually increased during postpartum period till 60 days postpartum (Table 2). The plasma iron concentrations in conceived and non-conceived buffaloes were almost similar. Benedito *et al.* (1997) however reported increase in iron level during pregnancy.

The mean plasma copper concentrations gradually increased from day 60 to day 15 prepartum, dropped significantly on the day of calving, and again rose to the prepartum levels by day 15-30 postpartum and then fluctuated non-significantly. The increasing trend noted in plasma copper concentrations around pre- and postpartum days 15 and 30 in this study could be due to parental injection of micro-minerals containing copper on day 60 prepartum and on the day of calving. There was no significant difference in plasma copper concentration in pregnant and non-pregnant buffaloes. The present findings corroborated with Khasatiya *et al.* (2005<sup>b</sup>) and Butani *et al.* (2009) that copper did not differ significantly between conceived and non-conceived groups. However, Saxena and Gupta (1995) reported significantly higher plasma copper concentration in crossbred cows that conceived within 120 days postpartum as compared to non-conceived cows (128.39 vs 100.13 µg/dl), while Benedito *et al.* (1997) documented an increased copper levels during pregnancy. The periparturient profile of micro-minerals noted during transition period was also in line with the findings of Theodore (2015) in crossbred cows.

“The results of the present study showed that the postpartum fertility was not directly correlated to peripartum plasma profile of biochemical and endocrine profile, except estradiol, of optimally managed buffaloes.”

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**Conflict of Interest:** All authors declare no conflict of interest.

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