ESTRUS INDUCTION AND FERTILITY RESPONSE IN POSTPARTUM ANOESTRUS SURTI BUFFALOES TREATED WITH NORGESTOMET EAR IMPLANTS ALONE AND IN COMBINATION WITH PMSG

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ABSTRACT

The study was conducted on eighteen postpartum anoestrus (> 45 days postpartum) Surti buffaloes, divided into three equal groups (6 in each) to evaluate the efficacy of Norgestomet ear implant/Crester alone (Gr-I) and in combination with PMSG (Gr-II). The buffaloes in Group-I and Group-II were implanted with Crestar ear implant for 9 days with 2 ml i/m injection of Crestar solution (estradiol valerate) on the day of implant insertion. In Group-II, additionally 500 IU PMSG was given i/m on the day of implant removal. The buffaloes in Group-III served as untreated anoestrus controls and were given 5 ml normal saline i/m as a placebo treatment on days 0 and 9. The estrus was induced in all buffaloes in Gr-I and 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 intensity of estrus differed significantly ($P<0.05$) among these three groups. The conception rate at induced estrus was highest (66.67 %) in Gr-II followed by Gr-I (33.33 %) and Gr-III (16.67%). The mean of number of services per conception did not differ significantly among the treatment and control groups. The service periods of the anoestrus buffaloes under Gr-I and II were significantly ($P<0.01$) shorter than in control Gr-III. It could be concluded that Norgestomet ear implant alone and in combination with PMSG can be successfully used to induce fertile estrus in the postpartum anoestrus Surti buffaloes.

Key words: Buffaloes, Postpartum anoestrus, Norgestomet ear implant, PMSG, Estrus, Fertility.

INTRODUCTION

In India, the incidence of anoestrus has been reported between 9.09 and 82.50 per cent in buffaloes (Kumar et al., 2014). To improve reproductive efficiency in anoestrus animals, several estrus induction and ovulation protocols have been developed. Estrus can be induced, using various hormones that act on the hypothalamo-pituitary-ovarian axis. These procedures are based on manipulating the corpus luteum, either to induce premature luteolysis using prostaglandins or to prolong the luteal phase using progestagens. Such approaches have many advantages and are increasingly being used in modern animal husbandry practices, particularly in buffaloes which are known to have anoestrus and silent estrus. Hence, the present investigation was carried out to study the estrus induction and fertility response in postpartum anoestrus Surti buffaloes treated with Norgestomet ear implants alone and in combination with PMSG.

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MATERIALS AND METHODS

The study was conducted on eighteen anoestrus (inactive ovaries) Surti buffaloes between 45 to 120 days postpartum maintained at Livestock Research Station, NAU, Navsari, Gujarat, over a period of six months from November, 2013 to April, 2014. They were randomly divided into three equal groups each of 6 buffaloes and were treated as under.

The buffaloes in Group I and II were implanted with silastic 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 together 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 the thumb. The buffaloes in Group-II received additional injection of Folligon (500 IU, PMSG- Pregnant Mare Serum Gonadotrophin, Intervet) on day 9, immediately after implant removal, while the buffaloes in Group-III (anoestrus control) were injected with 5 ml normal saline i/m as placebo treatment on days 0 and 9.

Estrus detection was done by visual observation and parading a teaser bull at every 6 hr interval following removal of implant. The onset of estrus was calculated from the time of implant removal to the appearance of first estrus signs. Duration of estrus was calculated as an interval from the onset of estrus to cessation of estrus as decided by first acceptance till the non-acceptance of teaser by the female. The intensity of the estrus was measured by assigning the numerical values (score) to a specific set of behavioural expression by the females and their interaction with the teaser/breeding bull (Dhali *et al.*, 2006). The buffaloes exhibiting estrus were bred either naturally or by using artificial insemination. The buffaloes which did not return to estrus following breeding were confirmed for pregnancy per-rectum 60 days later. The buffaloes that failed to conceive at the induced estrus and returned to estrus were rebred at subsequent estrus.

The data collected were analyzed following standard statistical methods (Steel and Torrie, 1981). The animals among different groups were ranked for intensity of estrus on five point scale (Very strong-5, Strong-4, Moderate-3, Weak-2 and Very weak-1) and for conception rate on four point scale (First service-4, Second service-3, Third service-2 and Non-concieved-1). Kruskal-Wallis sum rank test of significance was used for comparing the intensity of estrus and conception rates among the groups.

RESULTS AND DISCUSSION

In the present study, none of the implants was lost from any of the buffaloes treated with Crestar ear implant during the period of treatment due to proper fixation in the ear pinnae. This finding was in agreement with several earlier reports (Singh *et al.*, 2002; Chaudhari *et al.*, 2012).

The cent per cent buffaloes responded to treatment with induction of estrus within 2 to 3 days following removal of Crestar ear implant in Group I and II, while the buffaloes from the control Group III were observed in estrus between days 28 and 34 following last placebo treatment. The earlier researchers have also used Norgestomet ear implant alone or in combination with PMSG with similar estrus induction response in postpartum anoestrus buffaloes (Patel *et al.*, 2003; Nayak *et al.*, 2009; Malik *et al.*, 2010). Following removal of implant, the resumption of follicular development and maturation might be due to flux of the gonadotropin from the pituitary. However, Cavalieri and Fitzpatrick (1995) opined that the effectiveness of Norgestomet for inducing behavioral estrus in cows is due to the combined effect of progestogen priming on the brain and the direct effect of both exogenously administered estradiol and the high endogenous estradiol on the hypothalamus. Pregnant mare serum gonadotrophin is strong stimulator of ovarian activity because of its predominant FSH like activity. PMSG prevents and reverses the process of atresia in small follicles. The use of PMSG along with Norgestomet ear implant may be useful as PMSG is known to increase blood

estrogen level and in turn leads to induction of behavioral estrus signs (Singh *et al.*, 2004).

The mean intervals of onset of induced estrus following removal of Crestar ear implant in the treatment Group I and II were observed to be 2.56 ± 0.34 days and 2.40 ± 0.29 days, respectively, while the mean interval in control Group III was found to be 30.33 ± 0.95 days, which differed significantly ($p<0.01$) from treatment groups. These observations in Group I and II closely corroborated with previous reports of Nayak *et al.* (2009) and Dodamani *et al.* (2011) in postpartum anoestrus buffaloes. Exogenous administration of progesterone mimics the luteal phase of the estrus cycle by exerting negative feedback effect over hypothalamus and pituitary for LH release. Upon withdrawal of Crester, the concentration of progesterone declines abruptly and onset of normal follicular phase of the estrus cycle follows by estrus and ovulation within 2 to 8 days after end of treatment (Kumar *et al.*, 2014). The better induction of estrus in Norgestomet plus PMSG treated buffaloes might be through the synergistic effect of exogenous Gonadotropin on folliculogenesis, oocyte maturation and development of corpus luteum (Agarwal *et al.*, 2001).

The mean duration of estrus was found to be shortest in control Group III (18.67 ± 0.77 hrs), followed by Group I (22.17 ± 0.65 hrs) and Group II (25.50 ± 0.76 hrs), which differed significantly ($p<0.01$) among each other, and compared well with the reports of Selvaraju and Rajasundaram (2001) and Chaudhari *et al.* (2012) in cattle. Significantly longer estrus duration in Norgestomet plus PMSG treated group could be attributed to longer biological half life of PMSG resulting in higher concentration of estradiol in the circulation for a longer duration.

The number of the buffaloes that exhibited weak, normal/moderate, and strong estrus intensity in Group I were 1, 4 and 1, respectively. The corresponding figures for Group II were 0, 3 and 3, and for control Group-III 4, 2 and 0, respectively. None of the animals showed very weak or very strong estrus. On analysis of these qualitative data with Kruskal-Wallis sum rank test, the mean rank was found to be highest 13.25 in Group II followed by 9.92 in Group I and 5.33 in Group III. The mean rank of intensity of estrus differed significantly ($p<0.05$) among three groups. These findings of intensity of estrus in Group I were in agreement with 16.67 per cent reported by Chaudhari *et al.* (2012) in the delayed pubertal Kankrej heifers following Norgestomet treatment. Moreover, intermediate type of estrus intensity following Norgestomet treatment in postpartum anoestrus Jersey crossbred cows reported as 62.50 per cent by Selvaraju and Rajasundaram (2001) corroborated with the result of present study. Similarly the percentage of weak intensity of estrus was reported as 14.30 per cent by Nayak *et al.* (2009) and 25.0 per cent by Selvaraju and Rajasundaram (2001). The response of varying intensity of estrus with Norgestomet treatment might be due to variable ability of individual animal to recover from the influence of potent suppression of pituitary function. The intense manifestation of estrus observed in Norgestomet ear implant plus PMSG treated group might be due to the effect of PMSG on multiple follicular maturation resulting in higher endogenous estradiol production responsible for the improved response.

The number (and per cent) of buffaloes that were pregnant in Group I at 1st, 2nd and 3rd cycle were 2 (33.33 %), 3 (75.00 %) and 0 (00.00 %), respectively. The corresponding values for Group II were 4 (66.67 %), 2 (100.00 %) and 0 (00.00 %), and for control Group III 1 (16.67 %), 1 (20.00 %) and 1 (25.00 %), respectively. Analysis of these qualitative data on first, second and third service conception rate with Kruskal-Wallis sum rank test revealed the highest mean rank of 12.83 in Group II followed by 9.67 in Group I and 6.00 in control Group III, the difference was found to be non-significant among the three groups. The findings on first service conception rate in Group-I are little lower than 40.0 per cent obtained previously by Agarwal *et al.* (2001) following use of Norgestomet ear implant in postpartum anoestrus cows. The findings in Group-II were in close agreement with 68 per cent observed by Malik *et al.* (2010) in postpartum

anoestrus buffaloes. Moreover, the second service conception rate was in close agreement with Agarwal *et al.* (2001). Chaudhari *et al.* (2012) reported 50 per cent second service conception rate in cows.

The overall conception rate of three cycles/ services was maximum in Group II, 6/6 (100.00 per cent) followed by Group I, 5/6 (83.33 per cent) and control Group III, 3/6 (50.00 per cent). These findings in Group I and II are in agreement with reports of Agarwal *et al.* (2001) and Markendeya and Bharkad (2004) in postpartum anoestrus cows. However, little lower percentage of overall conception rate (75%) was reported by Nath *et al.* (2004) with Norgestomet plus PMSG treatment in anoestrus cows.

The mean number of services required per conception was 1.60 ± 0.24, 1.33 ± 0.21 and 2.00 ± 0.58 in Group I, II and III, respectively, which did not differ significantly. These observations in treatment groups are in agreement with average figure of 1.33 reported by Soni (2014) in Surti buffaloes, whereas Markendeya and Bharkad (2004) reported 1.85 services per conception in Deoni cows following Crestar ear implant treatment.

The mean intervals (days) from calving to conception (service period observed were 73.40 ± 0.24, 66.67 ± 4.70 and 106.67 ± 7.51 days) in three groups, respectively. The effect of both the treatments was significant compared to control group. These observations on service period recorded in Group-I and II were in agreement with values reported previously (Soni, 2014) in buffaloes following progestagens plus GnRH treatment.

It could be concluded that Norgestomet ear implant alone and in combination with PMSG could successfully induce fertile estrus in the postpartum anoestrous buffaloes, hence can be advocated for the treatment of anoestrus buffaloes under the field conditions to augment their fertility.

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REFERENCES

Agarwal, S.K., Shankar, U., Kumar, S. and Mohan, G. (2001). Ovarian cyclicity and progesterone profile in postpartum anoestrus cattle using a synthetic progestogen, norgestomet regime. *Ind. J. of Anim. Sci.,* **71**(12): 1120-1123.

Cavalieri, J. and Fitzpatrick, L.A. (1995). Oestrus detection techniques and insemination strategies in Bos indicus heifers synchronized with norgestomet-estradiol. *Austr. Vet. J.,* **72**(5): 177-182.

Chaudhari, C.F., Suthar, B.N., Sharma, V.K., Dabas, V.S., Chaudhari, N.F. and Panchasara, H.H. (2012). Estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant. *Vet. World,* **5**(8): 453-458.

Dhali, A., Mishra, D.P., Karunakaran, M., Mech, A. and Rajkhowa, C. (2006). Influence of plasma estradiol 17-β and progesterone levels on estrous behaviour in mithun (*Bos frontalis*). *Appl. Anim. Behav. Sci.,* **98**(1-2): 1-10.

Dodamani, M.S., Tandle, M.K., Mohteshamuddin, K. and Honnappagol, S.S. (2011). Induction of Fertile estrus in true anoestrus by re-utilization of Crestar implants in She buffales. *Vet. World,* **4**(1): 28-30.

Kumar, P.R., Singh, S.K., Kharche, S.D., Chethan, S.G., Behera, B.K., Shukla, S.N., Kumar, H. and Agarwal, S.K. (2014). Anestrus in cattle and buffalo: Indian perspective. *Adv. Anim. Vet. Sci.,* **2**(3): 124-138.

Malik, R.K., Singh, P., Sharma, R.K., Singh, I. and Tuli, R.K. (2010). Estrus and fertility response of postpartum anestrus Murrah buffaloes to Crestar and OvSynch treatment regimens. *Ind. J. Anim. Sci.,* **80**(10): 982-85.

Markendeya, N.M. and Bharkad, G.P. (2004). Controlled breeding with norgestomet ear implant for induction of postpartum estrus in Deoni cows. *Ind. J. Anim. Reprod.*, **25**: 53-54.

Nath, H.C., Dutta, D.J., Dutta, A. and Biswas, R.K. (2004). Reproductive performances of Crestar and PMSG administered to postpartum anoestrus cows. *Ind. J. Anim. Res.*, **38**(1): 50-52.

Nayak, V., Agrawal, R.G., Shrivastava, O.P. and Thakur, M.S. (2009). Induction of estrus in true anestrus buffaloes using Crestar implant alone and in combination with PMSG. *Buffalo Bull.*, **28**(2): 51-54.

Patel, D.M., Sarvaiya, N.P., Patel. A.V., Parmar, A.P. and Dugwekar, Y.G. (2003). Induction of estrus and hormonal profile in buffalo treated Norgestomet ear implant. *Ind. J. Anim. Reprod.*, **24**(1): 67-68.

Selvaraju, S. and Rajasundaram, R.C. (2001). Effect of GnRH on fertility response in norgestomet primed postpartum anoestrous cows. *Indian Vet. J.*, **78**: 325-326.

Singh, A., Saxena, M.S. and Prasad, S. (2004). Efficacy of Crestar® and its combination with Folligon® on postpartum anoestrus in buffaloes. *Ind. J. Anim. Reprod.*, **25**(1): 43-44.

Singh, U., Singh, I. And Khan, S.K. (2002). Influence of season, age & postpartum interval on reproductive parameters of norgestomet treated anoestrus zebu cattle. *Ind. J. Anim. Reprod.*, **23**: 113-116.

Soni, D.K. (2014). Management of Post-partum Inactive Ovaries in Surti Buffaloes with GnRH Analogue, Toldimphos Sodium and Vitamin A, D3, E Preparation and its Effect on Biochemical Profile. M. V. Sc. Thesis submitted to the 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.