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

S.C. Parmar, C.T. Khasatiya, K.K. Tyagi, J.K. Chaudhary and R.V. Patel

Department of Animal Reproduction, Gynaecology & Obstetrics,

Vanbandhu College of Veterinary Science and Animal Husbandry,

Navsari Agricultural University, Navsari, Gujarat, India

Received 12-01-2015 Accepted 20-3-2015

Corresponding author : dr.sanjayparmar@yahoo.com

ABSTRACT

The study was conducted on eighteen postpartum anoestrus Surti buffaloes to evaluate the effect of Norgestomet ear implant alone and in combination with PMSG treatment on estrus induction and serum progesterone and œstradiol-17β profile. The buffaloes in Group-I and Group-II were treated with Crestar ear implant for 9 days together with 2 ml i/m injection of Crestar solution on the day of implant insertion. In Group-II, additionally 500 IU PMSG was given i/m on the day of implant removal, whereas the buffaloes in Group-III received 5 ml normal saline i/m on day 0 and 9 as a placebo and served as anoestrus control. The estrus was induced in cent per cent of buffaloes in Gr-I and Gr-II with mean estrus induction intervals of 2.56 ± 0.34 and 2.40 ± 0.29 days, respectively and the conception rate at induced estrus was highest (66.67 %) in Gr-II followed by Gr-I (33.33 %). In the Norgestomet treated groups a significant decreasing trend of endogenous progesterone concentration was observed at different time intervals of ear implant insertion. The mean serum oestradiol-17β levels gradually increased from the day of implant insertion to the day of induced estrus in the treatment groups and the values were found to be the highest on the day of estrus in the treatment Group-II followed by Group-I and the least in control Group-III. 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 follicular activity and ovulation in anoestrus buffaloes.

KEY WORDS: Postpartum anoestrus, Surti buffaloes, Norgestomet, Oestradiol-17â, PMSG, Progesterone

INTRODUCTION

Progesterone in cyclic animals acts as a regulator of diestrus period, because as soon as the corpus luteum fails to secrete progesterone, development of follicles begins under pituitary FSH release leading to pro-estrus 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) at certain threshold level induces the preovulatory luteinizing hormone (LH) surge resulting in ovulation of Graafian follicle (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 and progesterone in general and progesterone in particular helps in assessing the efficacy of hormone preparations or devices like CIDR, Crestar etc used for inducing cyclical activity in experimental or clinical animals. This study was aimed to evaluate the effect of Norgestomet ear implant alone and in combination with PMSG on estrus induction and serum progesterone and oestradiol profile in anoestrus Surti buffaloes.

MATERIALS AND METHODS

Experimental animals:

The study was conducted on eighteen anoestrus (inactive ovaries) Surti buffaloes from 45 to 120 days postpartum maintained at University farm, Navsari, Gujarat from November, 2013 to April, 2014. All these buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. Estrus occurrence was detected daily in them with the help of teaser bull parading in morning and evening hours. The animals with smooth inactive ovaries (no palpable follicle or corpus luteum) on twice per rectal palpation 11 days apart were considered as postpartum anoestrus buffaloes. They were then randomly divided into 3 equal groups each of 6 buffaloes.

The buffaloes in Group-I & Group-II were implanted with siliastic Crestar ear implant (3.3 mg Norgestomet, Intervet) subcutaneously in the middle of the outer surface of the ear pinnae with the help of special applicator along with i/m injection of 2 ml Crestar solution containing 3 mg Norgestomet and 5 mg oestradiol valerate. After nine days, the implants were removed by nicking the skin at the outer end of the implant and expressing it with thumb. Buffaloes in Group-II also received additional Injection of 500 IU PMSG (Folligon, Intervet) on day 9, immediately after implant removal, while buffaloes in Group-III served as control and were given 5 ml normal saline i/m as placebo treatment on day 0 and 9. The animals detected in estrus were inseminated/bred naturally and in non-return cases pregnancy was confirmed per rectum 60 days later.

Blood collection:

Approximately 10 ml blood samples were collected in the vaccutainers without anticoagulant from all those selected animals on day 0 (prior to treatment), 5 (during treatment), 10 (after treatment) and on day of estrus by jugular vein puncture. The serum was separated out after clotting of blood by centrifugation at 3000 rpm for 15 minutes and stored at –20°C in deep freezer until analyzed.

Hormone assay:

Serum progesterone concentrations were measured by using a commercially available Enzyme Immunoassay Kit (DSI S.R.L. Saronno (VA), Italy). Serum œstradiol-17β concentrations were measured by using a commercially available EIA kit (Diagnostics Biochem., Canada, Inc.). A standard curve was obtained by plotting the concentrations of the standard versus the absorbance. The sensitivities of progesterone and oestradiol-17β kits were 0.5 nmol/l and 10 pg/ml, respectively. The intra- and inter-assay coefficients of variation were 4.6 and 5.3 per cent for the progesterone, and 9. and 10.1 per cent for the oestradiol-17β, respectively.

Statistical analysis:

The data on hormone profile were suitably tabulated and analyzed following standard statistical methods of ANOVA and DNMRT (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The cent per cent buffaloes exhibited estrus in Gr-I and Gr-II with mean estrus induction intervals of 2.56 ± 0.34 and 2.40 ± 0.29 days, respectively. These intervals were significantly ($P<0.01$) shorter as compared to control Gr-III (30.33 ± 0.95 days). The duration of estrus differed significantly ($P<0.01$) being longest (25.50 ± 0.76 hrs) in Gr-II followed by Gr-I (22.17 ± 0.65 hrs) and the least in control Gr-III (18.67 ± 0.77 hrs). The conception rate at induced estrus was highest (66.67 %) in Gr-II followed by Gr-I (33.33 %) and the least (16.67%) in control Gr-III, indicating that the Norgestomet with PMSG could be the protocol of choice as compared to Norgestomet alone in treating postpartum anoestrus condition in buffaloes. These findings are in closely corroborated with the earlier reports of Yadav *et al.* (2001), Patel *et al.* (2003) and Malik *et al.* (2011) in anoestrus

buffaloes.

Serum Progesterone (P_4) Profile:

The mean serum progesterone concentrations (ng/ml) at different time intervals in anoestrous treated and control groups of animals are presented in Table 1. The serum progesterone concentrations did not show any significant variation among the three groups of anoestrus buffaloes on any of the days or intervals studied, except on day 10th post-treatment wherein control group had significantly higher P_4 than the treated groups. Similarly, the progesterone levels did not differ significantly between day 0 and 5 in any of the groups, but the values decreased significantly ($P<0.01$) thereafter on day 10 and further on the day of induced estrus in both the treatment Groups. The sudden drop in the serum progesterone levels at 10th day, i.e. 3rd day after withdrawal of Crestar ear implant played significant role in the early induction of estrus in the treatment groups, while still elevated level in the control group might be responsible for delaying the onset of estrus in that group.

The initial mean serum progesterone levels of buffaloes revealed that the ovaries were acyclic without palpable structure when examined per rectally. The mean serum P_4 levels prior to insertion of implant in the treatment and control groups were nearly at basal level (0.68 ± 0.03 to 0.71 ± 0.05 ng/ml) confirming the anoestrus state in Surti buffaloes. These findings corroborated with the reports of Tiwary (2010) and Soni (2014) in anoestrus buffaloes and of Agarwal *et al.* (2001) and Selvaraju and Rajasundaram (2001) in anoestrus cows.

Table 1: Serum Progesterone (ng/ml) and Oestradiol-17β (pg/ml) profile at different time intervals in anoestrus treated and control groups of buffaloes (Mean ± SEM)

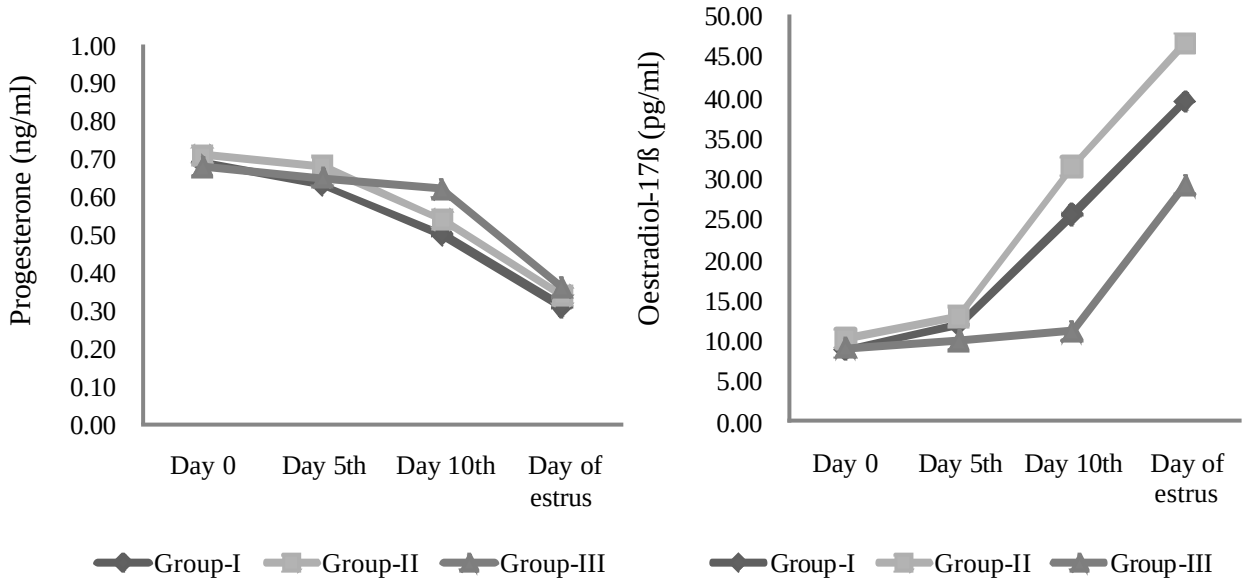
Serum Hormone	Time intervals / Days	Group-I	Group-II	Group-III	*F value*
Progesterone	0 day (pre treatment)	0.69 ± 0.02^{y}	0.71 ± 0.05^{y}	0.68 ± 0.03^{x}	0.29
	5th day (during treatment)	0.63 ± 0.03^{y}	0.68 ± 0.04^{y}	0.65 ± 0.02^{x}	0.82
	10th day (post treatment)	$0.50 \pm 0.04_{a}^{x}$	$0.54 \pm 0.02_{a}^{x}$	$0.62 \pm 0.02_{b}^{x}$	5.58*
	Day of estrus	0.31 ± 0.04^{w}	0.34 ± 0.04^{w}	0.36 ± 0.02^{w}	0.45
	F value	24.46**	22.86**	36.95**	
Oestradiol-17ß	0 day (pre treatment)	08.85 ± 0.59^{w}	10.28 ± 1.08^{w}	09.07 ± 1.18^{w}	0.61
	5th day (during treatment)	11.89 ± 1.16^{w}	12.96 ± 0.82^{w}	10.02 ± 0.77^{w}	2.55
	10th day (post treatment)	$25.52 \pm 1.94_{b}^{x}$	$31.47 \pm 1.34_{c}^{x}$	$11.16 \pm 0.76_{a}^{w}$	53.18**
	Day of estrus	$39.47 \pm 1.93_{b}^{y}$	$46.64 \pm 1.94_{c}^{y}$	$29.18 \pm 0.80_{a}^{x}$	28.47**
	F value	85.83**	157.02**	114.78**	

* $p < 0.05$,** $p < 0.01$; Group-I =Norgestomet, Group-II =Norgestomet + PMSG, Group-III = noestrus Control.
Means bearing different superscripts within a column (wxy) / a row (abc) differ significantly ($p < 0.05$).

The present findings on serum progesterone concentrations in anoestrus Surti buffaloes observed on the day of estrus in the treatment and control groups were in agreement with that reported by Gupta et al. (2010) in buffaloes and by Chaudhari (2005) and Selvaraju and Rajasundaram (2001) in cows treated with Norgestomet ear implant. The non-significant difference observed in the

progesterone concentration between treatment and control groups on the day of estrus supported the earlier observations of Agarwal et al. (2001) with similar treatment protocols.

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



There was a significant decreasing trend of endogenous progesterone (P4) concentration at different time intervals of ear implant insertion and removal (Fig. 1), which supported the findings of previous studies (Hoagland and Barnes, 1984; Pinheiro et al., 1998 and Nath et al., 2003). According to a few workers (Nath et al., 2003) 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 6th day of treatment and on the day of implant removal. Moreover, failure of elevation in serum P4 in Norgestomet treated averse the observations of Barnes et al. (1981) that RIA is unable to detect the Norgestomet a synthetic P4 in the serum samples because it does not cross react with natural progesterone used in RIA.

Serum Oestradiol-17β (E2) Profile:

The serum oestradiol-17β concentration did not show any significant difference among the three groups of anoestrus buffaloes on day 0 (i.e. prior to treatment) and on 5th day (during treatment). However, the values on 10th day (i.e. post-treatment) and on the day of estrus revealed significant difference ($p<0.05$) among the three groups, values being higher in treated groups. The oestradiol-17β levels did not show any significant difference between day 0 and 5 within the Group-I, II and III and between 0, 5th and 10th day in Group-III, but the values increased markedly ($p<0.01$) thereafter on 10th day and on the day of induced estrus in the Group-I & Group-II and on the day of estrus in the control Group-III. The estradiol-17β concentrations prior to insertion of implant and in control group were at basal level (08.85 ± 0.59 to 10.28 ± 1.08 pg/ml) confirming the anoestrus state in these buffaloes. These findings were in agreement with the reports of Dugwekar et al. (2008), who reported the levels of oestradiol-17β as 10 pg/ml in postpartum anoestrus Jaffarabadi buffaloes, whereas higher concentrations of 13.90 ± 0.92 to 18.33 ± 2.05 pg/ml were reported previously (Gupta et al., 2010 and Nath et al., 2003) in postpartum anoestrus buffaloes and cows before insertion of Norgestomet ear implant. The non-significant difference observed in the oestradiol-17β concentration between treatment and control groups on the day prior to treatment

with Norgestomet alone or in combination with PMSG supported the earlier observation of Nath et al. (2003).

Significantly ($p<0.05$) higher mean serum oestradiol-17β concentrations observed in treatment Gr-I and II on 10th day and on the day of induced estrus might probably be due to exogenous oestradiol administered which probably enhances the recruitment and growth of new follicular waves by encouraging gonadotropin secretion and the effect is 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 progesterone causes atresia of antral follicles and recruitment of a new cohort of follicles 4 to 5 days after administration (Vasconcelos et al., 1994). The gradual rising trend in mean serum oestradiol-17β levels found from the day of implant insertion to the day of induced estrus (Fig. 1) was in agreement with Nath et al. (2003). The oestradiol-17β concentrations on the day of induced estrus in treatment groups were in agreement with those reported by Dugwekar et al. (2008) and Gupta et al. (2010) in buffaloes and by Singh et al. (1998) in zebu cows.

The higher level of serum oestradiol-17β concentration on the day of estrus in treatment Group-II as compared to treatment Group-I and control Group-III might be due to administration of an extra injection of Folligon (PMSG) that causes release of FSH and LH from anterior pituitary and helps to stimulate estrogen synthesis in developing follicle and increasing levels of circulating estrogen (Bitt and Roche, 1980). It has been reported that cows treated with Norgestomet have an increased frequency of LH pulses and elevated circulating concentrations of oestrdiol-17β, which 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 concentration of oestrdiol-17β was 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 anoestrous condition could be done accurately by rectal palpation in large animals but to arrive at true status it should be coupled with estimation of P4 profile (concentration below 1 ng/ml reflects that the animal is in inactive ovarian condition). Thus, early detection and hormonal treatment of anoestrus condition can be planned to improve reproductive efficiency in those buffaloes. The linear increasing trend of serum oestrdiol-17β concentration observed over the period of time with Crestar ear implant alone and in combination with PMSG indicated resumption of ovarian activity and ovulation in treated animals.

ACKNOWLEDGEMENT

We thank the staff of Dept. of Physiology & Biochemistry and Dept. of Animal Nutrition, Vanbandhu College of Veterinary Sci. and AH, NAU, Navsari for cooperation and technical help extended in preservation of samples and performing ELISA.

REFERENCES :

Agarwal, S.K., Shankar, U., Kumar, S. and Mohan, G. (2001). Indian J. Anim. Sci., 71(12): 1120-1123.

Barnes, M.A., Kazmer, G.W. and Berley, S.T. (1981). Theriogenology, 16: 13-25.

Bitt, J.H. and Roche, J.F. (1980). Induction and synchronization of ovulation In: Reproduction in farm animals, 4th eds. Publ. Lea and Febiger, pp. 546-559.

Chaudhari, C.F. (2005). Serum biochemical and hormonal studies on induced estrus in delayed pubertal Kankrej heifers. M.V.Sc. Thesis submitted to the Sardarkrushinagar Dantiwada Agricultural

University, Sardarkrushinagar, Gujarat, India.

Dugwekar, Y.G., Sarvaiya, N.P., Patel, M.D., Tajne, K.R. and Shah, R.R. (2008). Indian J. Anim. Reprod., 29(2): 177-180.

Fanning, M.D., Spitzer, J.C., Burns, G.L. and Plyler, B.B. (1992). J. Anim. Sci., 70: 1352-1356.

Garcia, A. and Salaheddine, M. (2001). Reprod. Dom. Anim., 36: 301-307.

Garcia-Winder, M., Lewis, P.E., Townsend, E.C. and Inskeep, E.K. (1987). J. Anim. Sci., 64: 1099-1109.

Gupta, V., Thakur, M.S. Agrawal, R.G. Quadri, M.A. Shukla, S.N. (2010). Buffalo Bull., 29(3): 172-179.

Hafez, E.S.E. (1980). Reproduction in Farm Animals, 4th edn. Lea and Febiger, Philadelphia.

Hoagland, T.A. and Barnes, N.A. (1984). Theriogenology, 22(3): 247-257.

Lyimo, Z.C., Nielen, M., Ouweltjes, W., Kruip, T.A.M. and Van Eerdenburg, F.J.C.M. (2000). Theriogenololy, 53: 1783-1795.

Malik, R.K., Singh, P., Sharma, R.K., Singh, I., Phulia, S.K. and Tuli, R.K. (2011). Indian J. Anim. Sci., 81(7): 687-690.

Nath, H.C., Dutta, D.J., Dutta, A. and Biswas, R.K. (2003). Indian J. Anim. Sci., 73(10): 1102-1104.

Patel, D.M., Sarvaiya, N.P., Patel. A.V., Parmar, A.P. and Dugwekar, Y.G. (2003). Indian J. Anim. Reprod., 24(1): 67-68.

Pinheiro, O.L., Barros, C.M., Figueiredo, R.A., Do Valle, E.R. Encarnacao, R.O. and Padovani, C.R. (1998). Theriogenology, 49: 667-681.

Roberson, M.S., Wolfe, M.W., Stumpf, T.T., Kittok, R.J. and Kinder, J.E. (1989). Biol. Reprod., 41: 997-1003.

Selvaraju, S. and Rajasundaram, R.C. (2001). Indian J. Anim. Sci., 71: 241-244.

Singh, B., Dixit, V.D., Georgie, G.C., Lohan, I.S. and Dixit, V.P. (1998). International J. Anim. Sci., 13(2): 181-186.

Soni, D.K. (2014). Management of Post-partum Inactive Ovaries in Surti Buffaloes with GnRH Analogue, Toldimphos Sodium and Vitamin AD3E Preparation and its Effect on Biochemical Profile. M.V.Sc. Thesis, Navsari Agricultural University, Navsari, Gujarat, India.

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

Tiwary, V.P. (2010). Studies on anestrus in Murrah buffaloes during summer and response to insulin and dextrose pretreatment in Ovsynch protocol. M.V.Sc. & A.H. Thesis, I.G.K.V.V., Anjora, Durg (C.G), India.

Vasconcelos, J.L.M., Pursley, J.R. and Wiltbank, M.C. (1994J. Anim. Sci., 72(1): 89(Abstr.).

Yadav, K.V.S., Ansari, M.R. and Kumaresan, A. (2001). Indian J. Anim. Reprod., 22: 113-15.

❑