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Serum Progesterone, Oestradiol-17β, FSH and LH levels in Anestrus Surti buffaloes treated with Ovsynch alone and in combination with PRID

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Abstract

This study was carried out on 18 postpartum anestrus Surti buffaloes, divided into three equal groups (6 in each) to evaluate the serum progesterone, oestradiol-17β, FSH and LH concentrations (by ELISA) before, during and after Ovsynch and Ovsynch + PRID protocols. Six anestrus buffaloes each were treated with standard Ovsynch protocol (T1) and Ovsynch along with PRID (Progesterone releasing intravaginal device) protocol (T2) with fixed-time AI (FTAI). Six anestrus buffaloes were kept as untreated control (T3). Blood samples were obtained from the animals on day 0 (before treatment), day 4 (during treatment), day 8 (after $PGF_2\alpha$ injection), day of induced estrus/FTAI and day 18 post-AI (after treatment). The mean serum progesterone level on day 4 was significantly ($p<0.01$) higher in the T2 group as compared to T1 and T3 groups, while the level on day 8 was significantly ($p<0.01$) higher in both the treatment groups (T1 & T2) as compared to control group, however there was no significant difference ($p>0.05$) on the day of estrus between three groups. The mean serum oestradiol-17β and follicle stimulating hormone (FSH) levels on 8th day were significantly higher ($p<0.01$) in both the treatment groups (T1 and T2) as compared to control group (T3). However, there was no significant difference on day 0, 4, day of estrus and day 18 post-AI between groups. The mean serum luteinizing hormone (LH) levels of acyclic buffaloes were not found significantly different ($p>0.05$) among days in any of the treatment and control groups. But the mean LH concentration on the day of estrus in the T1, T2 & T3 was significantly higher ($p<0.01$) and thereafter decreased markedly on 18th day post-AI in all the groups. The conception rates at FTAI/first estrus in three groups were 66.66%, 50.00% and 75.00%, respectively.

Key Word: Surti Buffalo, Progesterone, Oestradiol-17β, FSH and LH profile, Ovsynch and Ovsynch+ PRID protocol

Introduction

Anestrus is generally defined as the state of ovarian inactivity, reflected by complete sexual inactivity without manifestation of estrus. Postpartum anestrus is the condition in which both the ovaries are small, smooth, inactive with the absence of Graafian follicle or corpus luteum and characterized by cessation of sexual cycle and psychic manifestation of estrus. Indigenous cattle and buffaloes show higher incidence of anestrus as 25-67% than the exotic and crossbred cattle as 2-10% (Pandit,

2004). The period of postpartum anestrus is usually longer in buffaloes than in cattle under comparative management conditions (Azawi *et al.,* 2012). There are numerous causes of anestrus e.g. poor nutrition, season, acquired abnormalities of female reproductive tract, hormonal imbalance etc. Prolonged postpartum acyclicity. Anestrus are major sources of economic losses to buffalo breeders, as being the most common causes of infertility in buffaloes (Khan *et al.,* 2012). True anestrus condition may be a result of suppression of FSH release through the effect of lactation, nutrition and systemic diseases. Various hormonal preparations (Estrogen, PMSG and GnRH) are used in anestrus and subestrus buffaloes with variable success (Rathore *et al.,* 2006). The idea behind using these hormones is to stimulate follicular growth in ovaries producing endogenous estrogen which exert positive feedback on the anterior pituitary function in turn induces estrus. Hence, this study was planned to evaluate the comparative efficacy of Ovsynch protocol alone and in combination with PRID to see their influence on conception rate and serum hormonal profile in anestrus Surti buffaloes.

Materials and Methods

The study was carried out on 18 Surti buffaloes, having inactive ovarian condition between 45 and 120 days postpartum maintained at Livestock Research Station, Navsari Agricultural University, Navsari, Gujarat. All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. Estrus occurrence was detected daily with the help of teaser bull parading during morning and evening hours. The animals which were not exhibiting overt signs of estrus during routine heat detection program were segregated and subjected to rectal palpations. The animals with smooth ovaries (no palpable structure over ovary i.e. follicle or corpus luteum) were selected for re-examination 11 days later to ascertain their acyclic nature and considered as postpartum anestrus buffaloes. They were divided into three equal groups of six animals each at random and were managed as under.

Treatment Protocol

In group T1 (Ovsynch protocol), the six true anestrus buffaloes were administered i/m with Injection of Busereline acetate, GnRH analogue, 10 µg (Pregulate, 2.5 ml) on day 0, Injection Cloprostenol sodium, $PGF_2\alpha$ analogue, 500 µg (Pregova, 2 ml) on day 7 and second Injection of Busereline 10 µg on day 9 followed by FTAIs twice on day 10. In group T2 (Ovsynch + PRID protocol), six true anestrus buffaloes were inserted intra-vaginally with PRID (0.96 g of progesterone; Triu-B, Virbac Animal Health India Pvt Ltd) for 7 days together above Ovsynch protocol. While in group T3 six anestrus buffaloes kept without hormone therapy served as control.

Buffaloes in spontaneous or induced estrus were inseminated using good quality frozen-thawed semen. Animals detected in estrus subsequent to FTAI were re-inseminated on next cycle and in non-return cases pregnancy was confirmed per rectally 60 days of last AI.

Collection of Blood Samples

Blood samples (5-6 ml) were collected aseptically from all animals on day 0 (prior to treatment), day 4 (during treatment), day 8 (after cloprostenol inj.), and day of estrus / FTAI and on day 28 (18^{th} day post-AI) by jugular vein puncture. The serum clotting vaccutainers containing blood samples were kept in slanting position at room temperature for 1-2 hours, centrifuged at 3000 rpm for 15 minutes and serum stored at –20°C until analysis.

Serum progesterone and oestradiol-17β concentrations were measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits of Diagnostic Automation/ Cortez Diagnostics, Inc., California, USA. Serum FSH and LH concentrations were measured by standard ELISA technique using assay kits of MyBioSource, Inc., USA. Statistical analysis was carried out using SPSS software version 20.0. The test of significance among and within the groups for hormonal profile was made by analysis of variance (ANOVA) and DMRT at 5 % level of significance.

Results and Discussion

Progesterone and Oestradiol-17β Profile

The mean serum progesterone and oestradiol-17β concentrations (ng/ml) at different time intervals in acyclic treated and control groups of animals are presented in Table 1. Statistical analysis did not reveal significant differences in the values of both these hormones among the three groups of buffaloes at day 0. Moreover, the mean serum progesterone level of T2 group at day 4 was significantly ($p<0.01$) higher as compared to T1 and T3 groups and the mean serum progesterone levels of T1 and T2 groups at day 8 were significantly higher ($p<0.01$) as compared to T3 group. Again on the day of estrus the serum progesterone concentration did not show significant difference among the groups. These levels again increased significantly at day 28 among all the groups without significant group variations. This might be due to estrus being ovulatory with development and maintenance of CL and establishment of pregnancy in some animals in each group.

The serum progesterone levels did not differ significantly between days 0, 4, 8 and day of estrus in T1 and T3 groups, and between days 0, 8 & day of estrus in T2 group. Significantly ($p<0.01$) higher mean serum progesterone level (2.28 ±0.32 ng/ml) recorded at 4th day in T2 might be due to the continuous release of the exogenous progesterone from the PRID. In T1 and T2 groups, the rise in mean serum progesterone level noted at day 8 might be due to luteinization of some of the growing follicles and/or ovulation of dominant follicle and formation of CL under the influence of GnRH that may not be fully regressed by $PGF_2\alpha$ on 8th day.

The serum oestradiol-17β concentrations also did not reveal any significant differences among three groups at day 0, 4, on the day of estrus/AI and at day 28. However, the values at day 8 were significantly ($p<0.01$) higher in treatment groups than in control. Likewise, the levels did not show any significant ($p>0.01$) difference at day 0 and 4 in treatment groups T1 & T2 and at day 0, 4 and 8 in control group T3, but the values increased markedly in all three groups at day 8. The oestradiol-17β concentration increased abruptly and significantly ($p<0.01$) on the day of estrus and thereafter decreased markedly on 28th day in all the groups.

The basal mean concentrations of serum progesterone and oestradiol prior to treatment in all the groups confirming anestrus status of buffaloes. These findings corroborated with reports of Buhecha *et al*. (2016), Soni *et al*. (2015) and Savalia *et al.* (2014) in CIDR/Triu-B and Ovsynch treated anestrus buffaloes. In the present study, apparently ($p>0.01$) higher mean serum progesterone concentrations were observed on day 8 with Ovsynch and Ovsynch + PRID protocol as compared to day 0 values. While, significantly ($p<0.01$) higher mean plasma progesterone concentrations were recorded by Nakrani *et al.* (2014), Savalia *et al.* (2014) and Buhecha *et al*. (2016) with Ovsynch, CIDR and Triu-B protocols. The mean serum progesterone levels obtained on the day of induced estrus with two protocols were also more or less similar to those reported by above researchers.

The mean serum oestradiol-17β concentrations on the day of induced estrus in treatment and control groups were in agreement with Batra and Pandey (1983) in Murrah buffaloes and to some extent with Rajesha *et al.* (2001) and Parmar *et al.* (2015) in anestrus Surti buffaloes. Whereas lower mean serum oestradiol-17β concentrations were reported by Tiwari *et al.* (2014) and higher mean concentrations by Gupta *et al.* (2010) in Murrah buffaloes.

FSH and LH Profile

The mean serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations at different time intervals in acyclic treated and control groups of animals are presented in Table 2. Statistical analysis did not show significant differences in FSH levels among the three groups of anestrus buffaloes at day 0, 4, on the day of estrus/AI and at day 28. However, the mean values at day 8 were significantly ($p<0.01$) higher in treatment group T1 and T2 than control group. This might be attributed to starting of 2nd follicular wave under the influence of GnRH in treatment-T1

Table 1: Serum progesterone and oestradiol-17β concentrations at different time intervals in acyclic treated and control groups of buffaloes (Mean ± SEM)

Groups/ Treatment	0 day (before treatment)	4th day (during treatment)	8th day (after $PGF_2\alpha$ inj.)	Day of estrus / AI	28th day (post treatment)	F value	P value
Progesterone (ng/ml)							
T1 (Ovsynch)	0.36 ± 0.09^{w}	$0.37 \pm 0.10_{a}^{w}$	$1.20 \pm 0.18_{b}^{w}$	0.39 ± 0.05^{w}	2.71 ± 0.66^{x}	10.24**	0.000
T2 (Ovsynch + PRID)	0.53 ± 0.10^{w}	$2.28 \pm 0.32_{b}^{y}$	$1.36 \pm 0.17_{b}^{wx}$	0.51 ± 0.09^{w}	1.69 ± 0.51^{xy}	6.95**	0.001
T3 (Control)	0.50 ± 0.04^{w}	$0.40 \pm 0.08_{a}^{w}$	$0.41 \pm 0.06_{a}^{w}$	0.31 ± 0.10^{w}	1.77 ± 0.21^{x}	29.43**	0.000
F value	1.08	28.87**	11.38**	1.41	1.08	--	--
P value	0.364	0.000	0.001	0.270	0.366	--	--
Oestradiol-17β (pg/ml)							
T1 (Ovsynch)	11.08 ± 0.88^{w}	12.08 ± 0.70^{w}	$18.08 \pm 0.67_{b}^{x}$	29.66 ± 2.30^{y}	18.25 ± 0.77^{x}	35.79**	0.000
T2 (Ovsynch + PRID)	10.08 ± 0.47^{w}	11.16 ± 0.65^{w}	$18.66 \pm 1.99_{b}^{x}$	31.75 ± 1.59^{y}	17.16 ± 1.01^{x}	45.79**	0.000
T3 (Control)	10.83 ± 0.51^{w}	11.08 ± 0.53^{w}	$11.66 \pm 0.44_{a}^{w}$	31.12 ± 2.65^{y}	16.50 ± 1.96^{x}	44.84**	0.000
F value	0.63	0.76	9.81**	0.27	0.53	--	--
P value	0.542	0.483	0.002	0.764	0.596	--	--

Means bearing different superscripts within a row (between time intervals; w,x,y) and those with subscripts within a column (between the groups; a,b) differ significantly ($p<0.05$). * $p<0.05$ & ** $p<0.01$

group and/or removal of progesterone block that lead to earlier folliculogenesis in treatment-T2 group. Likewise, the serum FSH levels did not vary significantly between day 0, 4, 8 and 28 in the treatment group T1 and control group T3, and between day 0, 4 and 28 in treatment group T2. Though, the mean FSH concentration on the day of estrus increased significantly ($p<0.01$) and thereafter decreased on day 28 in all the groups.

The mean serum LH levels of acyclic buffaloes did not differ significantly ($p>0.05$) at any day between treatment and control groups. The serum LH levels also did not show significant ($p>0.05$) variations between day 0, 4, 8 and 28 any of the groups, but the concentration on the day of estrus increased significantly ($p<0.01$) and thereafter decreased markedly to the basal levels on day 28 (day 18 post-AI) in all three groups, because of increased level of oestradiol from the mature Graafian follicle in the blood that switched on the ovulatory LH surge and thereafter LH was found at basal level during the pregnancy period due to most of the animals settled during first induced/ natural estrus.

The mean serum FSH & LH levels before treatment in all three groups were lowest and increased at subsequent periods till day of estrus. This might be due to activation of ovary by initial GnRH injection that started follicular wave at different period of time in an individual animal mingling that mean levels. The basal mean serum LH concentrations observed prior to treatment in all groups were in agreement with the report of Singh and Madan (2000). The peripheral serum LH remained at basal levels (1.36 ± 0.23 to 1.43 ± 0.20) prior to treatment (0 day) to 8th day (after $PGF_2\alpha$ inj.). While on the day of estrus it was observed significantly ($p<0.01$) higher (18.06 ± 3.22 to 21.68 ± 3.20 ng/ml) in both the treatments (T1 & T2) and control (T3) groups, suggestive of a pre-ovulatory LH surge might be occurred. Similar baseline values around 0.72-3.00 ng/ml during a major part of the estrus cycle with peak values of 20-40 ng/ml on the day of estrus have been observed by Heranjal *et al.* (1979) and Avenell *et al.* (1985).

The peripheral serum FSH concentrations found to be highest on the day of estrus were in agreement with peak concentrations ranging from 52.9 (Heranjal *et al.,* 1979) to 57.9 ng/ml (Galhotra *et al.,* 1985) on the day of estrus reported by previous workers. However, as compared

Table 2: Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations at different time intervals in acyclic treated and control groups of buffaloes (Mean ± SEM)

Groups/ Treatment	0 day (before treatment)	4th day (during treatment)	8th day (after $PGF_2\alpha$ inj.)	Day of estrus / AI	28th day (post treatment)	F value	P value
Follicle Stimulating Hormone (FSH, ng/ml)							
T1 (Ovsynch)	11.01 ± 0.81^{w}	11.40 ± 0.59^{w}	$15.51 \pm 0.68_{b}^{w}$	50.66 ± 3.86^{x}	11.20 ± 0.59^{w}	88.71**	0.000
T2 Ovsynch + PRID)	10.96 ± 0.89^{w}	10.98 ± 0.67^{w}	$16.40 \pm 0.63_{b}^{x}$	52.51 ± 2.75^{y}	11.46 ± 0.48^{w}	171.78**	0.000
T3 (Control)	10.83 ± 0.63^{w}	10.88 ± 0.54^{w}	$10.98 \pm 0.42_{a}^{w}$	50.40 ± 2.70^{x}	11.45 ± 0.78^{w}	227.15**	0.000
F value	0.01	0.20	23.96**	0.12	0.06	--	--
P value	0.986	0.818	0.000	0.885	0.937	--	--
Luteinizing Hormone (LH, ng/ml)							
T1 (Ovsynch)	1.40 ± 0.24^{w}	1.43 ± 0.18^{w}	1.41 ± 0.26^{w}	18.06 ± 3.22^{x}	1.75 ± 0.21^{w}	25.83**	0.000
T2 Ovsynch + PRID)	1.43 ± 0.20^{w}	1.40 ± 0.13^{w}	1.40 ± 0.15^{w}	21.68 ± 3.20^{x}	1.88 ± 0.18^{w}	39.15**	0.000
T3 (Control)	1.36 ± 0.23^{w}	1.40 ± 0.19^{w}	1.38 ± 0.23^{w}	18.45 ± 2.23^{x}	1.60 ± 0.29^{w}	78.77**	0.000
F value	0.02	0.01	0.00	0.43	0.37	--	--
P value	0.979	0.988	0.994	0.658	0.697	--	--

Means bearing different superscripts within a row (between time intervals; w,x,y) and those with subscripts within a column (between the groups; a,b) differ significantly ($p<0.01$). ** $p<0.01$

to present findings, much higher FSH levels as 57 to 65 ng/ml and 70 to 80 ng/ml have been observed during the beginning of estrous cycle by others (Razdan *et al.,* 1982; Palta and Madan, 1997).

As circulating concentrations of progesterone decrease, LH pulse frequency increases followed by a rapid increase in follicular estradiol secretion. Following FSH binding to membrane receptors on granulosa cells there is an increase in aromatase activity, which converts androgens to estradiol. Increased circulating concentrations of estradiol initiate estrous behavior and induce the preovulatory gonadotropin surge, which is essential for ovulation. In addition, estradiol can act within granulosa cells to increase LH receptor concentration and thereby prepare the preovulatory follicle to respond to the gonadotropin surge (Richards, 1980).

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Conflict of Interest: Authors declare no conflict of interest for this research work.

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