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SERUM PROGESTERONE AND OESTRADIOL-17β PROFILE IN NORGESTOMET PRIMED POSTPARTUM SILENT ESTRUS SURTI BUFFALOES

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The study was conducted on eighteen postpartum silent oestrus Surti buffaloes to evaluate the efficacy of Norgestomet ear implants alone and in combination with $PGF_2\alpha$. The buffaloes in Group-I & Group-II were implanted with Crestar for 9 days along with 2 ml injection of Crestar solution given on the day of implant insertion. In Group-II, additionally 500 µg Cloprostenol was given on one day before implant removal. Whereas, the buffaloes in Group-III served as silent oestrus control group and 5 ml normal Saline was given on 0 day and on 8th day as a placebo treatment. In the Norgestomet treated groups the significant decreasing trend of endogenous progesterone (P_4) concentration observed at different time intervals of ear implant inserted followed by Norgestomet injection given treatment groups of animals. Oestradiol-17β levels of the blood serum did not show any significant difference ($p > 0.05$) at 0 day, 5th day within the Group-I, Group-II & Group-III. But the mean values of Oestradiol-17β markedly increased thereafter at 10th day and on the day of estrus in the Group-I, Group-II & Group-III and showed significant difference ($p < 0.05$) during that various time interval within that groups and also reflected the same picture in the overall mean value of serum Oestradiol-17β at different time intervals. Drastic increasing level of Oestradiol-17β might have influenced the earlier follicular activity followed by early estrus in the treatment groups, while steady increase in Oestradiol-17β in control group might have showed late follicular activity in that group lead to late estrus.

Key words: Norgestomet, Oestradiol-17β, $PGF_2\alpha$, Postpartum Silent estus, Progesterone

Progesterone in cyclic animals acts as a regulator of dioestrus period, because as soon as the corpus luteum fails to secrete progesterone, development of follicles begins leading to pro-oestrus phase. The immediate precursor for progesterone is pregnenolone, which is derived from cholesterol, which in turn is synthesized from acetyl-CoA (Hafez, 1980). Estrogens are hormones produced by the ovary and are transported in the body by binding proteins. Estrogens play a key role in the regulation of the endocrine and behavioral events associated with the estrous cycle. Estrogens act on the Central Nervous System in order to induce behavioural estrus in females and the most important of these hormones is estradiol. Oestradiol-17β (E_2) induces the preovulatory luteinizing hormone (LH) surge as an "all or nothing" event. After a certain threshold of Oestradiol-17β (E_2) is reached there will be a LH surge, which will result in ovulation (Lyimo *et al.*, 2000). The gonadal hormones are often measured in farm animals to assess the ovarian status or cyclical activity of breeding females. Measurement of reproductive hormones, estrogen & progesterone in general and progesterone in particular helps in assessing the efficacy of preparation or device used for assessing the stage of cyclical activity in experimental female animal.

MATERIALS AND METHODS

The study was conducted on eighteen silent estrus (Suboestrus) Surti buffaloes from 45 to 120 days postpartum. All these buffaloes

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had normal calving and subsequent normal genital health as assessed Gynaeco-clinically. Oestrus occurrence was detected daily in them with the help of teaser bull parading in morning and evening hours. The animals which were not exhibiting overt signs of oestrus during routine heat detection program were segregated and subjected to rectal palpation. The animals with palpable structures either corpus luteum (CL) or follicle, on either of the ovaries were selected for another palpation after eleven days apart to ascertain their cyclic nature and considered as silent heat (suboestrus) buffaloes.

Grouping of experimental animals: The buffaloes in Group-I (T1) & Group-II (T2) were implanted with siliastic Crestar ear implant (3.3 mg Norgestomet, Intervet International B.V. Boxmeer, Netherlands) subcutaneously in the middle of the outer surface of the ear pinnae with the help of special applicator along with injection of 2 ml Crestar solution (Intervet International B.V. Boxmeer, Netherlands) containing 3 mg Norgestomet and 5 mg Oestradiol Valerate given immediately after inserting implant. After nine days in situ position; the implants were removed by nicking the skin at the outer end of the implant and expressing it with thumb. In addition to Crestar ear implant & injection of Crestar solution, the buffaloes in Group-II (T2) were also received Injection Pragma (500 µg Cloprostenol, Intas pharmaceuticals Ltd, Ahmedabad, India.) on day 8, a day before ear implant removal and the buffaloes in Group-III (T3) were served as control and given 5ml normal saline as Placebo treatment on 0 (zero) day and 8th day.

Blood collection: Approximately 10 ml blood samples were collected from all those selected animals on 0 day (prior to treatment), 5th day (during treatment), 10th day (after treatment) and on day of estrus aseptically by jugular vein puncture. The schedule of the blood sampling was made in order to know the probable changes in hormonal, metabolic and trace elements profile before implant insertion, after implant insertion when implant was kept in situ, after removal of implant and during induced estrus. The vaccutainers4 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 labeled sterilized 5 ml plastic storage vials at –20°C in deep freezer until analysis.

Hormone assay: Serum Progesterone concentrations were measured by using a commercially available Progesterone Enzyme Immunoassay Kit (DSI S.r.l. Saronno, Via A. Volonterio, Italy). Serum Oestradiol-17β concentrations were measured by using a commercially available EIA kit (Diagnostics Biochem Canada Inc., Canada). A standard curve was obtained by plotting the concentration of the standard versus the absorbance. The validity tests and standardization of the ELISA was performed by preparing the standard curve and working out the sensitivity, intra and inter assay variation for all the assays. The sensitivities of Progesterone and Oestradiol-17β kit were 0.5 nmol/l and 10 pg/ml, respectively. The intra and inter assay coefficients of variation were 4.6 per cent and 5.3 per cent for the Progesterone kit, respectively and 9.3 per cent and 10.1 per cent for the Oestradiol-17β kit, respectively.

Statistical analysis: The data collected were suitably tabulated and analyzed following standard statistical methods shown by Steel and Torrie (1981). The animals were divided into three groups using completely randomized design (CRD) technique. The test of significance among and within the groups for serum Progesterone and Oestradiol-17β concentrations were made by analysis of variance (ANOVA) and the mean differences between and within the groups were tested using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Serum Progesterone (P_4) profile:

Statistical analysis of the data generated in respective of the treatment on the progesterone concentration (ng/ml) on the blood serum, did not show any significant difference ($p > 0.05$) among the three groups of suboestrus Surti buffaloes under study at 0 day (before treatment) 5th day (during treatment), Moreover, the mean serum

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Table 1. Serum Progesterone (P_4) level (ng/ml) pattern at different time intervals / days in silent oestrus treated and control groups of animals (Mean ± SEM)

Time intervals / Days	Group-I	Group-II	Group-III	*F value*	*P value*
0 day (pre treatment)	$2.89 \pm 0.14_a^z$	$3.16 \pm 0.18_a^y$	$3.22 \pm 0.19_a^y$	1.009	0.388
5th day (during treatment)	$1.24 \pm 0.09_a^y$	$1.23 \pm 0.11_a^x$	$1.77 \pm 0.30_a^x$	2.593	0.108
10th day (post treatment)	$0.76 \pm 0.08_{ab}^x$	$0.62 \pm 0.07_a^w$	$1.22 \pm 0.25_b^x$	4.178*	0.036
Day of estrus	$0.41 \pm 0.02_a^w$	$0.36 \pm 0.02_a^w$	$0.45 \pm 0.04_a^w$	2.345	0.130
F value	139.83**	125.435**	36.95**		
P value	0.000	0.000	0.000		

Means bearing different superscripts within a column (between time intervals) and means bearing different subscripts within a row (between the groups) differ significantly ($p < 0.05$). * $p < 0.05$ & ** $p < 0.01$

Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + $PGF_2\alpha$), Group-III = T3 (Silent oestrus Control)

progesterone value at 10th day (post treatment) between T1 & T2 (treated groups) as well as between treatment (T1) group & control (T3) group did not differ significantly ($p > 0.05$). However, the mean serum progesterone level of control (T3) group at 10th day of treatment was differed ($p < 0.05$) significantly as compared to treatment (T2) group. Again on the day of estrus the progesterone concentration of the buffalo serum did not show significant difference ($p > 0.05$) among three groups of suboestrus Surti buffaloes.

Like this way, when the statistical analysis of the data compared within the group at different time intervals, the progesterone levels of the blood serum varied significantly ($p < 0.05$) between 0 day, 5th day, 10th day and on the day of estrus in Group-I, between 0 day, 5th day & 10th day in Group-II, and between 0 day, 5th day & on the day of estrus in control Group-III but did not varied significantly ($p > 0.05$) at 10th day and on the day of estrus and 5th day and 10th day in the treatment (T2) group &control Group-III, respectively. Again on the day of estrus the mean levels of progesterone markedly decreased and showed significant difference ($p < 0.05$) with different time interval within that respective treatment Group-I, treatment Group-II & control Group-III and differed within that group at various time intervals.

The sudden drop and fluctuated nature of progesterone on the 5th day (during treatment) and 10th day (post treatment) after withdrawal of Crestar ear implant may have played contributory role in the early induction of estrus in the treatment (T1 & T2) groups while still fluctuated and steady decrease level between 5th day (during treatment) and 10th day (post treatment) in the control (T3) group might be responsible and made them prepare late and delaying in the onset of estrus in that group.

The mean serum progesterone levels of silent oestrus in Surti buffaloes revealed that the ovaries were cyclic with palpable structure when examined per-rectally and was further confirmed by serum progesterone estimation. The mean serum progesterone concentration in the present study prior to insertion of implant in the treatment and control groups were at subluteal level (2.89 ± 0.14 to 3.22 ± 0.19 ng/ml) confirming the silent oestrus state in Surti buffaloes. These findings were in corroborated with 2.90 ± 0.46 ng/ml and 3.05 ± 0.63 ng/ml (ranging from 0.31 to 5.86 ng/ml) reported by Dugwekar *et al.* (2008) in Jafarabadi buffaloes and Jain (1994) in suboestrus crossbred cows, respectively.

The mean serum progesterone concentration in silent oestrus Surti buffaloes (range from 2.89 ± 0.14 to 3.22 ± 0.19 ng/ml) prior to insertion of implant in the treatment and control groups which was slightly lower as

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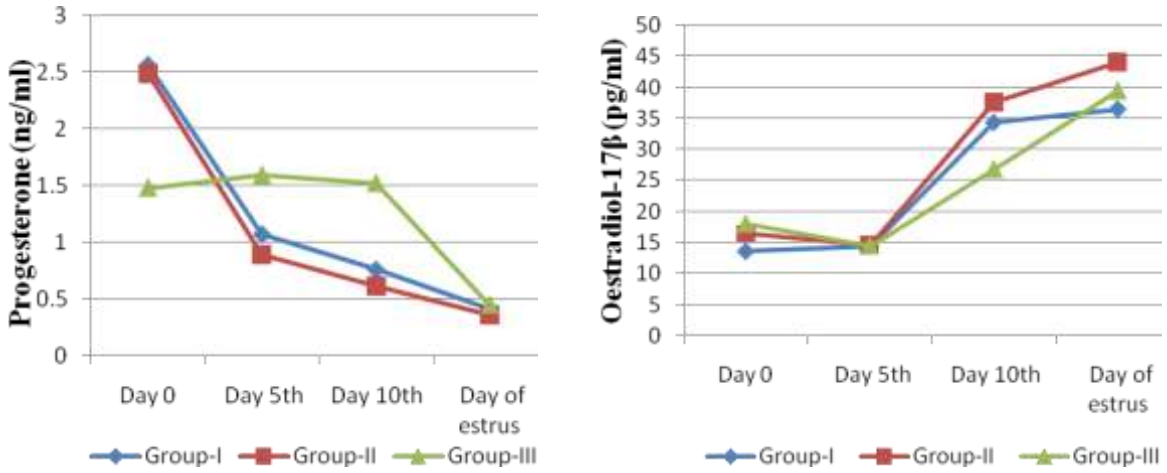

Fig. 1. Serum Progesterone (P_4) concentrations (ng/ml) and serum Oestradiol-17β (E_2) concentrations (pg/ml) of silent oestrus Surti buffaloes in different groups at different time intervals

compared to 3.29 ± 0.23 to 4.13 ± 0.15 ng/ml and 3.36 ± 0.50 ng/ml reported by (Rede, 2014 and Butani *et al.*, 2011), respectively in suboestrus Surti buffaloes. Moreover, serum progesterone concentration as above 1 ng/ml reported by Ullah *et al.* (2006) in cyclic or suboestrus Nilli-Ravi buffaloes; 1.3 ng/ml reported by Hoagland and Barnes (1984) in postpartum cyclic beef cows; 1.30 ± 0.04 ng/ml reported by (Khasatiya, 2003 and Khasatiya *et al.*, 2006) in postpartum suboestrus Surti buffaloes; 1.39 ± 0.13 ng/ml reported by Mondal and Prakash (2003) in suboestrus cows and 2.68 ± 0.75 ng/ml reported by Chede *et al.* (1992) in Cyclic Berari (Nagpuri) buffaloes. As compare to present finding, very low progesterone concentration observed as 0.10 ± 0.01 ng/ml (before insertion of implant) by Chaudhari (2005) in the delayed pubertal Kankrej heifers and 0.54 ± 0.01 ng/ml by Sharma *et al.* (1999) in suboestrus buffaloes-heifers and 0.71 ± 0.03 ng/ml by Ghuman *et al.* (2010) in cycling buffaloes.

The non-significant difference was observed in the progesterone concentration between treatment Group-I (0.41 ± 0.02 ng/ml), treatment Group-II (0.36 ± 0.02 ng/ml) and control Group-III (0.45 ± 0.04 ng/ml) on the day of estrus with Norgestomet ear implant alone and in combination with $PGF_2\alpha$. These findings were also well supported by Fanning *et al.* (1992), who also reported non-significant difference in progesterone concentration on the day of estrus with Norgestomet ear implant alone and in combination with $PGF_2\alpha$.

In the Norgestomet treated groups (T1 & T2) the significant decreasing trend of endogenous progesterone (P_4) concentration observed at different time intervals of ear implant inserted followed by Norgestomet injection given treatment groups of animals. (fig. 1). This conclusive statement supported well by Hoagland and Barnes (1984), Cavalieri *et al.* (1998) and Pinheiro *et al.* (1998). They also reported significant decline in serum progesterone concentration from the day of insertion of implant to the day 5^{th} and day 9^{th} (day of removal). According to (Nath *et al.*, 2003^{b}) this may be due to the leuteolytic effect of estradiol valerate used in the implant. Whereas, Hoagland and Barnes (1984) opined that the endogenous progesterone secretion was inhibited by Norgestomet ear implant. However, Fanning *et al.* (1992) reported almost constant mean progesterone concentration after 6^{th} day of treatment and on the day of implant removal. Moreover, Norgestomet treated groups revealed no more concentration of progesterone in the serum during hormonal estimation, though it has progesterone analogue effect. This finding avers the observations of Barnes *et al.* (1981), who opined that the lack of changes of serum progesterone concentration from the day of implant

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Table 2. Serum Oestradiol-17β (E_2) level (pg/ml) pattern at different time intervals / days in silent oestrus treated and control groups of animals (Mean ± SEM)

Time intervals / Days	Group-I	Group-II	Group-III	*F value*	*P value*
0 day (pre treatment)	$13.59 \pm 1.30_a^w$	$16.39 \pm 1.98_a^w$	$18.04 \pm 2.32_a^w$	1.380	0.282
5th day (during treatment)	$14.44 \pm 0.58_a^w$	$14.53 \pm 1.11_a^w$	$14.50 \pm 0.98_a^w$	0.002	0.998
10th day (post treatment)	$34.29 \pm 0.62_b^x$	$37.57 \pm 0.60_b^x$	$26.86 \pm 1.99_a^x$	19.186**	0.000
Day of estrus	$39.60 \pm 1.63_a^y$	$44.14 \pm 0.66_b^y$	$36.42 \pm 0.96_a^y$	11.227**	0.001
F value	141.875**	149.279**	34.212**		
P value	0.000	0.000	0.000		

Means bearing different superscripts within a column (between time intervals) and mean bearing different subscripts within a row (between the groups) differ significantly ($p < 0.05$). * $p < 0.05$ and ** $p < 0.01$

Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + $PGF_2\alpha$), Group-III = T3 (Silent oestrus Control)

insertion to removal may be due to the inability of the RIA to detect the Norgestomet in the samples because Norgestomet does not cross react with progesterone of RIA.

***Serum Oestradiol-17β (E_2) profile*:**

The mean serum Oestradiol-17β concentration (pg/ml) did not showed any significant difference ($p > 0.05$) among the three groups of suboestrus surti buffaloes under study at 0 day (before treatment), 5th day (during treatment). Moreover, the mean serum Oestradiol-17β value at 10th day (post treatment) between T1 & T2 (treatment) groups did not varied ($p > 0.05$) significantly but each treatment (T1, T2) varied ($p < 0.05$) with control (T3) group. On the contrary, significantly higher ($p < 0.05$) mean serum concentration of Oestradiol-17β was observed in treatment (T2) group on the day of estrus as compared to treatment (T1) group and control (T3) group, but revealed at par between treatment (T1) group & control (T3) group and reflected the same picture in the overall mean value of serum Oestradiol-17β among the three groups.

The mean serum Oestradiol-17β concentration in the present study prior to insertion of implant in treatment groups as well as in control group were at basal level (13.59 ± 1.30 to 18.04 ± 2.32 pg/ml) confirming the silent oestrus state in these buffaloes. The findings about mean serum Oestradiol-17β levels of active ovaries in Surti buffaloes revealed that the ovaries were cyclic with palpable structure when examined per-rectally and was further confirmed by serum Oestradiol-17β estimation.

These findings were in agreement with the reports of Chede *et al.* (1992), who reported the levels of Oestradiol-17β as 14.98 ± 1.74 pg/ml in cyclic (Birari) Nagpuri buffaloes. Whereas, as compared to present values, lower Oestradiol-17β concentration ranging from 8.6 ± 0.49 pg/ml and 12.15 ± 0.89 pg/ml reported by (Singh *et al.*, 1998 and Bonia and Goswami, 2011), respectively in buffaloes and crossbred cows before insertion of Norgestomet ear implant. In the present study, non-significant difference was observed in the Oestradiol-17β concentration between treatment Group-I (13.59 ± 1.3 pg/ml), treatment Group-II (16.39 ± 1.98 pg/ml) and control Group-III (18.04 ± 2.32 pg/ml) on the day prior to treatment with Norgestomet ear implant alone and in combination with $PGF_2\alpha$. These findings were also well supported by Nath *et al.* (2003[a]), who also reported non-significant difference in mean serum Oestradiol-17β concentration on the day prior to treatment with Norgestomet ear implant.

Moreover, Oestradiol -17β levels of the blood serum did not show any significant difference ($p > 0.05$) at 0 day (pre treatment) and 5th day (during treatment) within the

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Group-I (Norgestomet), Group-II (Norgestomet + $PGF_2\alpha$) and Group-III (Silent oestrus control). However, the mean values of Oestradiol-17β markedly increased thereafter at 10th day (post treatment) and on the day of estrus in the Group-I (Norgestomet), Group-II (Norgestomet + $PGF_2\alpha$) & Group-III (Silent oestrus control) and showed significant difference ($p < 0.05$) during that various time interval within that groups might be due to exogenous estradiol treatment enhances the growth of the recruitment of new follicular waves by encouraging gonadotropin secretion and the effect was found to be most consistent when combined with progesterone. Termination of follicular wave results in emergence of a new follicular wave 3 to 5 days later to ensure presence of a new growing dominant follicle at the termination of progestin treatment (Garcia and Salaheddine, 2001) and administration of estradiol combined with the progesterone treatment causes atresia of antral follicles and recruitment of a new cohort of follicles 4 to 5 days after administration (Vasconcelos *et al.,* 1994). Same phenomenon may be somewhat attributed to in control groups after rectal palpation and placebo treatment.

The mean serum Oestradiol-17β levels gradually increased from the 5th day of implant insertion to the day of induced estrus in the treatment groups and the values were found highest on the day of estrus within the treatment Group-II (Norgestomet + $PGF_2\alpha$) followed by treatment Group-I (Norgestomet) and control Group-III (Silent oestrus control). Gradual rising trend in mean serum Oestradiol-17β levels found here from the 5th day of implant insertion to the day of induced estrus might have influenced the follicular activity followed by early estrus in the treatment (T1 & T2) groups while steep and fluctuated increase in Oestradiol-17β in control (T3) groups might have showed follicular activity in that group lead to two animals came in estrus earlier while rest were came little bit late in estrus

The mean serum Oestradiol-17β concentration in the present study on the day of induced estrus in treatment groups (range from 39.60 ± 1.63 to 44.14 ± 0.66 pg/ml) were in agreement with 40.20 ± 19.68 pg/ml reported by Dugwekar *et al.* (2008) in Jafarabadi buffaloes and 41.02 ± 11.11 pg/ml reported by Chede *et al.* (1992) in cyclic (Birari) Nagpuri buffaloes and 41.50 ± 3.08 pg/ml reported by Bonia and Goswami, (2011) in crossbred cow, respectively. Similarly, the mean serum Oestradiol-17β concentration on the day of estrus in control group was 36.42 ± 0.96 pg/ml (ranging from 34.06 - 39.74 pg/ml). Our this findings were some extent corroborated with mean range (16.33 ± 4.67 to 34.32 ± 8.77 pg/ml) of Rajesha *et al.* (2001) in buffaloes. However, these findings were slightly higher as compare with the findings of Batra and Pandey (1983), who reported 30 - 35 pg/ml range of serum Oestradiol-17β concentration in Murrah buffaloes. Whereas, as compare to present values, lower mean Oestradiol-17β concentration as 16.19 ± 8.50 pg/ml reported by Dugwekar *et al.* (2002) in Jafarabadi buffaloes; 19.23 ± 2.14 pg/ml reported by Dhali *et al.* (2005) in Mithun (Bos frontalis); 19.32 ± 3.73 pg/ml and 19.50 ± 5.51 pg/ml reported by (Bachlaus *et al.,* 1979 and Singh *et al.,* 2001), respectively in buffaloes. Moreover, slightly higher serum Oestradiol-17β concentration reported as 59.93 ± 7.29 pg/ml by Kandiel *et al.* (2014) on the day of estrus in Egyptian buffaloes.

The Oestradiol-17β concentration on the day of estrus differs significantly within and between the treatment Group-I (Norgestomet), treatment Group-II (Norgestomet + $PGF_2\alpha$) and control Group-III (Silent oestrus control). The higher level of serum Oestradiol-17β concentration on the day of estrus was found to be 44.14 ± 0.66 pg/ml in treatment Group-II as compare to treatment Group-I (39.60 ± 1.63 pg/ml) and control Group-III (36.42 ± 0.96 pg/ml). The differences in concentrations of Oestrdiol-17β (E_2) in circulation in buffaloes treated with synthetic progestins may be caused by a greater LH pulse frequency which in turn may alter ovarian follicular development and further increase concentrations of Oestrdiol-17β (E_2).

It has been reported that cows treated with Norgestomet have an increased frequency of LH pulses and elevated circulating concentrations of Oestrdiol-17β (E_2), which

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are associated with increased size, estrogenic capacity and number of LH receptors of the largest ovarian follicle (Garcia-winder *et al.*, 1987). Roberson *et al.* (1989) reported that concentrations of Oestrdiol-17β (E_2) were higher and the onset of the preovulatory surge of LH was earlier after removal of the source of progesterone.

It could be concluded that the diagnosis of silent estrus condition could be done accurately by rectal palpation in large animals but to arrive at true nature it should be coupled with estimation of P_4 profile. Hence, early detection and hormonal treatment of silent estrus condition can be planned to improve reproductive efficiency in those buffaloes. The linear increasing trend of mean serum Oestrdiol-17β concentration observed over the period of time with Crestar ear implant alone and in combination with PMSG treatment indicated resumption of ovarian activity and ovulation with high value in the treatment groups might be attributed to resumption of ovarian follicular activity.

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