The Indian Journal of Animal Reproduction; 27 (2): 1-8; Dec. 2006

Research Article

Reproductive performance and plasma profile of alkaline phosphatase, progesterone and thyroid hormones in postpartum fertile and infertile Surti buffaloes*

C.T. KHASATIYA[1], A.J. DHAMI[2], N.P. SARVAIYA[3], V.B. KHARADI[4] AND F.S. KAVANI[5]

Deptt. of Animal Reproduction, Gynaecology & Obstetrics, Gujarat College of Veterinary Science and Animal Husbandry, GAU, Anand Campus, Anand - 388 001

Received : February 28, 2004
Accepted : November 12, 2006

ABSTRACT

The reproductive performance, AKP and plasma endocrine profile was studied in fertile (n=12) and infertile (n=11) Surti buffaloes at weekly interval from the day of calving till 15 weeks postpartum. The period for initiation of postpartum ovarian follicular activity, first oestrus postpartum, days open and calving interval were significantly shorter ($P < 0.01$) in fertile group of buffaloes than the infertile ones. The plasma AKP activity was consistently higher in fertile than the infertile buffaloes, and it showed a gradual increasing trend from calving till 15th week postpartum, but without significant weekly variation in both the groups. The mean P_4 levels varied significantly between weeks postpartum only in fertile group. The values of 0.81 ± 0.18 ng/ml on the day of calving dropped to base line and remained low until 8 weeks postpartum and then showed increasing trend as the weeks proceed to reach the highest value ($P < 0.01$) of 2.77 ± 0.27 ng/ml at 15th week post-partum, while in the infertile group the levels fluctuated non-significantly and the values were higher at week 6th, 9th and 13th with fall in-between, suggesting anoestrus/suboestrus condition, repeat cycles or early embryonic death in some animals. The levels of both T_3 and T_4 were highest on the day of calving, dropped by 3-4 weeks postpartum and remained low thereafter. Further, the levels of T_4 were apparently higher in infertile than the fertile group at all intervals with significant difference in the overall means. The P_4 levels had significant correlations with T_3 (0.75) and with resumption of follicular activity, first heat postpartum, days open and calving interval (-0.58, -0.72, -0.87, -0.85) in fertile group. The levels of T_3 showed significant negative correlations with FOPP, days open and calving interval (-0.55, -0.85, -0.81) only in fertile group, while T_4 had negative correlation with services per conception (-0.74) in fertile group. AKP had no significant correlation with any of the reproductive or hormonal traits.

Key words: AKP, fertile-infertile buffaloes, progesterone, postpartum period, thyroid hormone

The river buffaloes in India have established themselves superior to crossbred cows in many respects. However, their low reproductive rate and problems of silent / subestrus, anestrus, seasonality in breeding and extended calving interval are the major constraints of economic importance. Maintenance of regular calving interval of desirable length depends upon the ability of the buffalo to initiate ovarian activity and conceive within the shortest possible time after calving. Although majority of cows and buffaloes resume ovarian cycle within first month postpartum (King *et al.*, 1976; Shah, 1999), some may have very long postpartum period of ovarian acyclicity. Such cyclic aberrations can be very well characterized by serum profile of certain hormones and metabolites (Larson *et al.*, 1980; Barkawi *et al.*, 1986; Shah, 1999). Moreover, in recent years considerable interest has grew towards reproductive endocrinology as a means to identify specific reproductive problems and to adopt appropriate therapeutic measures for augmenting bovine fertility (Darwash and Lamming, 1997). Alkaline phosphatase influences steroid hormone production and thereby number of events of reproductive

* A part of Ph.D. Thesis of first author approved by GAU, Sardarkrushinagar – 385 506, India.

[1] Asstt. Res. Sci., Cattle Breeding Farm, JAU, Junagadh, Gujarat
[2] Res. Sci. & Head, Livestock Research Station, AAU, Anand
[3] Asstt. Res. Sci., Reproductive Biology Research Unit, AAU, Anand
[4] Assc. Res. Sci. Livestock Research Station, NAU, Navsari, Gujarat
[5] Prof. & Head, Dept. of ARGO, AAU, Anand

physiology (Vadodaria *et al.*, 1978). Thyroid hormones play an important role in regulating metabolic rate and reproduction through their action at cellular level and by influencing the pituitary-adrenal relation (Nalbandov, 1970). However, the literature available on weekly monitoring of hormonal and enzyme profile of postpartum fertile and infertile buffaloes is meager. Hence, the objective of this study was to investigate if the weekly profile of AKP, P_4 or thyroid hormone can be of any predictive value of postpartum fertility status of buffaloes.

MATERIALS AND METHODS

This study was carried out at Livestock Research Station of the University at Navsari, from August 2001 to January 2003, on 23 normally calved postpartum Surti buffaloes from day of calving till 105 days postpartum. The animals were fed green fodder, hay, compounded concentrate and mineral mixtures as per the routine of the farm through out the experimental period. They were subjected to weekly rectal palpation to evaluate the uterine involution and postpartum ovarian activity. Oestrus was detected daily from day 40 postpartum onwards with the help of a teaser bull. The animals were bred by natural service and were palpated per rectum for pregnancy 45-60 days later. The buffaloes that conceived normally within 105 days postpartum were considered as normal fertile (n=12) and those that did not conceive as infertile (n=11). The infertile anoestrus and suboestrus buffaloes were treated with i/m injection of GnRH (5 ml Receptal) or PGF_2 alpha (5 ml Lutalyse) at 15th week postpartum and were followed clinically till all of them settled. Heparinzed jugular blood samples were collected from all animals at weekly interval from the day of calving to at least 15th week postpartum and plasma samples were used for estimation of AKP and hormone P_4, T_3 and T_4 profile.

The plasma AKP activity was determined using standard assay kits and autoanalyser. Plasma progesterone was estimated by RIA technique of Kubasic *et al.* (1984). The sensitivity of the assay was 30 pg/ml and intra- and inter-assay variance 4.49 and 7.54 per cent, respectively. Plasma tri-iodothyronine (T_3) and thyroxine (T_4) were estimated using RIA technique described by BARC, Mumbai. The sensitivity of T_3 assay was 0.24 ng/ml and that of T_4 assay 0.5 ng/ml. The data were analysed statistically using CRD, DNMRT and 't' test, and were correlated with reproductive traits (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The findings on reproductive performance and weekly plasma profile of AKP, P_4 and thyroid hormone in fertile and infertile groups of buffaloes are presented in Table 1. The weekly P_4 profile of individual fertile and infertile buffaloes is illustrated in Figure 1.

Reproductive Performance

The mean values for the initiation of postpartum ovarian follicular activity (49.08 ± 5.49 vs 92.18 ± 12.21 days), first oestrus postpartum (69.00 ± 7.36 vs 141.82 ± 21.32 days), days open (77.83 ± 6.40 vs 155.91 ± 26.28 days) and calving interval (388.92 ± 6.13 vs 466.45 ± 26.39 days) were significantly shorter/lesser ($P < 0.01$) in fertile group of buffaloes than the infertile ones, although the time required for uterine involution (30.00 ± 1.36 vs 33.73 ± 1.65 day) did not differ. Agarwal *et al.* (1979) also reported similar findings. The early initiation of follicular activity was associated with rising P_4 levels 2-5 weeks onwards postpartum and had significant positive correlation with the first heat postpartum ($r = 0.64$), days open ($r = 0.70$) and calving interval ($r = 0.71$). The earlier rise in P_4 level was in turn associated with reduced ($P < 0.05$) period for resumption of follicular activity ($r = -0.58$), first heat postpartum ($r = -0.72$), days open ($r = -0.72$) and calving interval ($r = -0.85$).

Bekana *et al.* (1996) observed occurrence of first ovulation between 19-29 days in 70 per cent of the cows through progesterone analysis. The pre-oestrus rise (> 1 ng/ml) in progesterone recorded for a shorter duration in some fertile buffaloes under study (Fig. 1) agreed with the findings of Pahwa and Pandey (1983) and Tiwari and Pathak (1995) probably due to luteinization of large follicles forming subnormal short-lived corpora lutea. The variation between fertile and infertile buffaloes could be due to the difference in their endocrine status. Our results indicate that in some infertile buffaloes follicular

Table 1: Weekly plasma profile of alkaline phosphatase and certain hormones in naturally fertile and infertile (by day 105 postpartum) Surti buffaloes

Weeks post-partum	AKP (IU/L)		Progesterone (ng/ml)		Tri-iodothyronine (ng/ml)		Thyroxine (ng/ml)	
	Fertile (n=12)	Infertile (n=11)	Fertile (n=12)	Infertile (n=11)	Fertile (n=12)	Infertile (n=11)	Fertile (n=12)	Infertile (n=11)
0	113.85±31.08	101.27±9.91	0.81±0.18^{cd}	0.73 ± 0.26	0.89 ± 0.08	1.35 ± 0.33^{a}	17.63 ± 1.97	21.06 ± 1.94
1	114.58±20.32	109.51±16.84	0.58±0.18^{d}	0.47 ± 0.17	0.78 ± 0.08	0.92 ± 0.15^{bc}	13.85 ± 1.61	17.66 ± 1.55
2	118.15±22.27	111.27±14.32	0.61±0.17^{d}	0.47 ± 0.24	0.88 ± 0.12	1.22 ± 0.41^{ab}	17.82 ± 0.90	18.73 ± 1.27
3	123.52±26.75	107.62±10.43	0.65±0.17^{d}	0.51 ± 0.18	0.69 ± 0.08	0.65 ± 0.07^{bc}	16.63 ± 1.10	18.02 ± 1.18
4	122.17±26.48	118.32±11.82	0.87±0.29^{cd}	0.52 ± 0.12	0.64 ± 0.04	0.90 ± 0.19^{bc}	14.78 ± 1.41	20.58 ± 2.23*
5	140.93±32.03	116.49±10.38	0.61±0.22^{d}	0.79 ± 0.21	0.99 ± 0.21	0.86 ± 0.13^{bc}	17.28 ± 2.31	19.22 ± 1.81
6	141.86±35.65	112.71±10.84	0.66±0.16^{d}	1.02 ± 0.33	0.65 ± 0.09	0.65 ± 0.09^{bc}	16.87 ± 1.44	17.92 ± 1.80
7	146.44±31.53	123.83±12.42	0.64±0.27^{d}	0.82 ± 0.29	0.84 ± 0.09	0.63 ± 0.07^{c}	17.70 ± 1.51	16.29 ± 0.79
8	137.35±30.36	108.09±12.33	0.92±0.32^{cd}	1.15 ± 0.39	0.92 ± 0.14	0.72 ± 0.12^{bc}	16.67 ± 1.53	20.98 ± 2.49
9	145.42±25.72	120.56±11.10	1.22±0.40^{bcd}	1.11 ± 0.33	0.88 ± 0.11	0.74 ± 0.09^{bc}	17.98 ± 1.40	20.06 ± 1.32
10	162.89±30.64	126.98±13.55	1.34±0.48^{bcd}	0.68 ± 0.20	0.77 ± 0.10	0.85 ± 0.09^{bc}	17.23 ± 1.57	22.42 ± 2.01
11	151.26±22.86	122.06±15.13	1.37±0.39^{bcd}	0.66 ± 0.13	0.72 ± 0.07	0.80 ± 0.10^{bc}	19.07 ± 1.19	19.86 ± 1.48
12	174.94±30.91	120.25±16.36	1.79±0.41^{abc}	0.97 ± 0.28	0.77 ± 0.10	0.88 ± 0.12^{bc}	16.57 ± 1.33	19.40 ± 1.82
13	166.76±31.11	128.01±13.43	1.94±0.37^{ab}	1.53 ± 0.46	0.90 ± 0.10	0.80 ± 0.13^{bc}	18.78 ± 1.24	19.42 ± 2.53
14	167.54±29.60	124.37±19.85	2.07±0.24^{ab}	0.89±0.30**	0.77 ± 0.09	0.68 ± 0.08^{bc}	17.92 ± 1.37	19.04 ± 2.00
15	163.62±26.50	132.59±15.34	2.77±0.27^{a}	0.62±0.21**	0.90 ± 0.06	0.78 ± 0.15^{bc}	18.30 ± 1.92	19.35 ± 1.78
Overall	144.45±7.16	117.74±3.41**	1.18±0.07	0.81±0.07**	0.81 ± 0.03	0.85 ± 0.04	17.19 ± 0.38	19.37±0.45**

* $P < 0.05$; ** $P < 0.01$ between groups within the trait.
Means bearing superscript in common within a column do not differ significantly ($P > 0.05$).

ctivity appeared nearly the same period as in fertile uffaloes, but the maturation of follicle and it's ovulation was definitely affected indicating interference with LH surge. Shah (1999) also found significantly earlier occurrence of first oestrus postpartum in fertile than the infertile group of buffaloes (46.88 ± 3.30 and 130.25 ± 35.62 days). The interval for first heat postpartum had significant positive correlations with days open and calving interval (r = 0.82, 0.81). The prolonged period of days open (155.91 ± 26.28 days) leading to extended calving interval (388.92 ± 6.23 days) observed in infertile group of buffaloes compared to fertile ones might be due to delayed development and maturation of follicle, anoestrus, silent oestrus, ovulatory disturbance or higher percentage of embryonic mortality and return to oestrus in infertile group.

The extension of postpartum suboestrus or anoestrus period in buffaloes of infertile group agreed with that reported by Barkawi *et al.* (1995). This long infertile period was attributed especially to 3 animals out of 11 (Fig. 1). One animal (AN-18) did not show any ovarian activity till 181 days postpartum, another animal (AN-21) had exhibited first three heats regularly and then she did not reveal oestrus till 411 days, whereas, third animal (AN-23) had shown first heat on 22 days postpartum and then exhibited fertile heat at 154 days postpartum. However, the other 8 animals exhibited induced fertile heat within 35 days of GnRH or $PGF_2\alpha$ treatment (i.e. within 105-140 days postpartum) and hence to some extent reduced the average service period and calving interval of that group.

Alkaline Phosphatase Activity

The pooled mean AKP activity in fertile group of buffaloes was significantly higher ($P < 0.01$) than in infertile group (144.45 ± 7.16 vs 117.74 ± 3.41 IU/L). The weekly mean values fluctuated non-significantly between different intervals postpartum in both fertile and infertile groups and showed a gradual increasing trend similar to P_4 from calving till 15th week postpartum. The values of AKP at different postpartum intervals were higher in fertile than the infertile group (Table 1) and it

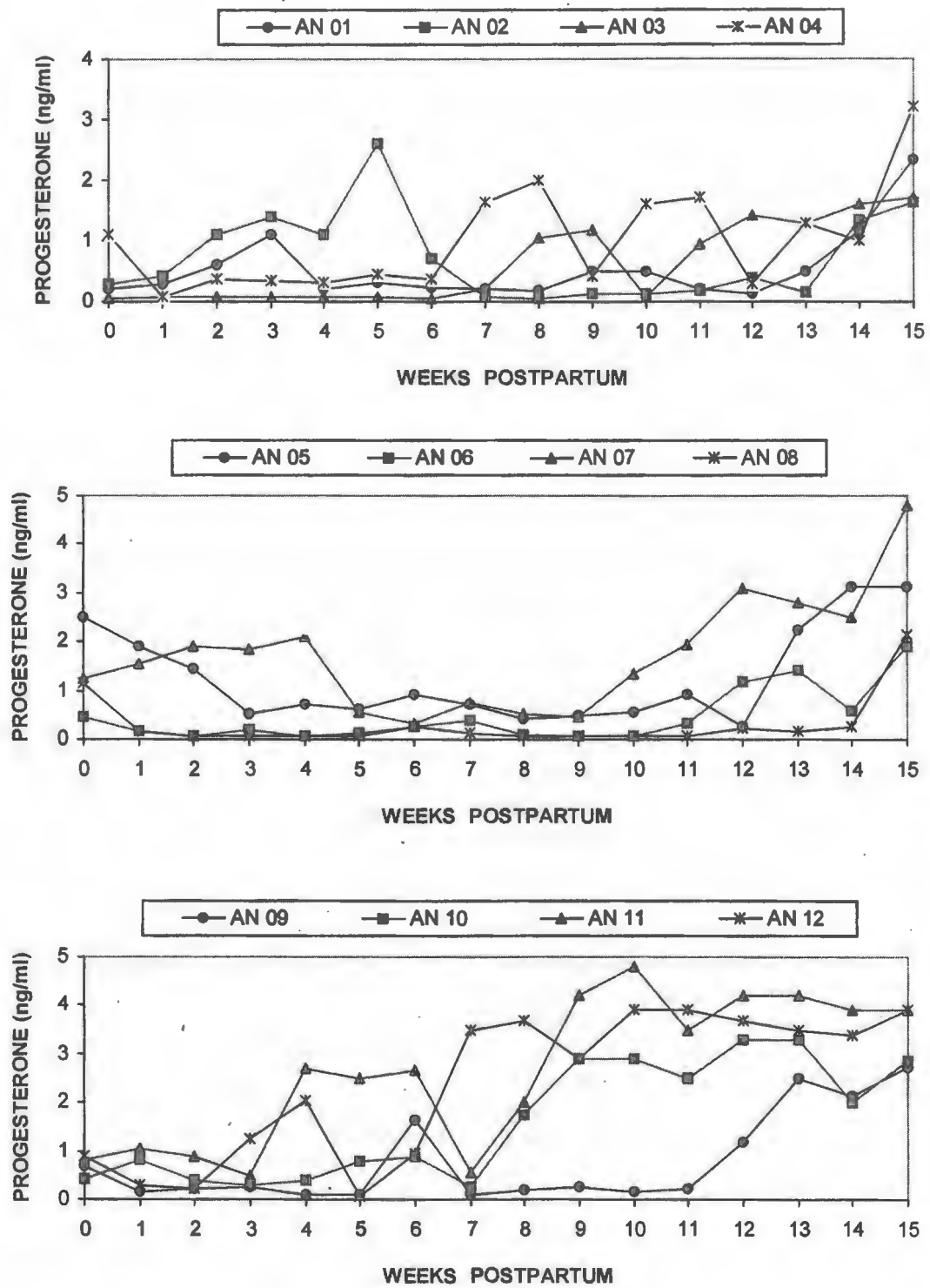

Fig. 1a: Weekly plasma progesterone profile of naturally *fertile (conceived within 15 weeks)* postpartum Surti buffaloes

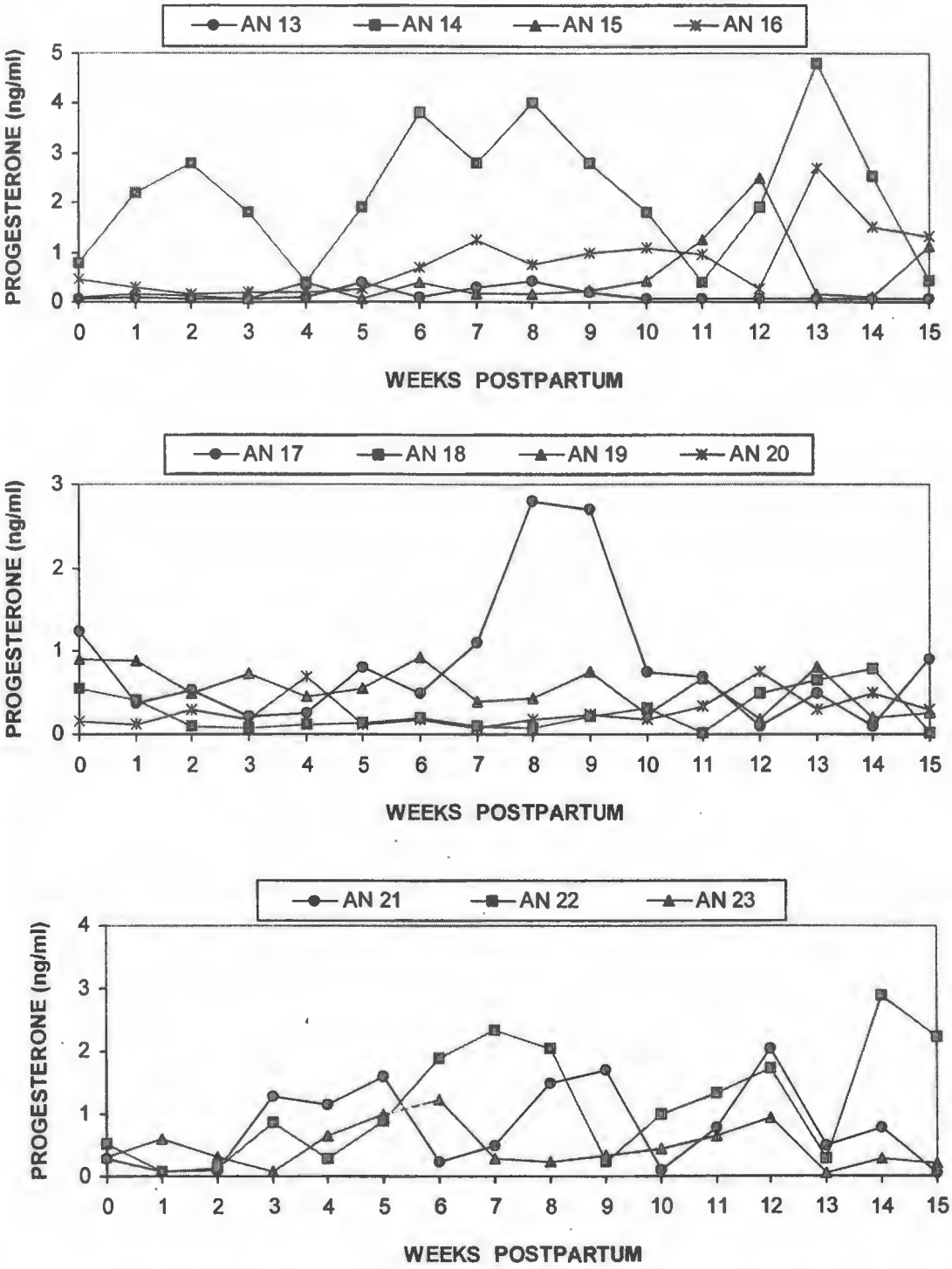

Fig. 1b: Weekly plasma progesterone profile of infertile (*non-conceived* by 15 week) postpartum Surti buffaloes

did not reveal any significant correlation with reproductive and hormonal traits studied. Noble *et al.* (1977) found higher AKP activity in buffaloes that came in heat before 30 days postpartum than those came late. However, Devraj (1982) did not find such variation in buffaloes till 38 days postpartum. Moreover, the positive association of serum AKP activity with thyroid function reported by Vadodaria *et al.* (1978) was not evident in the present study. The AKP is known for its role in number of important cellular processes such as transport of solute across the membranes of cells having secretory function, synthesis of phospholipids and RNA and in carbohydrate metabolism (Tewari and Bowrne, 1962).

Plasma Progesterone Profile

The mean plasma P_4 levels of buffaloes varied significantly between different weeks postpartum only in fertile group. The mean value of 0.81 ± 0.18 ng/ml found on the day of calving dropped to base line (0.58 ± 0.18 ng/ml) by 1 week postpartum, remained low till 8th week postpartum and then showed increasing trend as the weeks proceed to reach the highest value ($P < 0.01$) of 2.77 ± 0.27 ng/ml at 15th week postpartum. While in the infertile group, the levels fluctuated non-significantly through different weeks postpartum. The values were higher at 6th, 9th and 13th week postpartum with fall in-between; suggesting anoestrus/ suboestrus condition, repeat cycles or early embryonic death in some animals (Table 1). Further, the mean P_4 concentration was significantly higher ($P < 0.01$) in fertile than the infertile group only at 14th - 15th week postpartum. This trend of postpartum P_4 profile compared well with the reports of Perera *et al.* (1981), Tiwari *et al.* (1995) and Shah (1999). Tegegne *et al.* (1993) reported < 1 ng/ml level of P_4 in African Zebu cows until week 12 postpartum, while Jainudeen *et al.* (1981) recorded very low or negligible level of progesterone up to 60 to 90 days postpartum, particularly in anoestrus swamp buffaloes. The present findings indicate that overall reproductive efficiency is impaired due to deficiency of progesterone, which is essential for embryo survival. Further, non-conceived animals had a combined pattern of delayed rise and low level of P_4 during luteal phase indicative of insufficient luteal function than those conceived.

The progesterone levels had significant positive correlations with T_3 (0.75) and negative correlations with resumption of follicular activity, first heat postpartum days open and calving interval (-0.58, -0.72, -0.87, -0.85 in fertile group, while in infertile group no such correlations were seen. These correlations are highly conceivable and suggest their positive role in fertility enhancement. Further, significant negative correlation of P_4 with reproductive traits indicated that early rise in P_4 would reduce the interval for ovarian activity, first heat postpartum and thereby days open and calving interval in dairy animals.

In our study, most of fertile and some of infertile (Fig. 1) postpartum suckled buffaloes revealed pre-oestrus rise of progesterone ($\geq$ 1 ng/ml) between 14 and 56 days postpartum, while in the remaining fertile buffaloes (AN 5, 6, 7), P_4 levels remained >0.5 and < 1.0 ng/ml and they exhibited fertile oestrus thereafter. The pre-oestrus rise in P_4 was delayed in infertile buffaloes (AN 15, 18, 20), and occurred between 70 and 98 days postpartum. In general, it appeared that delay in progesterone rise in some of infertile buffaloes compared to fertile ones was responsible for longer service period. Shah (1999) observed pre-oestrus rise of progesterone between days 14 and 42 postpartum in 8 of 23 postpartum suckled Surti buffaloes, and this rise was delayed in infertile buffaloes from 42 and 84 days.

In the present study, anoestrus and suboestrus condition was diagnosed through rectal palpation and progesterone profile. The buffaloes with postpartum anoestrus (AN 18, 19, 23; Fig. 1) revealed basal to non-detectable plasma progesterone profiles throughout 105 days of weekly sampling and exhibited first heat thereafter (on 187, 181 and 154 days postpartum respectively). Similarly, one animal (AN 20) showed basal to detectable P_4 profile between day 77 and 91 postpartum and conceived at oestrus expressed on 112 days postpartum. Whereas, AN-21 revealed abnormal progesterone profile at various postpartum intervals indicating suboestrus condition and around weeks 15 postpartum it showed true anoestrus condition. Conception in this buffalo was delayed and it occurred on 411 days postpartum. Darwash and Lamming (1997)

observed that the cows with irregular progesterone patterns had longer intervals to start postpartum luteal activity, to first postpartum service and conception than in cows with normal progesterone pattern. The present findings in buffaloes also confirmed their observations.

Thyroid Hormones

The plasma concentrations of hormone T_3 and T_4 fluctuated non-significantly without any specific trend in both fertile and infertile groups with mean values of 0.81 ± 0.03 vs 0.85 ± 0.04 ng/ml, and 17.19 ± 0.38 vs 19.37 ± 0.45 ng/ml ($P < 0.01$), respectively. The levels of T_3 fluctuated non-significantly from 0.64 ± 0.04 to 0.99 ± 0.21 ng/ml in fertile group between different weeks postpartum, while in infertile group the fluctuation was significant (0.63 ± 0.07 to 1.35 ± 0.33 ng/ml). In general, the levels of both T_3 and T_4 were highest on the day of calving, dropped by 3-4 weeks postpartum and remained low thereafter (Table 1). These findings with respect to levels of T_3 and T_4 compared well with those of Deshmukh *et al.* (2001) in lactating crossbred cows. However, Kumar *et al.* (1993) reported relatively higher circulating T_3 and T_4 levels in Murrah buffaloes during first 10 months of lactation (1.35 to 9.99 and 28.83 to 55.33 ng/ml). The present values and trend closely agreed to the report of Lohan *et al.* (1989), who also demonstrated mean T_4 value of 27.43 ng/ml on the day of calving and then low levels till day 21 postpartum (24.45 ng/ml) followed by a rising trend and comparatively elevated levels till day 97 postpartum and then again decline by day 116 in non-suckled Murrah buffaloes. In the present study, the levels of T_4 had significant negative correlation only with services per conception (-0.74), while T_3 showed significant positive correlations with plasma P_4 (0.75) and negative correlations with first oestrus postpartum, days open and calving interval (-0.55, -0.85, -0.81) only in fertile group. Mahendran *et al.* (2001) found positive association between the levels of T_3 and T_4 at the time of insemination and conception rates in bovines.

Thus, it is concluded that the consistent higher plasma AKP activity and lower T_4 levels observed through out the postpartum period in fertile buffaloes as compared to infertile ones suggested their positive role in fertility enhancement by increasing dephosphorylation and decreased basal metabolic rate. Moreover, the weekly P_4 profile could successfully be used as a guide to evaluate postpartum fertility status of buffaloes.

ACKNOWLEDGEMENT

We are thankful to Dr. P.M. Desai, Research Scientist and Head, LRS, GAU, Navsari, as well as Dr. Y.G. Dugwekar, Research Scientist, RBRU, Anand, and their staff for their kind permission, co-operation and help extended in for this work.

REFERENCES

Agrawal, K.P., Raizada, B.C. and Pandey, M.D. (1979). Post-parturitional changes in the ovary and related endocrine glands. Indian J. Anim. Sci., **49**: 2536.

Barkawi, A.H., Farghaly, H.M. and ElBorady, A.M. (1995). Effect of treatment with GnRH analogue on postpartum reproductive performance of suckling Egyptian buffalo cows. Buffalo J., **11**: 117123.

Barkawi, A.H., Shafie, M.M. Mekawy, Y. and Aboul-Ela, M.B. (1986). The use of serum and milk progesterone concentration to monitor postpartum ovarian activity. Buffalo J., **2**: 125-134.

Bekana, M., Jonsson, P. and Kindahl, H. (1996). Intrauterine bacterial findings and hormonal profiles in pospartum cows with normal puerperium. Acta Veterinaria Scandinavica, **37**: 251-263.

Darwash, A.O. and Lamming, G.E. (1997). Abnormal ovarian patterns as a cause of subfertility in dairy cows: protocols for early detection and treatment. Cattle Practice, **5**: 3-7.

Deshmukh, B.T., Nagvekar, A.S., Talvelkar, B.A., Dalvi, S.H. and Chinchkar, S.R. (2001). Effect of bovine somatotropin on blood serum minerals, thyroid hormones and reproductive performance of lactating crossbred cows. Indian J. Anim. Sci., **71**: 663-666.

Devraj, M. (1982). Blood Serum Profile in Calves and Postpartum Buffaloes (Surti Breed) with Associated Peridata to Reproductive Efficiency. Ph.D Thesis, Gujarat Agricultural University, Anand, India.

Jainudeen, M.R., Tan, H.S. and Bongso, T.A. (1981). Plasma progesterone profiles in relation to postpartum ovarian activity in the swamp buffalo. Paper presented at 2nd Research Coordinated Research Programme on the use of nuclear technique to improve domestic buffalo production in Asia. March 26, Bangkok, Thailand.

King, G.J., Hurnie, J.F. and Robertson, H.A. (1976). Ovarian function and oestrus in dairy cows during early lactation. J. Anim. Sci., **42**: 688-692.

Kubasic, N.P., Hallauer, G.D. and Brodows, R.G (1984). Evaluation of direct solid-phase RIA for progesterone, useful for monitoring luteal function. Clinical Chemistry, **30**: 284-286.

Kumar, R., Rajvir Singh, Rattan, P.J.S., Setia M.S., Singh, R. (1993). Circulating levels of plasma thyroid and adrenocorticoid hormones in Murrah buffaloes during first 10 months of lactation. Indian J. Anim. Reprod., **14**: 5-6.

Larson, L.L., Mabruck, H.S. and Lowry, S.R. (1980). Relationship between early postpartum blood composition and reproductive performance in dairy cattle. J. Dairy Sci., **63**: 283-289.

Lohan, I.S., Maker, M.L., Singal, S.P. and Razdan, M.N. (1989). Thyroid activities of prepartum and postpartum Murrah buffaloes (Bubalus bubalis). Indian Vet. J., **66**: 115-118.

Mahendran, G., Kumaresan, A., Varshnay, J.P., Ansari, M.R. and Pathak, M.C. (2001). Plasma progesterone, T_3 and T_4 levels at the time of insemination and conception rate in normal and repeat breeding buffaloes (*Bubalus bubaslis*). Indian J. Anim. Sci., **71**: 1164-1165.

Nalbandov, A.V.(1970). Reproductive Physiology. 2nd Edn., D.B. Taraporevala Sons & Co., Bombay.

Noble, D., Rao, M.V.N. and Bhosrekar, M.R. (1977). Some biochemical constituents in blood and mucus of buffaloes during different stages of oestrus. Indian J. Dairy Sci., **30**: 252-55.

Pahwa, G.S. and Pandey, R.S. (1983). Hormonal changes in postpartum blood plasma and milk of buffaloes (*Bubalus bubalis*). Anim. Prod., **37**: 237-246.

Perera, B.M.A.O., Abeygunawardena, H., Thamotharam, A., Kindahl, H. and Edquist, L.E. (1981). Peripartal changes of estrone, progesterone and prostaglandin in water buffalo. Theriogenology, **15**: 463-467.

Shah, R.G. (1999). Hormonl and biochemical profile in fertile and infertile postpartum Surti buffaloes. Ph.D. Thesis, Gujar Agricultural University, Anand, Indian.

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

Tegegne, A., Geleto, A. and Kassa, T. (1993). Short luteal phase and ovulations without oestrus in primiparous Barona (*Bos indicus*) cows in the central high land of Ethiopia. Anim Reprod. Sci., **31**: 21-31.

Tewari, H.B. and Bourne, G.H. (1962). J. Histochem. Cytoche **10**: 42 (c.f. Kabra, G. 1965. Studies on the ultra-struct localization of alkaline phosphatase in the ovary of dog Current Sci., **34**: 639-645.

Tiwari, S.R. and Pathak, M.M. (1995). Influence of suckling on postpartum reproduction performance of Surti buffalo Buffalo J., **11**: 213-217.

Tiwari, S.R., Pathak, M.M. and Patel, A.V. (1995). Study of steroi during postpartum period of Surti buffaloes in relation to suckling and milking practices. Indian J. Anim. Reprod., 16 58.

Vadodaria, V.P., Janakiraman, K. and Buch, N.C. (1978). Serum enzyme to reflect thyroid activity in Surti heifers in relation to oestrous cycle and breeding periods. Indian J. Physiol Allied Sci., **32**: 108-115.