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Estrus Induction Response Vis-a-Vis Serum Progesterone and estradiol 17-β Profile in Postpartum Subestrus Surti Buffaloes Primed with Heatsynch Alone and Heatsynch plus PRID Protocol

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Abstract

This study was conducted on 18 subestrus Surti buffaloes at University farm to estimate steroid hormone values before, during and after use of Heatsynch (T1) and Heatsynch+PRID (T2) protocols. In the Heatsynch protocol (n=6), buffaloes were administered intramuscularly with inj. Busereline - GnRH analogue on day 0, inj. Cloprostenol sodium-$PGF_2\alpha$ analogue on day 7 and inj. Estradiol Benzoate ($EB_$) on day 8. In the Heatsynch+PRID protocol (n=6), PRID was inserted intra-vaginally and kept in situ for 7 days with inj. Buserelineon day 0. The PRID was removed on day 7 together with inj. of Cloprostenol and inj. EB was administered on day 8. FTAI was done twice on day 10 in both the groups. No treatment was given to control (n=6) group (T3). The estrus response was 100% in all three groups, but the conception rate at first cycle/service was 50.00, 66.66 and 33.33%, respectively, in three groups. The mean plasma progesterone concentrations were significantly lower on day of estrus (0.26±0.05, 0.63±0.07and 0.58±0.08 ng/ml in T1, T2 and T3 respectively) as compared to the corresponding values on day 0, 4, 8 of treatment and 18 post-AI in all the three groups.The mean values of estradiol-17β markedly increased ($p<0.01$) on the day of estrus in treatment groups T1 & T2 (43.45±1.42 pg/ml & 42.86±1.53 pg/ml) than control group T3 (35.74±0.63 pg/ml) and thereafter markedly decreased at 18th day post-AI in all the groups (17.93±4.49; 16.34±2.14; 25.52±3.10 pg/ml) with non-significant difference.

Key words: Estrus synchronization, Serum progesterone-estrogen, Subestrus, Surti buffaloes.

Introduction

Subestrus is a condition in which genital organs are undergoing normal cyclical changes but behavioral signs of estrus are not manifested. Low estrogen in high yielding animals results insubestrus condition and low intensity of estrus behavior is directly related to its concentration (Lopez *et al*., 2004). Now a days, the problem of poor estrus exhibition and its detection in the buffaloes can be ameliorated and reproductive efficiency improved by various estrus synchronization strategies including progesterone releasing intra-vaginal device/sponges (PRID) and $PGF_2\alpha$ protocol etc. Generally estrus synchronization with hormone is achieved by two approaches. The

first approach is by controlling the luteal phase of the cycle either through the administration of prostaglandins (Brito *et al*., 2002) or use of progesterone analogues (De Rensis *et al*., 2005). The second and more recent approach of synchronization is by controlling the follicular development and ovulation using combinations of prostaglandins and/or progesterone (Neglia *et al*., 2003) with GnRH (Berber *et al*., 2002), hCG (Companile *et al*., 2007) or estradiol (Bartolome *et al*., 2004). Response to any therapy is generally better in animals with optimal nutritional status. Hence, this study was carried out to evaluate the effect of different estrus synchronization protocols with FTAI on estrus induction/fertility rate, and serum progesterone and estrogen in postpartum subestrus Surti buffaloes.

Materials and Methods

The work was carried out on 18 Surti buffaloes having subestrus (silent estrus) condition around day 45 postpartum over a period of seven months from October 2015 to April 2016.Animals were divided randomly into three equal groups, six in each. In the Heatsynch protocol (T1), six subestrus Surti buffaloes were administered i/m with inj. Busereline–GnRH analogue 0.01 mg (Inj. Pregulate, 2.5 ml) on day 0, inj. Cloprostenol sodium-$PGF_2\alpha$ analogue 500 μg (Inj. Pregova, 2 ml) on day 7 and inj. Estradiol Benzoate(EB) 0.5 mg (Inj. Pregheat, 1 ml) on day 8. In the Heatsynch+PRID protocol (T2), PRID (0.958 g of progesterone; Triu-B) was inserted intravaginally and kept insitu for 7 days, beside, Heatsynch protocol followed, by FTAI twice on day 10 in both the groups. The third group served as untreated subestrus control (T3). The buffaloes in estrus were decided by observing behavioural signs of estrus.

Blood samples were collected in vaccutainers from all animals on day 0 (prior to treatment), 4 (during treatment), 8 (after prostaglandin inj.), 10 (day of estrus) and 28 (day 18 post-AI) post-treatment by jugular vein puncture. The serum was separated after clotting of blood in 1-2 hrs by centrifugation at 3000 rpm for 15 minutes and stored at –20°C in deep freezer until analyzed. Serum progesterone and estrogen concentrations were measured by standard Enzyme Linked Immuno Sorbent Assay (ELISA) technique using assay kits and procedure described by Diagnostic automation/Cortez Diagnostics, Inc., California, USA.

The data were analysed for Mean ± SE, ANOVA and DNMRT using Statistical Package for Social Sciences (SPSS) software version 20.0.

Results and Discussion

Estrus Induction Response

The induction of overt estrus was cent per cent in all three groups. Moreover, all of the six buffaloes in treatment groups T1 & T2 came in heat with high estrus intensity within one or two days following i/m inj. of EB, while the buffaloes from the control groupT3 remained subestrus and showed estrus at 69 to 110 days (i.e. within 45 to 120 days postpartum) with apparently lower estrus intensity as compared to treatment groups.The conception rate at induced estrus/first cycle was 50.00, 66.66 and 33.33%, respectively, in Heatsynch (T1) Heatsynch+PRID (T2) and Control group (T3), respectively.

Serum Progesterone Profile

The mean serum progesterone concentration (ng/ml) at different intervals in subestrus treated and control groups of animals is presented in Table 1. The serum progesterone concentrations in all the silent estrus buffaloes prior to the treatment ranged from 1.25 to 3.00 ng/ml confirming cyclic/ subestrus condition.

The mean serum progesterone concentration did not show significant difference among three groups of subestrus buffaloes on day 0 (prior to treatment), 4 (during treatment) and 8 (after $PGF_2\alpha$ injection). The levels increased non-significantly at day 28 (day 18 post-AI) among all the three

Table 1:Serum progesterone (ng/ml) and estradiol-17β (pg/ml) pattern at different time intervals in treated and control groups of subestrus buffaloes (Mean±SEM)

Groups/ Treatments	0 day (before treatment)	4th day (during treatment)	8th day (after $PGF_2\alpha$ inj.)	10thday (day of Estrus/AI	28th day post-treatment (day 18 post-AI)
Progesterone (P_4)					
Group I	2.42 ± 0.25^{z}	2.46 ± 0.14^{z}	1.29 ± 0.20^{y}	$0.26\pm0.05_{a}^{x}$	2.32 ± 0.29^{z}
Group II	1.91 ± 0.17^{y}	2.32 ± 0.08^{y}	1.04 ± 0.18^{x}	$0.63\pm0.07_{b}^{x}$	2.23 ± 0.32^{y}
Group III	1.92 ± 0.16^{y}	1.96 ± 0.13^{y}	1.54 ± 0.28^{y}	$0.58\pm0.08_{b}^{x}$	1.86 ± 0.14^{y}
Estradiol-17β (E2)					
Group I	12.25 ± 1.27^{x}	$17.11\pm1.90_{b}^{x}$	$13.25\pm0.76_{a}^{x}$	$43.45\pm1.42_{b}^{y}$	17.93 ± 4.49^{x}
Group II	14.03 ± 2.37^{xy}	$12.32\pm0.89_{a}^{x}$	$17.88\pm1.10_{b}^{y}$	$42.86\pm1.53_{b}^{z}$	16.34 ± 2.14^{xy}
Group III	12.60 ± 1.65^{x}	$18.03\pm1.25_{b}^{y}$	$12.00\pm1.33_{a}^{wx}$	$35.74\pm0.63_{a}^{z}$	25.52 ± 3.10^{z}

Means bearing different superscripts within a row (x,y,z between time intervals) and within a column (a,b,c between the groups) differ significantly ($p<0.05$).

groups (2.32±0.29, 2.23±0.32 and 1.86±0.14 ng/ml) due to establishment of pregnancy and presence of CL in some animals. The mean progesterone levels of the blood serum increased non-significantly at 4th day (during treatment) and thereafter decreased significantly ($p<0.01$) at 8th day (a day after $PGF_2\alpha$ injection) in treatment groups T1 & T2, while non-significantly decreased in control group T3. Again on the day of estrus the concentration decreased significantly ($p<0.05$) in T1 and T3 groups and non-significantly in T2 group. Moreover, mean serum progesterone levels increased significantly ($p<0.01$) in all the groups at 18th day post-AI. The increased levels found at 4th day might be due to maturation of the CL under GnRH inj. in the treatment group T1 and/ or continuous release of the exogenous progesterone from the PRID (Triu-B) inserted in the treatment group T2.

These findings corroborated well with value 1.42 to 5.52 ng/ml reported by Rede *et al.* (2016) in subestrus Surti buffaloes. However, higher mean serum progesterone concentrations of 3.36±0.50 ng/ml (Butani*et al.,* 2011) and 2.89±0.14 to 3.22±0.19 ng/ml (Chaudhary *et al.,*2015) in subestrus postpartum Surti buffaloes, 2.90±0.46 ng/ml (Dugwekar *et al.,* 2008) in Jafarabadi buffaloes, and 4.70±1.27 ng/ml (Tiwary, 2010) in cyclic Murrah buffaloes have been documented.The present mean progesterone value of 0.49±0.05 ng/ml (0.26±0.05 to 0.63±0.07 ng/ml) on the day of estrus in the treatment and control groups of buffaloes agreed to some extent with Chaudhary *et al.* (2015) in Surti buffaloes, and Dugwekar *et al.* (2008) in Jafrabadi buffaloes. However much lower level at the time of estrus as 0.20 ng/ml was reported by Mirmahmoudi *et al*. (2014) following double synch protocol in cyclic Murrah buffaloes.

Serum Estradiol-17β Profile

The mean serum estradiol-17β concentration (pg/ml) also did not show significant difference among the three groups of subestrus buffaloes on day 0. However, the mean level in treatment group T2 at 8th day was significantly ($p<0.01$) higher than that of treatment group T1 and control group T3. Again, on the day of estrus, mean serum estradiol-17β concentrations in both treatment groups were significantly ($p<0.01$) higher as compared to control group. The mean levels again decreased non-significantly at day 28 among all the three groups (17.93±4.49, 16.34±2.14 and 25.52±3.10 pg/ml) due to ovulatory heat with settlement of pregnancy in some animals. The mean serum estradiol-17β concentration at 8th day **(**after $PGF_2\alpha$ injection) decreased non-significantly in group

T1, and significantly ($p<0.01$) in group T3, but increased significantly ($p<0.01$) in group T2. Again on the day of estrus, the mean concentration increased significantly ($p<0.01$) among all three groups.

The mean serum estradiol-17β concentration found on the day of induced estrus in treatment and natural estrus in control group closely agreed with reports of Chaudhary *et al*. (2015) in subestrus Surti buffaloes and Dugwekar*et al*. (2008) in postpartum Jafarabadi buffaloes, whereas higher mean concentration of 57.2±14.3 pg/ml was documented by Mirmahmoudi *et al*. (2014) using Heatsynch protocol in Murrah buffaloes. On the other hand, lower level on the day of estrus was reported as 19.50±5.51 pg/ml by Singh *et al.* (2001) in buffaloes, and 31.33±0.97 pg/ml by Caesar *et al*. (2013) using Heatsynch protocol in Kankrej cows.

Conclusion

The diagnosis of subestrus (silent estrus) condition could be done accurately by rectal palpation in large animals as serum P_4 concentration was above 1 ng/ml. Hence, early detection and hormonal treatment of subestrus condition can be planned to improve reproductive efficiency in those buffaloes. The linear increasing trend of mean serum estrdiol-17β concentration observed over the period of time with Heatsynch protocol alone and in combination with PRID treatment indicated resumption of ovarian follicular activity, ovulation and conception in these groups.

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Conflict of Interest: All authors declare no conflict of interest.

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