The Indian Journal of Animal Reproduction, 24(1): 16-21, June 2003

Biochemical and trace minerals profile in fertile and infertile postpartum Surti buffaloes

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Received: October 6, 2001
Accepted: April 22, 2002

ABSTRACT

Blood biochemical and trace minerals profile of 26 suckled postpartum Surti buffaloes (18 fertile, 8 infertile by day 90 pp) was studied at weekly intervals from the day of calving to at least 91 days postpartum and the findings were correlated to reproductive status. The levels of total protein and total cholesterol did not differ significantly between fertile and infertile buffaloes at postpartum stages. Only cholesterol increased significantly from the day of calving (109.86±15.46 Vs 125.76±8.14 mg%) to 4-6 weeks postpartum (199.97±13.63 Vs 207.62±20.13 mg%) in both the groups. The weekly profile of all macro minerals was higher in fertile than in infertile groups throughout the post-partum period studied with significant differences in the levels of calcium (total and ionized) and Ca:P ratio, which were significantly higher in fertile than the infertile group from 1st to 6th weeks postpartum, but not the inorganic phosphorus, and none of them differed significantly between different weeks post-partum. Plasma iron levels were highest on the day of calving in both the groups, which declined significantly by 1-2 weeks postpartum and again, rose to significant level by 11-12 weeks postpartum in fertile group. Zinc, copper and manganese levels did not show any specific trend or difference at any stage postpartum in either of the groups, and the values were higher at most intervals in fertile group as compared to infertile group, except copper, which was significantly higher in fertile than infertile group at 7-8 weeks post-partum. Moreover, none of the constituents showed significant variation between different days (0,7,14 & 21) of estrous cycle. The cholesterol had significant negative associations with total proteins, inorganic phosphorus and manganese. Total protein had positive relation with zinc and copper and negative relation with ionized calcium. Iron was positively correlated with zinc and copper had negative correlation with manganese.

Key words: Biochemical profile, macro-micro minerals, postpartum period, Surti buffalo

The blood biochemical and mineral profile during post-partum period has great relevance to future fertility in dairy animals. Cholesterol, a precursor of steroids, 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 oestrus. 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, 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. In recent years, the interest in nutritional significance of trace minerals in animal reproduction has markedly increased. Trace elements, viz. Cu, Co, Zn, Se and Mn are the integral parts of many metalloenzymes and carrier proteins and play an important role in body metabolism, protein synthesis, haemopoiesis, immuno competence 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

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relate the blood profile of these components with fertility status in postpartum buffaloes.

MATERIAL AND METHODS

This study was conducted on 26 postpartum Surti buffaloes, maintained at University livestock Research Station, Navsari over a period of six months from September 1997 to February 1998. The buffaloes were observed from the day of calving to day 91 postpartum. They were subjected to repeated rectal palpations to study the involution of uterus, regression of pregnancy CL, postpartum ovarian activity, detection of first postpartum oestrus and postpartum fertile oestrus (Devraj, 1982). All the postpartum buffaloes were checked for oestrus daily with the help of bull parading in morning and evening hours. The animals detected in oestrus were bred by natural service or by artificial insemination and were palpated per rectum for pregnancy diagnosis at 45 days post-breeding. The buffaloes conceiving within 91 days postpartum were identified as fertile group (18), while the non-conceiving buffaloes were classified as infertile group (8).

Blood samples were collected from all animals on day of calving and then at weekly interval till 91 day postpartum in heparinized vials. Those animals which exhibited oestrus signs in between the blood samples were collected on day 0 (oestrus), 7, 14 and 21 post-oestrus/post-breeding. Immediately after collection the blood samples were centrifuged for 15 minutes at 3,000 rpm and plasma samples separated out were stored at -20^{0}C with 1-2 drops of merthiolate (0.01%) till analysed. Total proteins and cholesterol were determined by using the methods of Lowry *et al.* (1951) and Schoenheimer and Sperry (1934), respectively. Calcium and inorganic phosphorus were estimated by standard procedures, and the amount of ionized calcium was estimated from the equation of Mc Lean and Hastings (1958). Plasma concentrations of trace minerals, viz. zinc, iron, copper and manganese were determined by atomic absorption spectrophotometer (Oser, 1979). The data on blood biochemical profiles and reproductive performance were analysed following standard statistical prodecures.

RESULTS AND DISCUSSION

The blood biochemical and macro-micro mineral profiles from calving to conception in fertile and infertile post-partum Surti buffaloes are shown in table 1 to 3.

Total cholesterol

The overall mean cholesterol levels of postpartum fertile (180.97±4.37 mg%) and infertile (188.25±6.30 mg%) buffaloes did not differ significantly. The cholesterol levels on the day of calving in fertile and infertile groups of buffaloes were 109.88±15.14 and 125.76±8.14 mg%, respectively, which increased significantly by 5-6 wks (199.97±13.63 mg%) and 3-4 wks (164.40±24.84 mg%) postpartum and reached to the highest concentration by 11-12 wks (239.01±16.87 mg%) and 7-8 wks (211.74±21.45 mg%) postpartum, in both the groups, respectively (Table 1). This increasing trend was in agreement with the reports of Bahga *et al.* (1980), Setia *et al.* (1992) and Jagdale *et al.* (1995) in dairy cows and buffaloes.

In our study, the cholesterol profile of fertile and infertile oestrous cycle neither differed significantly between different days post-breeding nor between groups. Although, higher total cholesterol during oestrus has been reported earlier in cows (Rao *et al.*, 1982) and buffaloes (Sarvaiya and Pathak, 1992). The total cholesterol had significant ($P<0.05$) positive correlations with the progresterone (0.16), Ca:P ratio (0.13) and ionized calcium (0.10), and negative correlations with estradiol 17B (-0.13), total protein (-0.16), inorganic phosphorus (-0.14) and manganese levels (-.013).

Total proteins

The mean total protein content was comparatively higher in fertile than in infertile group of buffaloes on the day of calving (10.02±0.27 vs 9.30±0.19 g%), and then in both the groups, it declined non-significantly during subsequent postpartum periods. The overall plasma protein levels in fertile and infertile groups were found to be 8.87±0.07 Vs 8.83±0.07 g% (Table 1). Agarwal *et al.* (1985) and Vohra *et al.* (1995) reported comparable findings for fertile/cycling animals. However, some workers reported lower protein levels for infertile/anoestrus/repeat breeder cows (Ramakrishna, 1997) and buffaloes (Sarvaiya and Pathak, 1992, El-Beley *et al.*, 1994).

The total protein levels (g%) of fertile vs infertile oestrous cycles on the day 0, 7, 14 and 21 did not reveal any significant difference between groups or between days post-breeding. These findings compared well with the reports of Devraj (1982) and Setia *et al.* (1992). The levels were neither influenced by feeding or suckling regimen nor by cyclic and acyclic nature of cows. The plasma protein levels had significant positive correlations

Table 1: Plasma biochemical profile from calving to conception in postpartum fertile and infertile Suti buffaloes (Mean ±E)

Weeks	Observations		Cholesterol (mg%)		Total proteins (g%)	
Postpartum	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Calving	6	2	109.88±15.46^{b}	125.76±8.14^{b}	10.02±0.27	9.30±0.19
1-2 weeks	17	8	135.77±8.54^{b}	150.72±23.16^{b}	9.32±0.24	9.04±0.29
3-4 week	24	10	149.01±8.98^{b}	164.40±24.84^{a}	9.08±0.23	8.90±0.36
5-6 week	26	10	199.97±13.93a	207.62±20.13a	9.31±0.23	8.89±0.32
7-8 week	24	10	197.50±12.28a	211.74±21.45a	9.33±0.23	8.87±0.23
9-10 week	22	10	199.04±13.04a	204.39±19.42a	8.92±0.23	8.83±0.31
11-12 week	16	10	239.01±16.87a	206.69±23.67a	9.02±0.33	8.78±0.23
13-14 week	14	10	232.00±14.96a	197.71±20.25a	8.78±0.32	8.89±0.25
Overall Pooled	247	118	180.97±4.37	188.25±6.30	8.87±0.07	8.83±0.07

Means with the column with different superscript differ significantly (P<0.05)

Table 2: Blood plasma concentration of macro-minerals from calving to conception in postpartum fertile and infertile Surti buffaloes (Mean ± SE)

Weeks Post	Observations		Calcium (mg%)		Inorganic phosphorus (mg%)		Ca:P ratio		Ionized calcium (mg%)	
Partum	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Calving	6	2	9.37 ±0.96	8.93 ±0.07	4.92 ±0.38	5.19 ±0.28	1.94 ±0.24	1.72 ±0.10	3.31 ±0.38	3.30 ±0.07
1-2 week	17	8	10.09* ±0.49	8.02 ±0.27	5.74 ±0.28	4.45 ±0.25	1.87 ±0.16	1.50 ±0.10	3.76* ±0.19	3.02 ±0.15
3-4 week	24	10	9.60* ±0.39	7.98 ±0.50	5.24 ±0.24	5.32 ±0.35	1.99* ±0.15	1.55 ±0.12	3.62* ±0.14	3.05 ±0.25
5-6 week	26	10	9.51* ±0.25	7.68 ±0.23	5.52 ±0.25	5.61 ±0.28	1.83 ±0.10	1.42 ±0.10	3.54* ±0.10	2.91 ±0.11
7-8 week	24	10	9.37 ±0.34	8.22 ±0.22	5.89 ±0.24	5.40 ±0.22	1.68 ±0.12	1.49 ±0.09	3.44 ±0.13	3.16 ±0.16
9-10 week	22	10	9.00 ±0.29	8.28 ±0.41	5.78 ±0.19	5.65 ±0.26	1.61 ±0.08	1.49 ±0.09	3.44 ±0.13	3.16 ±0.16
11-12 week	16	10	8.82 ±0.26	8.56 ±0.35	5.76 ±0.29	5.67 ±0.20	1.62 ±0.14	1.51 ±0.4	3.34 ±0.11	3.27 ±0.11
13-14week	14	10	9.21 ±0.41	8.66 ±0.24	5.89 ±0.31	5.54 ±0.24	1.62 ±0.10	1.59 ±0.08	3.55 ±0.16	3.30 ±0.14
Overall Pooled	247	118	9.45 ±0.10	9.08 ±0.15	5.86* ±0.07	5.62 ±0.07	1.69 ±0.03	1.64 ±0.03	3.62 ±0.04	3.48 ±0.06

* P<0.05 between groups within the row/period.

with the plasma copper (0.13), and negative correlation with the ionized calcium (-0.42).

Macro minerals

The mean levels of calcium, phosphorus, ionized calcium and Ca:P ratio in fertile and infertile groups fluctuated non-significantly from the day of calving to different weeks postpartum. However, the levels of calcium and ionized calcium in fertile buffaloes were found to be significantly higher than in infertile group during 1-2, 3-4 and 5-6 wks postpartum and Ca:P ratio during 3-4 wks postpartum. The overall inorganic phosphorus level of fertile group was also significantly higher than that of infertile group, but the calcium, ionized calcium and Ca:P ratio did not differ between groups (Table 1). The present trend of calcium and inorganic phosphorus levels from calving to early

Table 3 Plasma trace mineral concentrations from calving to conception in postpartum fertile and infertile Surti buffaloes (Mean±SE)

Weeks Post	Observations		Iron (µg/ml)		Zinc (µg/ml)		Copper (µg/ml)		Manganese (µg/ml)	
	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile	Fertile	Infertile
Calving	6	2	1.33^{ab} ± 0.23	1.62^{a} ± 0.01	1.36 ± 0.17	2.01 ± 0.09	1.34 ± 0.03	1.50 ± 0.06	0.103 ± 0.02	0.120 ± 0.006
1-2 week	17	8	1.11^{b} ± 0.11	1.23^{b} ± 0.09	1.35 ± 0.14	1.52 ± 0.12	1.34 ± 0.07	1.30 ± 0.03	0.083 ± 0.01	0.086 ± 0.009
3-4 week	24	10	1.18^{b} ± 0.06	1.33^{ab} ± 0.15	1.27 ± 0.12	1.38 ± 0.06	1.23 ± 0.03	1.27 ± 0.05	0.068 ± 0.01	0.093 ± 0.01
5-6 week	26	10	1.17^{b} ± 0.03	1.32^{ab} ± 0.08	1.60 ± 0.14	1.37 ± 0.14	1.29 ± 0.03	1.08 ± 0.05	0.068 ± 0.01	0.108 ± 0.01
7-8 week	24	10	1.10^{b} ± 0.06	1.17^{b} ± 0.09	1.23 ± 0.08	1.39 ± 0.11	1.33 0.04	1.09 ± 0.03	0.054 ± 0.01	0.076 ± 0.01
9-10 week	22	10	1.26^{ab} ± 0.06	1.42^{ab} ± 0.16	1.48 ± 0.17	1.36 ± 0.13	1.34 ± 0.08	1.13 ± 0.04	0.065 ± 0.01	0.096 ± 0.01
11-12 week	16	10	1.59^{a} ± 0.13	1.29^{ab} ± 0.11	1.18 ± 0.07	1.50 ± 0.15	1.20 ± 0.06	1.15 ± 0.03	0.057* ± 0.01	0.139 ± 0.01
13-14week	14	10	1.43^{ab} ± 0.10	1.39^{ab} ± 0.11	1.23 ± 0.06	1.70 ± 0.18	1.15 ± 0.05	1.16 ± 0.01	0.070 ± 0.01	0.112 ± 0.01
Overall Pooled	247	118	1.26 ± 0.03	1.20 ± 0.04	1.28 ± 0.03	1.33 ± 0.04	1.24 ± 0.02	1.16 ± 0.02	0.08 ± 0.004	0.09 ± 0.006

Means within the column with different superscript differ significantly ($P<0.05$).
* $P<0.05$, significant between groups within the row/period.

postpartum period is comparable to the reports of Ward *et al.* (1953), Devraj (1982) and Sarvaiya and Pathak (1992) in cows and buffaloes.

The non-significant variation observed in calcium and phosphorus levels of fertile and infertile oestrous cycles indicated between groups and between different days post-breeding in Surti buffaloes also agreed with the report of Shrivastava *et al.* (1981) in Murrahs. Moreover in the present study, higher Ca:P ratios observed in fertile group during 3-4 wks and 5-6 and 7-8 wks postpartum were related with early ovarian activity and higher conception rate than in infertile buffaloes. The plasma Ca:P ratio had significant positive correlation with the calcium level (0.64) and negative correlation (-0.72) with the inorganic phosphorus. In the fertile group of buffaloes, the phosphorus had significant positive correlations with the initiation of ovarian activity (0.51) and service period (0.47), and negative correlations with the Ca:P ratio (-0.88) and estradiol 17B levels (-0.57), but not in infertile group. On the contrary, Marinov (1978) recorded positive correlation between Ca:P ratio and fertility, but did not observe any correlation of the inorganic phosphorus and calcium with the number of services required per conception and/or service period. While El-Belely *et al.* (1994) found negative correlation of inorganic phosphorus with the no. of services/conception.

Trace minerals

The mean plasma iron levels of both fertile and infertile groups was highest on the day of calving (1.33±0.23 and 1.62±0.01 mg/ml), which decreased significantly by 1-2 wks postpartum (1.11± 0.11 and 1.23±0.09 mg/ml) and maintained at that level up to 7-8 wks postpartum. Then it showed rising trend from 9-10 from 9-10 wks and reached to a peak value of 1.59±0.13 mg/ml by 11-12 wks postpartum only in fertile group (Table 3). The mean plasma zinc, copper and manganese levels also did not exhibit any significant variation during different weeks postpartum. Although the fertile group had significantly higher copper level overall (1.24± 0.02 mg/ml) and on 7-8 wks postpartum (1.33±0.04 mg/ml) than the infertile group (1.16±0.02 and 1.09 ±0.03 mg/ml, respectively), while manganese showed inversed trend with significant differences at weeks 11-12 (0.057 ±0.01 vs 0.139± 0.01 mg/ml). At all other wks postpartum, the differences were not significant between them (Table 3). These findings with respect to trend are in agreement with the report work in cyclic buffaloes (Khattab *et al.* 1995) and in dioestus, oestrus and pregnant cows (Reddy and Reddy, 1988, Singh *et al.*, 1991). Ramakrishna (1997) reported higher iron levels in normal cyclic cows as compared to non-cyclic or irregular breeders. Manickam *et al.* (1977) documented higher values of plasma zinc in regular breeders than repeat breeders. Saxena and Gupta (1995) also found higher copper levels in postparturient buffaloes and cows conceiving than non-conceiving within 120 days postpartum.

Ramakrishna (1997) also reported similar findings in bovines. The plasma zinc level of postpartum period had significant positive correlation with plasma iron (0.18) and total protein level (0.08), and negative correlation with ionized calcium (-0.52). Plasma copper had significant positive correlation with estradiol- 17 B (0.19) and total protein levels (0.13), and negative correlation with the manganese level (-0.12) and first heat postpartum (-0.34). This emphasized possible role of higher plasma copper level in reducing the interval for first heat postpartum. Copper levels in circulation appears to be influenced by hormones of reproduction and it's concentration in blood serves as an indicator of gonadal hormones and pituitary gonadotropins. The association of copper with resumption of ovarian activity and fertility has also been shown by Manickam *et al.* (1977). The manganese had significant negative correlations with the periods of uterine (-0.40) and cervical involution (-0.32).

The low level of iron could possibly result in improper tissue oxygenation to the uterus resulting in impaired nutrition in the uterus for the conceptus causing death of embryo. A deficiency of iron might also interfere with enzymatic reaction on the release of luteinizing hormone, which is highly essential for the maintenance of pregnancy in cows (Reddy and Reddy, 1988). Impairment of reproduction such as delayed/depressed oestrus and poor conception in cows and buffaloes has been reported due to deficiency of manganese in diet (Hidiroglou, 1979). The basal levels of manganese observed in this study might be suggestive of it's bio-utilization as an activator of various enzymes and it involvement in the lipid and carbohydrate metabolism.

It may concluded that the early rising trend of cholesterol with relatively higher values on different weeks postpartum noticed in fertile in comparison to infertile group was associated with earlier conception. Protein levels did not reveal such trend. Higher calcium and phosphorus levels, and Ca:P ratio (1.5 to 2.0) are required for efficient reproduction and any alteration in this is unfavourable to fertility. Plasma iron and copper levels were also higher during postpartum period in buffaloes having better fertility/reproductive efficiency.

ACKNOWLEDGEMENTS

We wish to thank Dr. P.M. Desai, Research Scientist and head of Livestock Research Station, Gujarat Agricultural University, Navsari, Dr. V.P. Vadodaria, Professor & head of Physiology Department and Dr. M.C. Desai, Principal, Veterinary College, Anand for their cooperation and permission granted to utilize the facilities under their control for this work.

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