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RESEARCH ARTICLE

Reproductive performance and progesterone profile in post-partum acyclic surti buffaloes

D.K. SONI, C.T. KHASATIYA[1], A.S. REDE[1], M.D. PATEL[2] AND S.S. CHAUDHARY[3]

Members of the Research Forum

Associate Author :

[1]Department of Veterinary Gynaecology and Obstetrics, Vanbandhu College of Veterinary Science and Animal Husbandry, Navsari Agricultural University, NAVSARI (GUJRAT) INDIA

[2]Livestock Research Station (NAU) NAVSARI (GUJRAT) INDIA

[3]Department of Physiology and Biochemisty, Vanbandhu College of Veterinary Science and Animal Husbundry, Navsari Agricultural University, NAVSARI (GUJRAT) INDIA

AUTHOR FOR CORRESPONDENCE :

D.K. SONI

Department of Veterinary Gynaecology and Obstetrics, Vanbandhu College of Veterinary Science and Animal Husbundry, Navsari Agricultural University, NAVSARI (GUJRAT) INDIA

Abstract : Postpartum acyclic surti buffaloes of an organized farm confirmed by twice per-rectal palpation 11 days apart from 45 days postpartum were treated with 5ml of inj. Buserelin acetate I/M route in first group (n=6) and 5ml of inj. Buserelin acetate I/M route along with 5 ml inj. Vit. AD_3E preparation and 15 ml inj. Toldimphos sodium preparation I/M route in second group (n=6) on 55 days postpartum after confirmation of acyclicity. Keeping 6 animals of same status as control to see the oestrus induction response and conception rate including weekly evaluation of blood progesterone profile, just before (0 day) treatment and 3 weeks after treatment (7^{th}, 14^{th} and 21^{st} day's post-treatment). The service period and oestrus induction interval in days was found significantly lower ($p<0.05$) in GnRH treated T_1 (71.17±4.42; 12.67±1.11 days) and T_2 (70.83±3.80; 12.33±1.11 days) groups as compared to control T_3 (94.50±5.43; 30.75±3.95 days) group under the study. It was observed that service period in the GnRH treated (T_1 and T_2) groups has been minimized up to 23 to 24 days *i.e.* one cycle earlier in treatment groups than that of T_3 control group. However, the number of services per conception did not differ significantly among all the experimental anoestrous treatment and control groups. Moreover, cent per cent conception rate in T_1 (GnRH alone) and T_2 (GnRH + Vit.+ P) groups as compared to 66.66 per cent conception rate in acyclic control group (T_3), respectively might be under the influence of various treatments during period (45 to 120 days) with overall 88.89 per cent (16/18) conception rate. GnRH treatment instituted (T_1 and T_2) groups revealed the increasing trend in progesterone concentration from 14^{th} and 21^{st} days post-treatment were increasing trend in progesterone concentration as (0.93±0.26 to 2.77±0.26 ng/ml and 1.39±0.30 to 3.16±0.30 ng/ml), respectively as compared to control (T_3) group. This might be due to use of GnRH treatment in that groups of animals postpartum leading to early resumption of ovarian follicular activity followed by conception. On the other hand, in control (T_3) group fluctuating trend (0.41±0.06 to 0.85±0.06 ng/ml) in progesterone concentration at different time (0 day, 7^{th} day, 14^{th} day and 21^{st} day) intervals was found, that could be attributed to because of late settling (after 20 days with normal saline and rectal palpation) of pregnancy in that group.

Key words : Acyclic surti buffaloes, Hormone therapy, Progesterone, Postpartum period

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INTRODUCTION

Reproduction efficiency of is the key for a profitable herd and in order to maximize the productive life of a buffalo cow, it should be bred within 80-90 days after parturition to produce a calf and start a new lactation every 13-13.5 months (El-Wishy, 2007). Moreover, longer inter-calving intervals in buffaloes are mainly due to prolonged postpartum anoestrum which is mainly attributed to ovarian inactivity (Hattab and Osman, 2000). Postpartum anoestrus is affected by several factors such as nutrition plane, milk yield, body condition score (BCS) at calving, suckling, parity, calving season and other factors as documented by (El-Wishy, 2007). Various hormonal treatments like GnRH, Estrogen and Progesterone either alone or in combination have been tried with variable success (Rao and Rao, 1984 and Singh *et al.,* 2004). Keeping all this in view, the study was undertaken on acyclic/inactive ovarian condition buffaloes to see the efficacy of GnRH alone and in combination with vitamin and phosphorus in relation to reproductive performance and progesterone profile.

RESEARCH METHODOLOGY

A study was carried out on 18 postpartum acyclic surti buffaloes of University farm, Navsari (Gujarat). All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. The animals were fed green fodder, hay and compounded concentrate, as per the standard feeding schedule followed on the farm. The animals had free access to drinking water. Oestrus occurrence was detected daily in them from day 40 postpartum onwards with the help of teaser bull parading in morning and evening hours and females are naturally served with known fertile buffalo bull or bred with good quality frozen semen through artificial insemination. They were palpated per-rectum for confirmation of pregnancy at 60 to 90 days post-breeding. During the course of study, 18 buffaloes beyond 45 days postpartum were identified through twice per-rectal palpation 11 days apart as acyclic or inactive ovarian condition. They were randomly divided into three equal groups consisting 6 surti buffaloes in each group *viz.*, T_1, T_2 and T_3 (Control). The buffaloes in T_1 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH analogue, I/M route); the buffaloes in T_2 group were treated with 5ml of inj. Buserelin acetate (20 µg, GnRH analogue, I/M route) + [inj. Vit. AD_3E preparation (5 ml, I/M route) + inj. Toldimphos sodium preparation (15 ml, I/M route)] and the buffaloes in T_3 group were kept as anoestrus control group. All these buffaloes were followed for oestrus induction response, reproductive performance for up to 120 days postpartum and weekly hormonal profile at least 3 weeks after treatment. Approximately 10 ml blood samples in serum clotting vaccutainers were collected from all those selected animals on 0 day (prior to treatment), 7^{th}, 14^{th} and 21^{st} days post treatment aseptically by jugular vein puncture. The vaccutainers containing blood samples were kept in slanting position at room temperature for 1-2 hours. Finally, serum was separated by centrifugation at 3000 rpm for 15 minutes and stored in properly labelled sterilized 4.5 ml plastic storage vials at –20°C in deep freezer until analysis. Serum progesterone concentration was measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and procedure described by Labour Diagnostika Nord GmbH and Co. K.G., Nordhorn.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Reproductive performance :

The service period and treatment to oestrus induction interval of the acyclic surti buffaloes in between T_1 (71.17±4.42; 12.67±1.11 days) and T_2 (70.83±3.80; 12.33±1.11 days) treatment groups did not differ significantly. However, the service period and treatment to oestrus induction interval of the inactive ovarian condition of surti buffaloes in T_1 (71.17±4.42; 12.67±1.11 days) and T_2 (70.83±3.80; 12.33±1.11 days) treatment groups differed significantly from T_3 control (94.50±5.43; 30.75±3.95 days) groups. It was observed that service period in the GnRH

treated (T_1 and T_2) groups has been minimized up to 23 to 24 days *i.e.* one cycle earlier in treatment groups than that of T_3 control group. These finding is corroborated with the findings of Khasatiya (2003) in which he reported service period (69.93±4.29 vs 87.71±4.00 days) and there by calving interval (380.36±4.02 vs 400.28±3.97 days) both were shorten by one cycle in GnRH treated conceived than the control conceived buffaloes, respectively.

The service period and oestrus induction interval in days was found significantly lower in GnRH treated (T_1 and T_2) groups as compared to control (T_3) group under the study which clear cut showed the effect of GnRH treatment on ovary and earlier resumption of ovarian activities as compared to control group. Moreover, there was no significant difference found in service period and oestrus induction interval between T_1 (GnRH alone) and T_2 (GnRH + Vitamin AD_3E + Phosphorus combine) group. However, slightly lower service period and oestrous induction interval in days (70.83±3.80; 12.33±1.11) was found in T_2 group as compared to T_1 group (71.17±4.42; 12.67±1.11 days), which might be attributed to the influence of exogenous inorganic phosphorus and vitamins along with GnRH given to the animals in T_2 group. Earlier workers also have used vitamins (Kumar *et al*., 2010) and inorganic phosphorus (Giri and Yadav, 2001) as a therapy in the anoestrous cows and buffaloes with encouraging results in terms of induction of oestrous and conception rate.

Additional exogenous injection of inorganic phosphorus along with vitamins might have helped to resolve the problem of anoestrous (acyclic ovarian condition) in surti buffaloes in the study in group T_2. However, the number of services per conception did not differ significantly among all the experimental anoestrous treatment and control groups. Moreover, cent per cent conception rate in T_1 and T_2 groups as compared to 66.66 per cent conception rate in control (T_3) group, respectively might be under the influence of various treatments in above period (45 to 120 days)

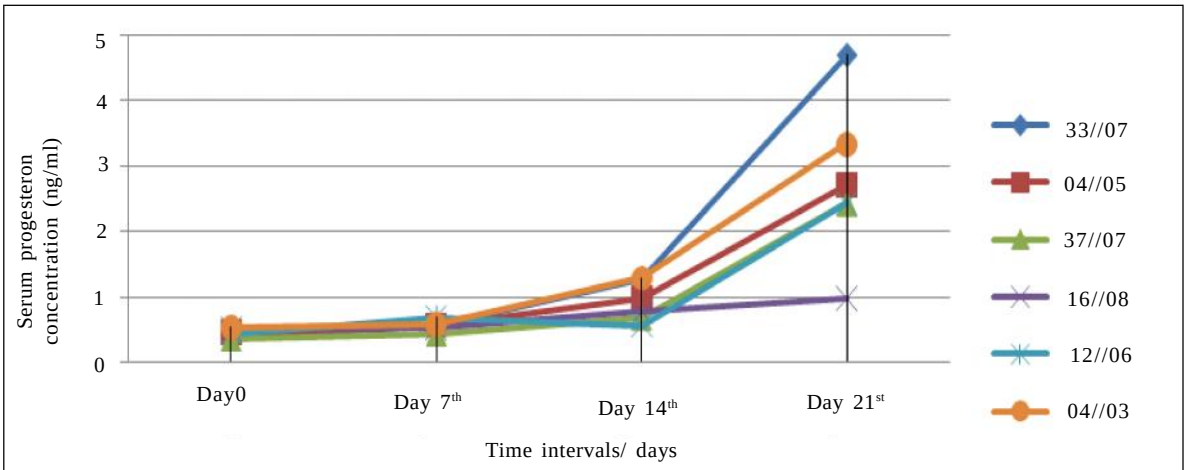


Fig. 1 : Serum progesterone concentration (ng/ml) of the acyclic surti buffaloes in T_1 (GnRH analogue) group at different time intervals (n=6)

Table 1 : Effect of different treatments on reproductive performance of postpartum anoestrus surti buffaloes (Mean±SE)

Treatment/ group (n=6)	Service period (days)	Number of services per conception	Treatment to oestrus induction interval (days)	Conception rate (%)	No. of animals responded to the treatment	No. of pregnant animals (n=6)
T_1	71.17±4.42[a]	1.33[a]	12.67±1.11[a]	100.00	6(100.00%)	6
T_2	70.83±3.80[a]	1.33[a]	12.33±1.11[a]	100.00	6(100.00%)	6
T_3 (Control)	94.50±5.43[b]	1.50[a]	30.75±3.95[b]	66.66	4(66.66%)	4
Overall	76.88±3.66	1.38	17.06±2.29	88.89	16(88.89%)	16/18

Means bearing different superscripts within a column (group) differ significantly ($p< 0.05$) Group-I = T_1 (GnRH analogue)
Group-II = T_2(GnRH analogue + Vit.+ P) Group-III = T_3 (Acyclic Control)

with overall 88.89 per cent (16/18) conception rate. That is why phosphorus and vitamin therapy could have frequently been used as an initial package of practices in the treatment of anoestrus in the field conditions to augment fertility in those animals. Moreover, injectable inorganic phosphorus might have corrected the marginal deficiency arising from intake of phosphorus to promote gonadal and genital activity.

Serum progesterone profile :

The mean progesterone concentration prior to treatment (0 day) were found to be 0.45±0.26 ng/ml in group T_1 (GnRH alone), 0.50±0.30ng/ml in group T_2 (GnRH +Vit+ P) and 0.41±0.06ng/ml in control (T_3) group, which was found to be below 1 ng/ml during the sampling period.

As compared to above finding, lower progesterone concentration in anoestrus as 0.289 ± 0.029 ng/ml, below 0.25 ng/ml and 0.1 ±0.00 ng/ml reported by (Yadav *et al.*, 1995; Ullah *et al.*, 2006 and Ghuman *et al.*, 2010), respectively. Slight higher progesterone concentration 0.67± 0.02 ng/ml and 0.91±0.04 ng/ml reported by Tiwary (2010) in murrah buffaloes and Khasatiya (2003) in surti buffaloes, respectively. Whereas, these findings were well comparable with those of Butani *et al.* (2011) and Sharma *et al*. (1999), they reported progesterone concentration 0.36±0.08 ng/ml and 0.46±0.00 ng/ml, respectively. In addition to this, Narayana *et al*. (1984) reported serum P_4 value less than 1.0 ng/ml in 2 to 6 month postpartum anoestrus condition of cattle and buffaloes. Exceptionally one buffalo showed progesterone concentration more than 1.0 ng/ml without a preceding palpable follicle or corpus luteum and one cow showed an oestrous cycle without a preceding palpable follicle or corpus luteum.

GnRH treatment instituted (T_1 and T_2) groups revealed the increasing trend in progesterone concentration from 14th and 21st days of intervals from the conception in which progesterone concentration were found (0.93±0.26 to 2.77±0.26 ng/ml and 1.39±0.30 to 3.16±0.30 ng/ml), respectively. This might be due to use of GnRH treatment in that

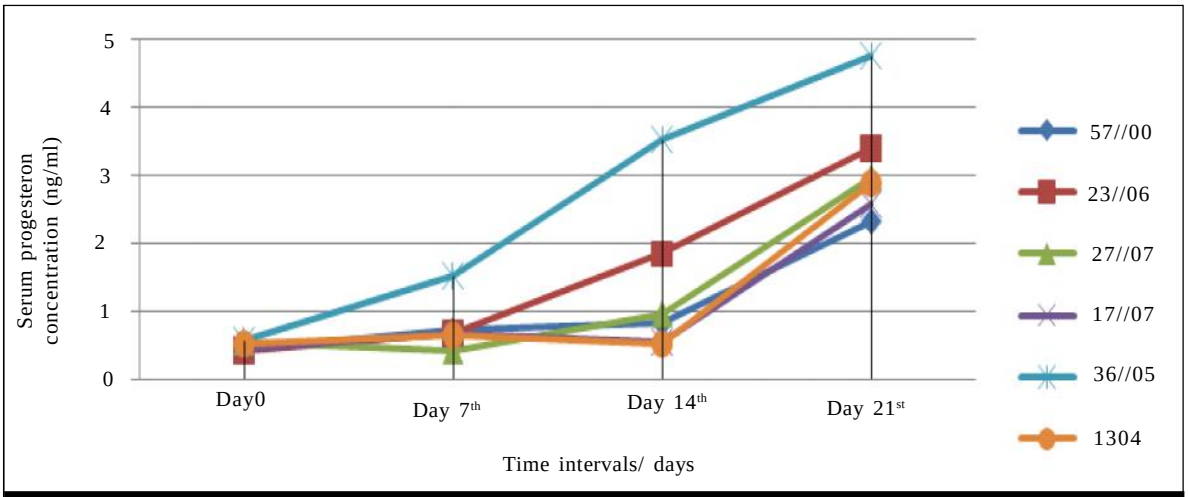


Fig. 2 : Serum progesterone concentration (ng/ml) of the acyclic surti buffaloes in T_2 (GnRH analogue + Vit.+ P) group at different time intervals (n=6)

Table 2 : Serum progesterone concentration (ng/ml) pattern at different time intervals / days in acyclic treated and control groups of animals (Mean±SE)

Time intervals/ days	Groups / treatments (n=6)		
	T_1	T_2	T_3 (Control)
0 day	$0.45\pm0.26_{ab}^{w}$	$0.50\pm0.30_{b}^{w}$	$0.41\pm0.06_{a}^{w}$
7th day	$0.56\pm0.26_{a}^{w}$	$0.79\pm0.30_{a}^{w}$	$0.57\pm0.06_{a}^{w}$
14th day	$0.93\pm0.26_{a}^{w}$	$1.39\pm0.30_{a}^{w}$	$0.70\pm0.06_{a}^{wx}$
21st day	$2.77\pm0.26_{a}^{x}$	$3.16\pm0.30_{a}^{x}$	$0.85\pm0.06_{b}^{x}$

Means bearing different superscripts within a column (group) differ significantly ($p<0.05$). Means bearing different subscripts within a row (between the groups) differ significantly ($p<0.05$)

groups of animals postpartum leading to early resumption of ovarian follicular activity followed by conception. On the other hand, in control (T_3) group fluctuating trend (0.41±0.06 to 0.85±0.06 ng/ml) in progesterone concentration at different time (0 day, 7th day, 14th day and 21st day) intervals was found, that could be attributed to because of late settling of pregnancy in that group.

Statistical analysis of the data generated in respect of the treatment on the progesterone concentration of the blood serum did not show any significant difference among the three groups of acyclic surti buffaloes under study at 7th day and at 14th day post-treatment. Moreover, the mean serum progesterone values at 0 day (prior to the treatment) and 21st day (post-treatment) between treated T_1 and T_2 groups did not differ significantly ($p<0.05$). However, the mean serum progesterone levels of T_2 group differed significantly ($p<0.05$) from control group at 0 day. While T_1 and T_2 groups both were differ significantly ($p<0.05$) from control at 21st day post treatment.

It is well known fact that after conception followed by recognition of pregnancy progesterone concentration increased due to active CL and in non-conceptional cases progesterone drops after 17th day of cycle, this concluding statement supported by (Barkawi *et al.*, 1986 and Komoto *et al.*,1991). Many earlier workers also reported peripheral

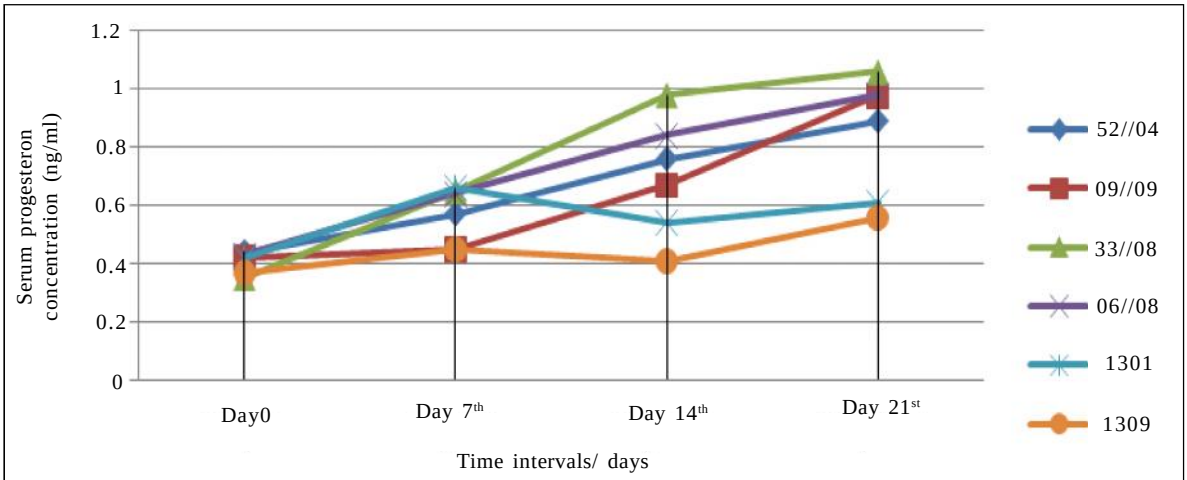
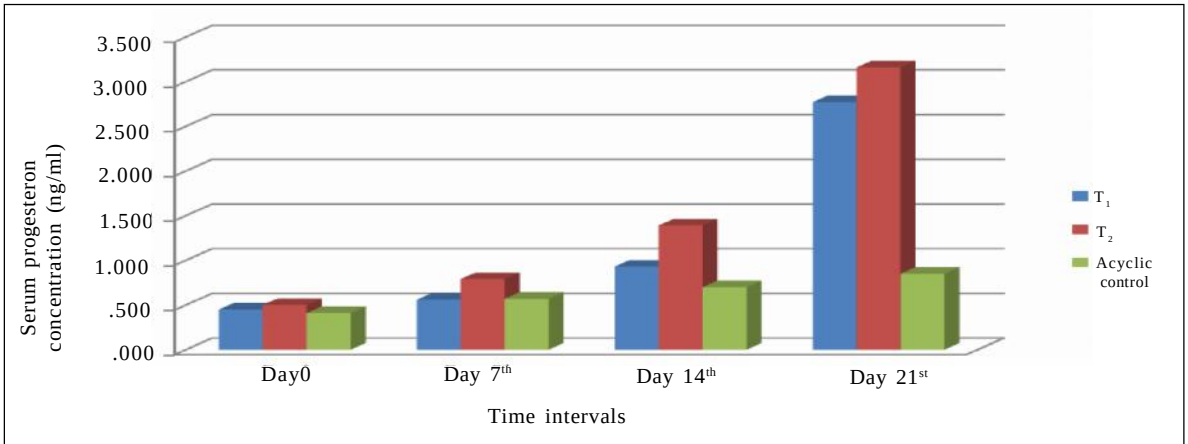


Fig. 3 : Serum progesterone concentration (ng/ml) of the acyclic surti buffaloes in T_3 (Acyclic control) group at different time intervals (n=6)



Group-I = T_1(GnRH analogue), Group-II = T_2(GnRH analogue + Vit.+ P) and Group-III = T_3 (Acyclic control)

Fig. 4 : Serum progesterone concentration (ng/ml) of the acyclic surti buffaloes from groups different time intervals

progesterone level was found to be minimal on the day of oestrus ranging from 0.10 to 0.50 ng/ml (Sharma *et al*., 1999 and Mondal *et al.*, 2007), which was rise to peak concentrations of 1.6 to 3.6 ng/ml on days 13 to 15 of the cycle or even on day 17 before declining to basal levels at the onset of next oestrus.

This study revealed that per rectally we could palpate the smooth ovaries in large animals but could not say regarding its viability as 100 per cent accurate, for that ultrasonography followed by estimation of progesterone have been the best tool for further accurate diagnostic confirmation. There is dire need for correlation of repeated per-rectal palpation, ultrasonography and progesterone measurement for critical differential diagnosis of true anestrous from suboestrus condition.

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