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Research Article

# Studies on postpartum biochemical and hormonal profile of fertile and infertile oestrous cycles in Surti buffaloes

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## ABSTRACT

A comparative study on plasma profile of biochemical, metabolic and hormonal constituents and macro-micro minerals was studied on different days of fertile and infertile cycles (20 each) in 40 postpartum Surti buffaloes of an organised farm. The animals detected in oestrus after day 50 of normal calving were bred by natural service and heparinized jugular blood samples were collected on the day of oestrus/breeding (day 0) and subsequently on 7th, 14th and 21st day for estimation of plasma profiles of various constituents. Pregnancy was confirmed per rectum 45 days post-service in non-return cases. The plasma profile revealed that the progesterone level was significantly higher ($P < 0.05$) in fertile than the infertile cycle on day 14 (2.14±0.21 vs 1.20±0.19 ng/ml) and day 21 (2.57±0.21 vs 0.42±0.04 ng/ml), but not at oestrus (0.37±0.02 vs 0.39±0.05 ng/ml) or day 7 (1.29±0.08 vs 1.12±0.11 ng/ml) post-breeding. Moreover, the infertile buffaloes had a combined pattern of delayed rise and low level of $P_4$ indicative of insufficient luteal function. The glucose levels were apparently higher and riglycerides lower ($P > 0.05$) in fertile than the infertile cycle at most intervals, while total protein content was significantly higher ($P < 0.05$) on day 7 and 21, and AKP on all days post-service in fertile than the infertile cycles, but did not vary between days/stages within the cycle. Further, the average plasma profile of thyroid hormones ($T_3$, $T_4$), total cholesterol, calcium, phosphorus as well as micro-minerals, viz., Zn, Fe, Cu, Co and Mn neither varied significantly between different days nor between groups/cycles at any of the intervals post-breeding, probably due to uniform balanced ration/nutrition and supplementation of mineral mixtures in daily ration of these animals under organized farm management.

**Key words** : Hormone profile, metabolic profile, mineral profile, oestrous cycle, Surti buffalo

One of the major factors of economic importance in buffalo reproduction is the postpartum fertility. Any deviation or prolongation in the breeding rhythm results into a progressive economic loss through widening of days open, dry period and calving interval, and reduced calf crops and lactations. Nearly 2/3rd of buffalo population usually return to oestrus after breeding for one or the other reasons (Panchal *et al.*, 1992; Shah, 1999). Efforts should therefore, be made to enhance fertility in such animals by narrowing down their service period ideally to 60-90 days and calving interval of 12-13 months through clinical and laboratory diagnosis of fertile oestrus. Earlier workers have shown direct relationship of certain hormonal and blood biochemical parameters with this phenomenon in bovine (Erb *et al.*, 1976; Dhoble and Gupta, 1981; Buyanov *et al.*, 1983; Shelton *et al.*, 1990; Panchal *et al.*, 1992; Shah, 1999). Similarly, deficiencies or excess of certain macro-micro minerals in blood adversely affect the normal reproductive rhythm (Hidiroglou, 1979; Kumar and Sharma, 1991; Shah, 1999). However, most of these studies were based on limited number of parameters. Hence, this study was aimed to evaluate the possibility of predicting postpartum fertile and infertile oestrus through exhaustive analysis of blood profile of various biochemical, metabolic, hormonal and mineral constituents in buffaloes.

## MATERIALS AND METHODS

Postpartum Surti buffaloes (n = 59) of Livestock Research Station, GAU, Navsari (Gujarat) maintained under identical nutritional and managerial practices and fed green fodder, hay, compounded concentrate (Sumul Dan) and mineral mixture as per the standard feeding schedule were incorporated in the study. The buffaloes had calved normally and had subsequent normal genital health as assessed gynaeco-clinically. They were also monitored through

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periodical rectal palpation and weekly blood sampling from the day of calving till 15 weeks postpartum.

Oestrus occurrence was detected daily from day 50 postpartum onwards with the help of teaser bull parading in morning and evening hours. The animals detected in oestrus were bred by natural service and were palpated per rectum for pregnancy 45-60 days post-service. During the period of 15 weeks postpartum, with weekly jugular blood sampling, some animals came in heat naturally and conceived with first service only and some returned to oestrus for 2 or 3 times before settling. From these, the data of 20 animals that conceived and another 20 that did not conceive at particular oestrus coinciding with weekly blood sampling were used for comparing their blood profile on day 0 (oestrus), 7, 14, 21 post-breeding as fertile and infertile cycles.

The heparinized blood samples collected from jugular vein on specified days were centrifuged @ 2500 rpm for 20 min and the plasma samples were stored at -20°C till analyzed. The estimations of various metabolic (glucose, total protein, cholesterol, triglycerides) as well as macro-minerals (calcium, phosphorus, magnesium) profile and enzyme alkaline phosphatase (AKP) activity were done in plasma by using standard assay its and autoanalyzer (Photometer, BT 224, Biotechnica Instrumenta, Rome, Italy). Plasma profile of hormone progesterone ($P_4$), thyroxine ($T_4$) and tri-iodothyronine ($T_3$) was determined through standard RIA techniques of Kubasic *et al.* (1984) and BARC, Mumbai, respectively. The estimation of trace elements, viz., zinc, iron, copper, cobalt and manganese was done using wet oxidized plasma samples on an atomic absorption spectrophotometer (Oser, 1979).

The data were analyzed statistically using completely randomized design, Duncan's NMRT and Student's test to know the differences, if any, between days and between groups in various plasma constituents studied (Steel and Torrie, 1981).

**Tables 1. Postpartum plasma profile (Mean±SE) of various biochemical, enzymatic and hormonal constituents of fertile (F) and infertile (IF) oestrous cycle in Surti buffaloes**

| Attributes | Groups | Days post-breeding | | | |
|---|---|---|---|---|---|
| | | 0 (N = 20) | 7 (N = 20) | 14 (N = 20) | 21 (N=20) |
| Glucose (mg%) | F | 53.46±3.99 | 59.43±3.82 | 60.03±2.58 | 58.80±3.34 |
| | IF | 54.80±3.69 | 50.73±2.91 | 55.12±5.08 | 52.43±4.84 |
| Total protein (%) | F | 8.95±0.38 | 9.00±0.36** | 9.21±0.47 | 8.56±0.35* |
| | IF | 8.49±0.33 | 7.87±0.31 | 8.24±0.33 | 7.52±0.28 |
| Cholesterol (mg%) | F | 115.44±6.55 | 122.74±6.50 | 119.38±6.32 | 122.56±7.11 |
| | IF | 118.18±7.12 | 124.59±7.85 | 128.24±9.42 | 118.44±6.29 |
| Triglycerides (mg%) | F | 33.05±5.35 | 32.86±6.36 | 33.06±5.39 | 39.11±5.89 |
| | IF | 38.67±7.39 | 41.45±6.89 | 39.13±7.43 | 49.78±8.44 |
| AKP (IU/L) | F | 192.53±31.63* | 182.95±29.45* | 192.69±29.35* | 188.81±28.23* |
| | IF | 115.55±8.53 | 127.50±10.63 | 127.05±12.04 | 116.30±9.21 |
| $P_4$ (ng/ml) | F | 0.37±0.02[b] | 1.29±0.08[ab] | 2.14±0.21[a]*** | 2.57±0.21[a]*** |
| | IF | 0.39±0.05[b] | 1.12±0.11[a] | 1.20±0.19[a] | 0.42±0.04[b] |
| $T_4$ (ng/ml) | F | 20.01±1.12 | 19.70±1.77 | 19.03±1.16 | 18.75±0.82 |
| | IF | 18.28±1.46 | 15.60±1.16 | 16.71±1.33 | 17.01±1.19 |
| $T_3$ (ng/ml) | F | 0.69±0.07 | 0.84±0.06 | 0.91±0.12 | 0.81±0.07 |
| | IF | 0.81±0.08 | 0.84±0.08 | 0.84±0.07 | 0.81±0.07 |

N = Number of animals or observations; '0' = oestrus day
*P < 0.05; ** P < 0.01 between groups within the column.
Only the means of $P_4$ bearing different superscripts within the row differ significantly (P < 0.05) between days, but not of the other traits.

## RESULTS AND DISCUSSION

The levels of various blood biochemical, enzymatic, metabolic, hormonal and macro-micro mineral constituents obtained on day 0, 7, 14 and 21 post-breeding among fertile and infertile cycles of postpartum Surti buffaloes are presented in Tables 1 and 2.

(a) **Biochemical/metabolic and enzymatic profile** : Among the biochemical, metabolic and enzyme profile, the levels of plasma glucose were apparently higher and triglycerides lower at most intervals post-breeding in fertile than the infertile cycles, but did not differ significantly between groups or between days of the cycle. The total protein content was significantly higher ($P < 0.05$) on day 7 and 21 and AKP on all days post-service in fertile than the infertile cycles, though it did not differ significantly between days within the cycle. However, the cholesterol concentration did not reveal any specific trend between days or between groups (Table 1).

The present findings on blood glucose levels (52.43±4.84 to 60.03±2.58 mg%) at different days of cycle compared well with the repòrt of Dhoble and Gupta (1979), who recorded the mean values of 60.70, 59.30 and 55.63 mg/dl for oestrus, metoestrus and dioestrus phase of cycle in buffaloes. However, no literature was available to substantiate the present findings on triglyceride levels (32.86±6.36 to 41.45±6.86 mg%) during fertile and infertile cycles in postpartum buffaloes, and this tudy is perphaps the first of its kind in postpartum Surti buffaloes. However, Mesaric *et al.* (1997) found significantly higher serum triglycerides in pregnant-dry cows compared to early or mid-lactation phase.

The levels and trend of total protein (7.52±0.28 to 9.21±0.47 g%) observed on different days of fertile and infertile cycles in the present study closed coincided with the findings of Setia *et al.* (1992) and Shah (1999). However, Dhoble and Gupta (1981) observed relatively lower values of total protein at different phases of cycle (5.80±0.44 to

**Tables 2. Postpartum plasma profile (Mean±SE) of macro-micro constituents of fertile (F) and infertile (IF) oestrous cycle in Surti buffaloes**

| Attributes | Groups | Days post-breeding 0 (N = 20) | 7 (N = 20) | 14 (N = 20) | 21 (N=20) |
|---|---|---|---|---|---|
| Calcium (mg%) | F | 10.31±0.45 | 9.95±0.36 | 9.99±0.42 | 9.89±0.45 |
| | IF | 10.59±0.42 | 11.21±0.55 | 10.78±0.38 | 10.83±0.52 |
| Phosphorus (mg%) | F | 5.67±0.38 | 5.51±0.36 | 5.15±0.38 | 4.78±0.41 |
| | IF | 5.35±0.34 | 5.10±0.48 | 5.61±0.43 | 4.84±0.38 |
| Magnesium (mEq/L) | F | 4.03±0.21 | 4.11±0.25 | 4.44±0.25 | 4.11±0.20 |
| | IF | 4.05±0.21 | 3.85±0.21 | 4.04±0.20 | 3.88±0.20 |
| Zinc (ppm) | F | 1.47±0.10 | 1.64±0.13 | 1.58±0.13 | 1.44±0.13 |
| | IF | 1.92±0.23 | 1.96±0.24 | 1.88±0.24 | 1.71±0.20 |
| Iron (ppm) | F | 3.55±0.30 | 3.04±0.33 | 3.69±0.32 | 4.18±0.31 |
| | IF | 3.62±0.29 | 3.55±0.33 | 3.28±0.34 | 3.56±0.33 |
| Copper (ppm) | F | 1.45±0.11 | 1.48±0.10 | 1.55±0.09 | 1.42±0.09 |
| | IF | 1.62±0.07 | 1.56±0.08 | 1.55±0.08 | 1.55±0.10 |
| Cobalt (ppm) | F | 0.67±0.06 | 0.61±0.06 | 0.62±0.07 | 0.74±0.06 |
| | IF | 0.62±0.05 | 0.59±0.05 | 0.60±0.05 | 0.58±0.06 |
| Manganese (ppm) | F | 0.09±0.01 | 0.10±0.01 | 0.10±0.01 | 0.10±0.01 |
| | IF | 0.12±0.02 | 0.13±0.02 | 0.14±0.02 | 0.11±0.01 |

N = Number of animals or observations; `0' = oestrus day
None or the traits differed significantly between groups or between days within the group.

6.86±0.37 g%) and Pathak (1983) found non-significant rise in the protein concentration within one day of fertile heat (8.93±0.24 vs 7.87±0.37 g%) in buffaloes. Significantly higher ($P < 0.05$) levels of total protein in the luteal phase of fertile cycles as compared to infertile cycles, suggest a positive role of plasma protein levels in reproduction.

The present findings of almost identical plasma total cholesterol levels (115.44±6.55 to 128.24±9.42 mg%) observed at different days post-breeding in both fertile and infertile cycles closely agreed to the reports of Zala *et al.* (1972) and Pathak (1983) who found a transient increase in serum cholesterol on day 11 and 13 of cycle, as was seen on day 7 and 14 of infertile cycle in our study. This transient rise around mid-cycle may be due to initiation of follicular activity for next oestrus in infertile cycles, since cholesterol is a precursor for synthesis of sex hormones from the gonads. Devnathan *et al.* (1984) and Sarvaiya and Pathak (1991), however, recorded significantly higher cholesterol level during oestrus than the dioestrus phase in buffaloes. Further, the levels of cholesterol found on different days of cycle in present study were much lower than those 179 to 256 mg%) reported by Shah (1999) in Surti buffaloes.

The present findings of significantly higher ($P < 0.05$) AKP activity found at all stages of fertile cycles (*182.95±29.45 to 192.69±29.65 IU/L*) as compared to infertile ones (115.55±8.53 to 127.50±10.63 IU/L) agreed well with the report of Noble *et al.* (1977), who reported higher AKP activity at fertile heat in comparison to infertile heat. Vadodaria (1976), however, observed lower levels of serum AKP activity at oestrus than at peak luteal phase in Surti buffalo-heifers. Further, the trend of non-significant differences seen in AKP activity between different days/phases of both fertile as well as infertile cycles also compared well with the report of Roussel and Stallcup (1967) in dairy cows.

b) **Hormonal profile** : In present study, the progesterone levels were significantly higher ($P < 0.05$) in fertile than the infertile cycles on day 14 (2.14±0.21 vs 1.20±0.19 ng/ml) and day 21 (2.57±0.21 vs 0.42±0.04 ng/ml), but not at oestrus (0.37±0.02 vs 0.39±0.05 ng/ml) or day 7 (1.29±0.08 vs 1.12±0.11 ng/ml) post-breeding (Table 1). Further, the mean progesterone level in fertile cycle rose significantly by day 14 of the cycle as compared to that on day of oestrus and then remained almost at the same level till at least day 21 post-breeding. This was associated with presence of active CL on the ovary due to establishment of pregnancy and failure of return to oestrus. Whereas in infertile cycle the mean progesterone levels recorded on days 0 and 21 were at par and significantly lower/basal ($P < 0.05$) than those found on day 7 and 14 post-breeding. This trend suggested either anovulatory oestrus, failure of normal CL formation/luteal deficiency and conception failure or early embryonic mortality resulting into lowered $P_4$ profile and return to oestrus by day 21 post-breeding in infertile cycles as stated by Buyanov *et al.* (1983).

Our findings on plasma progesterone profile compared well with the reports of Shah (1999) in Surti buffaloes and of Erb *et al.* (1976) and Maurer and Echtemkamp (1985) in cows, wherein they found subnormal $P_4$ concentration from day 6 through 14 post-breeding in infertile cycles and in repeat breeder animals than those of fertiloe/conceived ones. Shelton *et al.* (1990) observed delayed and slow increase of post-ovulatory peripheral progesterone concentration in subfertile than in the normal fertile heifers indicating the possibility of luteal inadequacy. Lukaszewska and Hansel (1980), based on significantly higher plasma progesterone concentrations in conceiving than non-conceiving animals between days 10 and 18 of cycle, opined that embryo might be producing one or more luteotrophic substances that stimulate increased progesterone secretion by the CL beginning as early as day 10 of earlier (5.5 to 6 days) than in cattle, therefore, earlier maternal recognition and higher progesterone was observed on day 7 in conceived than non-conceived buffaloes. Panchal *et al.* (1992) and Shah (1999), however, found much higher concentration of progesterone till day 21 post-oestrus/post-breeding even in infertile cycles, almost at par with fertile ones, suggesting delayed embryonic mortality or longer luteal phase. Fertility of buffaloes under study was closely related with the progesterone profile post-service indicating that the overall reproductive efficiency in buffaloes is impaired due to deficiency of progesterone, which is essential for embryo survival.

The plasma $T_3$ (0.69±0.07 to 0.91±0.12 ng/ml) and $T_4$ (15.60±1.16 to 20.01±1.12 ng/ml) profile of fertile and infertile ccles neither varied significantly between different days post-breeding nor between cycles/groups at any of the intervals (Table 1). These findings agreed with the reports of Jindal *et al.* (1988) and Sharma *et al.* (1999), but the levels of $T_4$ in the present study were almost one-half than those reported by the later authors in cyclic buffalo-heifers during different phases of cycle. However, Sharawy *et al.* (1987) reported significantly higher levels of $T_3$ and $T_4$ during follicular phase than the luteal phase in normal cycle buffaloes. Sarvaiya *et al.* (1992) also observed little higher concentrations of both $T_3$ and $T_4$ at oestrus than at other phases of cycle suggesting that they do have some role in reproduction.

c) **Macro-minerals profile** : The average plasma profile of calcium (9.89±0.45 to 11.21±0.55 mg%), inorganic phosphorus (4.78±0.41 to 5.67±0.38 mg%) and magnesium (3.85±0.21 to 4.44±0.25 mEa/L) evaluated in fertile and infertile cycles neither differed significantly between different days post-breeding nor between cycles at any of the intervals (Table 2). Many workers have shown comparable results/values of calcium and/or phosphorus during different phases of oestrous cycle in cows and buffaloes (Khattab *et al.*, 1995; Shah, 1999). Sharma *et al.* (1999) also did not find significant variation in calcium, phosphorus and magnesium levels between different phases of normal cycle in buffaloes. Quayum (1977), however, found higher levels of both calcium and phosphorus at oestrus (12.49 and 5.78 mg%) than the dioestrus phase (10.35 and 2.40 mg%). Kumar and Sharma (1991) estimated serum phosphorus level to be higher at fertile than non-fertile oestrus in cows (6.44±0.42 and 4.98±0.08 mg/dl). Lower inorganic phosphorus level has been attributed as a cause of cyclic aberrations and infertility in bovines, but this was not evident in our study.

d) **Micro-minerals profile** : Like macro-minerals, the levels of micro-minerals viz., Zn, Fe, Cu, Co and Mn of fertile and infertile oestrous cycles also revealed non-significant variation between days post-service and even between groups/cycles. Although, the mean plasma zinc (1.44±0.13 to 1.96±0.24 ppm), copper (1.42±0.09 to 1.62±0.07 ppm) and maganese (0.09±0.01 to 0.14±0.01 ppm) levels were apparently higher at all intervals of infertile cycle than the fertile one (Table 2), whereas the values of iron (3.28±0.34 to 4.18±0.31 ppm) showed increasing trend in fertile cycle and was probably associated with establishment of pregnancy. The insignificant fluctuations observed in trace minerals profile between groups or between days post-breeding may be attributed to uniform balanced feeding and supplementation of mineral mixtures followed in daily ration of these animals on the farm.

Shah (1999) in a similar study in Surti buffaloes also observed comparable values and trend in fertile and infertile cycles for all trace elements, except iron, which was significantly higher in fertile cycle. Present findings further corroborated well with the reports of Singh *et al.* (1991) and Sharma *et al.* (1999) who did not observe significant difference in the concentrations of Fe, Cu and Zn between different phases of cycle in bovines. Reproductive failure may be induced by deficiencies of single or combined trace elements and by imbalances and concomitant infertility in cattle is believed to be associated with enzyme dysfunctions resulting from these deficiencies (Hidiroglou, 1979). In the present study, the levels of all trace elements were, however, well above (almost double) the critical limits suggested by Mc Dowell (1992), hence probably could not give any clue to distinguish fertile and infertile cycles.

It can be inferred that among various blood plasma constituents studied during different days/phases of oestrous cycle, only the levels of total protein, alkaline phosphatase and progesterone were significantly higher in fertile cycle and may be used as indicators/predictors, whether the cycle would be fertile or infertile post-breeding. The non-significant difference observed in the levels of most constituents between groups or between days post-breeding could be due to uniform balanced feeding and mineral supplementation followed on the farm.

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## REFERENCES

Buyanov, A.A., Lyutinskii, S.I., Stepanov, G.S. and Kogan, M.E. (1983). Blood progesterone after insemination in normal cows and cows with abnormal development of the corpus luteum. Anim. Breed. Abstr., **51**: 3552.

Devanathan, B., Neduncheralathan, D., Kathiresan, D. and Qyayanm, S.A. (1984). Serum cholesterol level in cycling and anoestrus Murrah buffaloes. Cheiron, **13**: 43-44.

Dhoble, R.L. and Gupta, S.K. (1979). A note on blood glucose level in relation to postpartum anoestrus in buffaloes. Indian J. Anim. Sci., **49**: 953-954.

Dhoble, R.L. and Gupta, S.K. (1981). Total plasma protein and haemoglobin status during oestrous cycle and anoestrus in postpartum buffaloes. Indian Vet. J., **58**: 544-547.

Erb, R.E., Garverick, H.A., Randel, R.D., Brown, B.L. and Challahan, C.J. (1976). Profiles of reproductive hormones associated with fertile and non-fertile inseminations of dairy cows. Theriogenology, **5** 227-242.

Hidiroglou, M. (1979). Trace element deficiencies and fertility in ruminants. A review. J. Dairy Sci., **62**: 1195-1206.

Jindal, S.K., Gill, S.P.S. and Rattan, P.J.S. (1988). Some hormonal profiles during oestrus cycle in buffaloes. 4th Annual Conference of SAPI, Makhdoom (UP), Sept. 24-26.

Khattab, R., Eltohamy, M.M., Mourad, K.A. and Youssef, R.H. (1995). Post partum ovarian cyclicity in relation to blood micro- and macro-elements in Egyptian buffaloes. Buffalo J., **11**: 61-70.

Kumar, S. and Sharma, M.C. (1991). Level of haemoglobin and certain serum biochemical constituents in rural cows during fertile and non-fertile oestruses. Indian Vet. J., **68**: 361-364.

Lukaszewska, J. and Hansel, W. (1980). Corpus luteum maintenance during early pregnancy in the cow. J. Reprod. Fert., **59**: 485-493.

Maurer, R.R. and Echtemkamp, S.E. (1985). Repeat breeder females in beef cattle : Influences and causes. J. Anim. Sci., **61**: 625-635.

McDowell, L.R. (1992). *Minerals in Animal and Human Nutrition*. Academic Press, Inc., London, UK.

Mesaric, M., Nemeck, M. and Zadnik, T. (1997). The variation of cholesterol and triglycerides in blood serum of dairy cows with regards to physiological time and feeding seasons. Zbornik veterinarske Fakultete Univerza Ljubljana, **34**: 59-65.

Noble, D., Rao, M.V.N. and Bhosrekar, M.r. (1977). Some biochemical constituents in blood and cervical mucus of buffaloes during different stages of oestrus. Indian J. Dairy Sci., **30**: 252-255.

Oser, B.L. (1979). *Hawk's Physiological Chemistry*. 14th edn., Mc Grew Hill Books Co., Bombay.

Panchal, M.T., Dhami, A.J., Mehta, V.M. and Kodagali, S.B. (1992). Plasma progesterone profile of fertile and infertile oestrus cycles in repeat breeding buffaloes. Indian Vet. J., **69**: 766-767.

Pathak, M.M. (1983). Blood Serum Profile in Pregnant Buffalo (Surti breed) during Different Stage of Pregnancy. Ph.D. thesis submitted to GAU, Anand, India.

Quayam, S.A. (1977). Level of calcium and phosphorus in blood and serum of the buffaloes. Cheiron, **8**: 164-167.

Roussel, J.D. and Stallcup, O.T. (1967). Blood serum enzymes within the oestrous cycle. J. Dairy Sci., **50**: 1341-1342.

Sarvaiya, N.P. and Pathak, M.M. (1991). Blood serum cholesterol in pubertal cycling and non-cycling Surti buffaloes. Indian J. Anim. Reprod., **12**: 167-169.

Sarvaiya, N.P., Pathak,M.M. and Patel, A.V. (1992). Study on circulating thyroid hormones in pubertal Surti buffaloe heifers (cycling and noncycling). Int. J. Anim. Sci., **7**: 211-213.

Setia, M.S., Duggal, R.S. and Singh, R. (1992). Biochemical constituents of blood in buffaloes and cows during late pregnancy and different stages of lactation - A longitudinal study. Buffalo J., **8**: 123-129.

Shah, R.G. (1999). Hormonal and Biochemical profile in fertile and infertile postpartum Surti buffaloes. Ph.D. thesis, Gujarat Agricultural University, Anand, India.

Sharawy, S.M., El-Azab, M.A., Labib, F.M. and Heshmat, H.A. (1987). Serum level of $T_3$ and $T_4$ in normal cycling buffaloes and buffaloes with inactive ovaries before and after PMSG treatment. J. Egyptian Vet. Med. Assoc., **47**: 299-306.

Sharma, K.b., Nayar Shashi, Malik, V.S., Singh, Rajvir and Sodhi, S.P.S. (1999). Levels of hormones and minerals in cyclic, anoestrus and suboestrus buffalo heifers. Indian J. Anim. Sci., **69**: 214-216.

Shelton, K., Gayeric De Abreu, M.F., Hunter, M.G., Parkinson, T.J. and Lamming, G.E. (1999). Luteal inadequacy during the early luteal phase of subfertile cows. J. Reprod. Fert., **90**: 1-10.

Singh, R., Singh, S.P.S., Singh, R. and Setia, M.S. (1991). Distribution of trace elements in blood, plasma and erythrocytes during different stages of gestation in buffalo (*Bubalus bubalis*). Buffalo J., **7**: 77-85.

Steel, R.G.D. and Torrie, J.H. (1981). *Principles and Procedures of Statistics, A Biometric Approach*. 2nd edn., McGraw Hill, Int. Book Agency, Singapore.

Yadodaria, V.P. (1976). Thyroid Activity in Relation to Reproductive Performance of Surti Buffalo (*Bubalus bubalis*). M.V.Sc. thesis, Gujarat Agril. Univ., Anand.

Zala, P.M., Janakiraman, K. and Memon, G.N. (1972). Ascorbic acid and cholesterol of blood and adrenals during oestrous cycle in Surti buffalo-heifers. Indian J. Exptl. Biol., **10**: 312-314.