

## Estrus Induction and Fertility Response in Postpartum Silent estrus Surti Buffaloes treated with Norgestomet Ear Implants alone and in Combination with PGF<sub>2</sub>α

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**Abstract:** The study was conducted on eighteen postpartum silent oestrus (> 45 days postpartum) Surti buffaloes, divided into three equal groups (6 in each) to evaluate the efficacy of Norgestomet ear implant alone (Gr.-I) and in combination with PGF<sub>2</sub>α (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 µg Cloprostenol was given i/m on one day before implant removal. The buffaloes in Group-III served as untreated silent oestrus controls and were given 5 ml Normal Saline i/m as a placebo treatment on 0 and 8<sup>th</sup> day. The estrus was induced in cent per cent of buffaloes in the Gr-I and Gr-II with mean estrus induction intervals of 2.17 ± 0.06 and 2.03 ± 0.05 days, respectively. These intervals were significantly (P<0.05) shorter as compared to control Gr-III (8.33 ± 2.01 days). The duration of estrus differed significantly (P<0.05) longest (29.17 ± 1.086 hrs) in Gr-II followed by Gr-I (27.83 ± 0.95 hrs) and the least in control Gr-III (19.33 ± 1.05 hrs). The intensity of estrus differed significantly (P<0.05) between treatment groups as well as between each treatment and control group. The conception rate at induced estrus was highest (66.67 %) in Gr-II followed by Gr-I (33.33 %). The overall conception rate of 3 cycles was found to be highest (83.33 %) in Gr-II followed by Gr-I (66.67 %) and the least in Gr-III (50.00 %). It could be concluded that Norgestomet ear implant alone and in combination with PGF<sub>2</sub>α can be successfully used to induce fertile estrus in the postpartum silent oestrus Surti buffaloes.

**Keywords:** Buffaloes, Estrus, Fertility, Norgestomet, PGF<sub>2</sub>α, Postpartum Silent estrus

### INTRODUCTION

Buffaloes play a prominent role in rural economy depends on livestock production in India. Among various problems Silent estrus is perhaps the most important factor leading to poor reproductive efficiency in buffaloes[1]. Moreover, problem of silent heat coupled with late maturity, poor expression of oestrus, irregular oestrous cycle, seasonality in breeding, low conception rate, long postpartum interval are some of the major constraints in buffalo productivity. Among this silent oestrus is again one of the major impediments in understanding reproductive parameters and assisted reproduction in this species[2]. Therefore, it is important to improve the level of fertility in dairy buffaloes to increase their reproductive efficiency. Suboestrus is a condition in which genital organs are undergoing normal cyclical changes but behavioral signs of oestrus are not manifested[3]. The intensity of heat signs in buffaloes is generally low and the incidence of suboestrus varied from 15 to 73 per cent [4]. To improve reproductive efficiency, several protocols of induction of estrus and ovulation 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. It has many advantages and become mandatory in modern animal husbandry practice, particularly in buffaloes which are known for anoestrus and silent estrus. Hence, the present investigation was carried out to study the estrus induction and fertility response in postpartum silent oestrus Surti buffaloes treated with Norgestomet ear implants alone and in combination with PGF<sub>2</sub>α.

### MATERIALS AND METHODS

The study was conducted on eighteen silent estrus (Suboestrus) Surti buffaloes from 45 to 120 days postpartum. All these buffaloes had normal calving and subsequent normal genital health as assessed Gynaecologically. 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 (500µg 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 8<sup>th</sup> day.

#### Estrus detection and Breeding

Induction of estrus among groups with & without treatments followed by onset of heat was calculated from the end of treatment (after removal of the implant on day 9) to the appearance of first (induced) estrus. Estrus detection was done by visual observation of estrus signs and parading the buffaloes with a teaser buffalo bull having good sexual drive at every 6 hour interval following removal of implant. The very first acceptance of male by the female was considered as the onset of estrus. Duration of estrus was calculated as an interval from the first acceptance of male by the female till the non acceptance of male by the female. i.e. onset of estrus to cessation of estrus. 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 accompanying or parading buffalo bulls as per Dhali *et al.* [5]. The buffaloes exhibiting estrus were bred either naturally or by using artificial

insemination, there after all the buffaloes were closely observed for re-occurrence of estrus. The buffaloes which failed to return in estrus following breeding were examined per-rectally on day 60<sup>th</sup> post breeding for confirmation of pregnancy. Percentages of buffaloes conceived at induced estrus were considered as first service conception rate. The buffaloes failed to conceive at the induced estrus and returned to estrus were rebred at subsequent (2<sup>nd</sup> or 3<sup>rd</sup>) estrus. Buffaloes conceived at 2<sup>nd</sup> and 3<sup>rd</sup> estrus following induced estrus plus buffaloes conceived at induced estrus were considered as overall conception rate.

#### Statistical analysis

The data collected were suitably tabulated and analyzed following standard statistical methods shown by Steel and Torrie [6]. The animals among different groups were ranked on five point scale (Very weak-1, Weak-2, Moderate-3, Strong-4 and Very strong-5) for intensity of estrus and on four point scale (First service-4, Second service-3, Third service-2 and Non conceived-1) for conception rate. The tests of significance among the groups for intensity of estrus and conception rate were tested using Kruskal-Wallis sum rank test.

## RESULTS AND DISCUSSION

The incidence of silent oestrus (suboestrus) was found to be 26.09 per cent in Surti buffaloes maintained at Livestock Research Station, Navsari Agricultural University, Navsari. These findings were some extent agreed with 27.55 per cent and 27.74 per cent (ranging from 16.13 to 39.34 Per cent) reported by Singh *et al.* [7]. in postpartum silent oestrus buffaloes and Gautum *et al.* [8]. in postpartum suboestrus buffaloes maintained at JNKVV, Jabalpur, respectively.

In the study, no implant (0.0 percent) was lost from any of the buffalo treated with Crestar ear implant in treatment groups (T1 & T2) before day 9 during the period of treatment. This finding was in agreement with Wishart *et al.* [9]. Logue *et al.* [10]. Singh *et al.* [7]. Singh *et al.* [11]. and Chaudhari [12]. The lack of loss of any implant might be due to its proper location of implants.

**Table 1. Effect of different treatments on Induction of overt heat of postpartum silent oestrus Surti buffaloes**

Parameters	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)	F value	P value
Induction of estrus / Buffaloes responded	6 (100)	6 (100)	0 (0)	--	--
Onset of estrus after withdrawal of implant (days) (Mean ± SEM)	2.17 ± 0.06 <sup>a</sup>	2.03 ± 0.05 <sup>a</sup>	8.33 ± 2.01 <sup>b</sup>	9.602*	0.002
Duration of estrus (hours) (Mean ± SEM)	27.83 ± 0.95 <sup>b</sup>	29.17 ± 1.08 <sup>c</sup>	19.33 ± 1.05 <sup>a</sup>	26.96**	0.000
Means bearing different superscripts within a row (between the groups) differ significantly (p <0.05). Figures in the parenthesis indicate percentage. Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + PGF <sub>2α</sub> ), Group-III = T3 (Silent oestrus control)					

### **Induction of estrus:**

The number of the buffaloes responded to treatment with induction of overt heat were cent per cent in the treatment Group-I and Group-II, as all of the six buffaloes in treatment groups came in heat within 2 to 3 days following removal of Crestar ear implant, while two buffaloes from the control Group-III came in heat within 2 to 3 days following second placebo treatment and rest remained silent oestrus during that period as compared to treatment groups and showed estrus symptoms thereafter at 8 to 14 days. The previous various research workers had also used Norgestomet ear implant alone or in combination with PGF<sub>2</sub>α and obtained cent per cent estrus induction response Chinchkar *et al.*, [13]. and Pawshe *et al.*, [14]. in postpartum cyclic buffaloes and in postpartum cyclic Nagpuri buffaloes in monsoon and summer season, respectively; [15]. in postpartum cyclic beef cows; Murugavel *et al.*, [16]. in suboestrus crossbred cows.

Norgestomet implant inhibits the secretion of FSH / LH by the pituitary. Following removal of implant resumption of follicular development and maturation might be due to flux of the Gonadotropin from the pituitary. However, Cavalieri and Fitzpatrick [17] 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. Prostaglandin F<sub>2</sub> alpha (PGF<sub>2</sub>α) and its analogue causes luteolysis and thereby return to the estrus. Prostaglandins act on luteal tissue of corpus luteum (CL) and remove that inert tissue, as a result of it rapid decline in progesterone production by the corpus luteum (CL) which in turn leads to new follicular waves followed by estrus signs.

### **Onset of estrus**

The mean interval of onset of induced estrus following removal of Crestar ear implant in the treatment Group-I & Group-II were observed to be 2.17 ± 0.06 days and 2.03 ± 0.05 days, respectively. While the mean interval of onset of natural estrus following second placebo treatment in control Group-III was found to be 8.33 ± 2.01 days. Statistical analysis of the above groups, it was observed the mean interval of onset of estrus was significantly longer (p <0.05) in terms of days in control Group-III (T3) as compared to treatment Group-I (T1) and treatment Group-II (T2). On the other hand, there was no significant (p >0.05) difference in the mean interval of onset of induced estrus observed between treatment Group-I & treatment Group-II.

The mean interval between Norgestomet ear implant withdrawal and onset of induced estrus observed as 57.7 ± 3.2 hours (ranging from 47.1 to 83.6 hours) and 62.5 ± 11.2 hrs (range of 41.1 to 91. hours) by Pinheiro *et al.* [18]. in Nelore breed (*Bos indicus*)

cows and heifers, respectively. The findings on onset of estrus in Group-II were in close agreement with 47.00 ± 5.7 hrs (ranged from 37 – 62 hrs) reported by Utage *et al.* (2011) in postpartum anoestrus buffaloes and 46.8 ± 7.0 hrs reported by Cavalieri *et al.* [19]. in (*Bos indicus*) heifer-cows and 49.8 ± 4.7 hours reported by Heersche *et al.* [20] in cyclic beef heifers. Whereas, Norgestomet ear implant plus PGF<sub>2</sub>α treated postpartum cyclic beef cows came in estrus within range of 44 – 48 hrs reported by Beal *et al.* [21]. within 48 hours reported by Joshi *et al.* [22]. in anoestrus Red-Kandhari cows and Joshi *et al.*, [23]. and Joshi *et al.*, [24]. in Red-Kandhari X Jersey crossbred cows and Red-Kandhari heifers, respectively.

PGF<sub>2</sub>α possesses luteolytic like biological action, the better induction of estrus in Norgestomet + PGF<sub>2</sub>α treated buffaloes might be through the synergistic effect of exogenous progesterone when sudden decline its level and luteolysis of the inert luteal tissue cause by PGF<sub>2</sub>α, oocyte maturation and follicular activity starts immediately after C.L regression. 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, the concentration of progesterone decline abruptly and onset of normal follicular phase of the estrus cycle followed by estrus and ovulation occurs within 2 – 8 days after end of treatment [59,60].

### **Duration of estrus**

The mean duration of estrus was found to be shortest in Group-III (19.33 ± 1.05 hrs), followed by Group-I (27.83 ± 0.95 hrs) and Group-II (29.17 ± 1.08 hrs). The mean duration of the induced and natural estrus in terms of hours differed significantly (p <0.05) in between treatment group (T1) & treatment group (T2) as well as between control group (T3) and each treatment (T1, T2) group. The significantly longer duration was observed in treatment Group-II as compared to Group-I and control Group-III may be augmented by PGF<sub>2</sub>α in which least basal level of progesterone was observed during estrus. The present findings on mean duration of estrus in Group-I (T1) were in agreement with Chede [25]. who reported mean duration of estrus as 28.35 ± 1.46 hrs following Norgestomet ear implant treatment in cycling Nagpuri buffaloes during summer season. The mean duration of estrus observed in Group-II (T2) was some extent corroborated with findings of Patil [26]. who reported 26.85 ± 2.99 and 27.22 ± 2.32 hrs in cyclic Nagpuri buffaloes treated with Lutalyse and Prosolvin, respectively. The mean duration of estrus observed in Group-III (T3) was some extent corroborated with 17.65 ± 4.47 hrs (ranged from 6 - 47 hrs) reported by Gill *et al.* [27]. in normal cyclic buffaloes. In the study, longer duration of estrus (hours) was found in treatment Group-I (T1) and treatment Group-II (T2) as compared to control Group-III (T3) may entirely be depended on

progesterone and progesterone plus PGF<sub>2</sub>α as compared to control group in which least basal level of

progesterone and higher level of oestradiol-17β was observed during estrus.

**Table 2. Effect of different treatments on intensity of estrus of postpartum silent oestrus Surti buffaloes**

Parameters / Intensity of estrus	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)
Very strong	0 (00.00)	0 (00.00)	0 (00.00)
Strong	1 (16.67)	4 (66.67)	0 (00.00)
Moderate / normal	3 (50.00)	2 (33.33)	3 (50.00)
Weak	2 (33.33)	0 (00.00)	3 (50.00)
Very weak	0 (00.00)	0 (00.00)	0 (00.00)
Mean Rank (K-W Test)	8.42 <sup>b</sup>	13.83 <sup>c</sup>	6.25 <sup>a</sup>
Chi-square value	7.37*		
P value	0.025		
Means bearing different superscripts within a row (between the groups) differ significantly (p < 0.05). Figures in the parenthesis indicate percentage. Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + PGF <sub>2</sub> α), Group-III = T3 (Silent oestrus control)			

### Intensity of estrus

The number of the buffaloes exhibited intensity of estrus during induced estrus was recorded Very strong, Strong, Moderate, Weak and Very weak in the treated Group-I of Surti buffaloes as 0 (0.0 per cent), 1 (16.67 per cent) 3 (50.00 per cent), 2 (33.33 per cent) and 0 (0.0 per cent) buffaloes, respectively. The corresponding figures for treated Group-II were 0 (0.0 per cent), 4 (66.67 per cent) 2 (33.33 per cent), 0 (0.0 per cent), and 0 (0.0 per cent), respectively. Similarly, the number of the buffaloes exhibited intensity of estrus during natural estrus was recorded in control Group-III of Surti buffaloes as 0 (0.0 per cent), 0 (0.0 per cent) 3 (50.00 per cent), 3 (50.00 per cent), and 0 (0.0 per cent), respectively.

Analysis of the above qualitative data with Kruskal-Wallis sum rank test. The mean rank was found to be highest 13.83 in Group-II (T2) followed by 8.42 in Group-I (T1) and 6.25 in Group-III (T3 - Silent oestrus control). The intensity of estrus was differed significantly (p < 0.05) in between treatment groups (T1 & T2) as well as between each treatment (T1, T2) group and control (T3) group. The findings of intense estrus (Group-I) were in agreement with 16.67 per cent reported by Chaudhari [12]. in the delayed pubertal Kankrej heifers following Norgestomet ear implant treatment. Moreover, intermediate type of estrus intensity following Norgestomet ear implant treatment in the delayed pubertal Kankrej heifers reported 50.0 per cent by Chaudhari [12] which was corroborated with result of the present study. Like this way, percentage of weak intensity of estrus reported as 30.60 per cent by Kadu and Chede [28] in summer anoestrus buffaloes and 33.60 per cent by Chaudhari [12] in the delayed pubertal Kankrej heifers following Norgestomet ear implant treatment.

The intense estrus (Group-II) was in agreement with 62.50 per cent reported by Joshi *et al.* [22] in true anoestrus Red kandhari cows treated with Synchronate-

B and PGF<sub>2</sub>α plus PMSG and 66.66 per cent reported by Jacob *et al.* [29]. in postpartum crossbred cows treated with PGF<sub>2</sub>α. Moreover, intermediate type of estrus intensity following Norgestomet ear implant and PGF<sub>2</sub>α treatment reported as 33.33 per cent by Utage *et al.* [30] in postpartum anoestrus buffaloes treated with Norgestomet and PGF<sub>2</sub>α plus GnRH and 37.50 per cent by Joshi *et al.* [22] in postpartum anoestrus Red Kandhari cows treated with Synchronate-B and PGF<sub>2</sub>α plus PMSG, respectively which was corroborated with result of the study. Like this way, percentage of weak intensity of estrus reported as 14.28 per cent by Jacob *et al.* [29]. in postpartum crossbred cows treated with PGF<sub>2</sub>α and 33.30 per cent, by Joshi *et al.* [23] in Red Kandhari X Jersey crossbreed cows treated with Synchronate-B and PGF<sub>2</sub>α plus PMSG and 54.55 per cent by Joshi *et al.*[24] in Red Kandhari heifers treated with Synchronate-B plus PGF<sub>2</sub>α and 60.00 per cent by Utage *et al.* [30] in postpartum anoestrus buffaloes following Norgestomet ear implant plus PGF<sub>2</sub>α treatment, respectively which was very high per cent of weak type of estrus as compared to the study.

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 suppressor of pituitary function [31]. The intense manifestation of estrus was observed in Norgestomet ear implant plus PGF<sub>2</sub>α treated group might be due to the effect of PGF<sub>2</sub>α on inert luteal tissue followed by multiple follicular maturation resulting in higher endogenous estradiol production responsible for the improved response that could be seen in the estrogen estimation as compared to other groups.

Norgestomet ear implant saturated with progesterone causes an increased circulatory concentration of progesterone exerted negative feedback on hypothalamus and anterior pituitary. Hence, result in favoring GnRH, FSH and LH storage.



Following termination of progesterone therapy, the rapid drop in circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone per se abolish, followed by FSH and LH release with subsequent resumption of ovarian cyclicity Zerbe *et al.*, [32]. Also, the increased

circulatory concentration of progesterone has sensitized the hypothalamic-pituitary system [33]. Likewise, progesterone increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of heat [34].

**Table 3. Effect of different treatments on Conception rate of postpartum silent oestrus Surti buffaloes**

Conception rate	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)
First service	2/6 (33.33)	4/6 (66.67)	0/6 (00.00)
Second service	2/4 (50.00)	1/2 (50.00)	2/6 (33.33)
Third service	0/2 (00.00)	0/1 (00.00)	1/4 (25.00)
Mean Rank (K-W Test)	9.67 <sup>a</sup>	12.58 <sup>a</sup>	6.25 <sup>a</sup>
Chi-square value	4.66		
P value	0.097		
Means bearing common superscripts within a row (between the groups) do not differ significantly (p >0.05). Figures in the parenthesis indicate percentage. Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + PGF <sub>2</sub> α), Group-III = T3 (Silent oestrus control)			

**Number of services per conception (conception rate):**

The number of buffaloes found absolute followed by subsequent pregnant in the treated Group-I of Surti buffaloes at 1<sup>st</sup> service were 2 (33.33 per cent), 2<sup>nd</sup> service were 2 (50.00 per cent) and 3<sup>rd</sup> service was 0 (00.00 per cent), respectively. The corresponding values for treated Group-II were 4 (66.67 per cent) at 1<sup>st</sup> service, 1 (50.00 per cent) at 2<sup>nd</sup> service and 0 (00.00 per cent) at 3<sup>rd</sup> service, respectively. Whereas, the corresponding values for control Group-III were 0 (00.00 per cent) at 1<sup>st</sup> service, 2 (33.33 per cent) at 2<sup>nd</sup> service and 1 (25.00 per cent) at 3<sup>rd</sup> service, respectively (Table 1)

Analysis of the above qualitative data influencing on first, second and third service per conception rate in each group was carried out with Kruskal-Wallis sum rank test. The mean rank was found to be higher (12.58) in treatment Group-II (T2) followed by (9.67) in treatment Group-I (T1) and (6.25) in control Group-III (T3), the mean rank difference was found to be non-significant (p >0.05) among the three groups by chi-square value. On the other hand, when mean of number of service per conception rate was calculated for each group and statistical analysis between each treatment and control group with DMRT (Table 2) non-significant difference (p >0.05) was observed between both treatment (T1 & T2) as well as between treatment Group-I (T1) & control Group-III (T3). While significant difference obtained between treatment Group-II (T2) & control Group-III (T3) might be attributed to total less number and none of the animal conceived at first observed estrus as compared to treatment Group-II (T2). On the other hand, more number of animals conceived at first service in Group-II (T2) and almost equal numbers conceived at first & second service in treatment Group-I (T1) as well as equal numbers conceived at second service in the treatment Group-I & control Group-III revealed non-significant (p >0.05) difference between that groups.

These first service conception rate were somewhat in agreement with 31.00 per cent reported by Hixon *et al.* [35]. in beef heifers treated with Synchronate-B. While little bit higher first service conception rate as 41.66 per cent reported by Kadu and Chede [28]. in summer anoestrus buffaloes following Synchronate-B treatment and 40.0 per cent and 44.0 per cent reported by Richards *et al.* [36]. in suboestrus crossbred beef cows during spring season and fall season, respectively following Norgestomet ear implant treatment. Whereas, the lower second service conception rate as 20.0 per cent and 28.57 per cent reported by Chaudhari *et al.* [37].and [38].Gopikrishnan *et al.*, 2004) in delayed pubertal Kankrej heifers and crossbreed heifers, respectively as compare to the result of present study. On the contrary, higher percentage of third service conception rate following Norgestomet ear implant treatment as 14.28 per cent reported by Gopikrishnan *et al.* [38]. in crossbreed heifers and 25.0 per cent reported by Chaudhari *et al.* [37]. in delayed pubertal Kankrej heifers as against 0.0 per cent third service conception rate in the present findings of postpartum Surti buffaloes.

The first service conception rate were higher when compared to findings as 44.44 per cent, 45.94 per cent, 47.0 per cent, 49.0 per cent and 55.0 per cent reported by Bormann, J. M [39] in crossbred Angus heifers; Sandor *et al.* [40] in cows; Galloway *et al.* [41] in anoestrus cows, Chupin and Pelot [42] in French Friesian cows and .Gauthier *et al.*, [58] in cows, respectively following Norgestomet plus PGF<sub>2</sub>α treatment. However, as compare to the present findings very low first service conception rate at induced estrus as 25.0 per cent, 30.0 per cent and 37.50 per cent reported by Joshi *et al.* [22] in true anoestrus Red Kandhari cows; Mares *et al.* [43] in beef cows and Selvaraju *et al.* [44] in crossbred repeat breeder cows, respectively treated with Norgestomet plus

PGF<sub>2</sub>α. There were no pertinent references came across or available with Norgestomet plus PGF<sub>2</sub>α treatment and second service conception rate. Hence, we were not in position to compare with other research workers. However in the present study, we found 50 per cent (one out of two remaining animals) pregnant at second service reflected higher percentage of figure that might be dependent only on two animals.

Out of total eighteen silent oestrus (suboestrus) Surti buffaloes in the treatment and control groups, six (33 per cent), five (28 per cent) and one (6 per cent) conceived at first, second service and third service, respectively. While six (33 per cent) buffaloes remained non-conceived from both the treatment and control groups up to 120 days. Out of total six silent oestrus

(suboestrus) Surti buffaloes in the T1 (Norgestomet) group, two (34 round percentage) and two (33 round percentage) conceived at first and second service, respectively. While two (33 round percentage) buffalo remained non-conceived up to 120 days. Out of total six silent oestrus (suboestrus) Surti buffaloes in the T2 (Norgestomet + PGF<sub>2</sub>α) group, four (67 per cent) and one (16 per cent) conceived at first and second service, respectively. While one (17 per cent) buffalo remained non-conceived up to 120 days. Out of total six silent oestrus (suboestrus) Surti buffaloes in the T3 (silent oestrus control) group, none (00.00 per cent), two (33 per cent) and one (17 per cent) conceived at first, second and third service, respectively. While three (50 per cent) buffaloes remained non-conceived up to 120 days.

**Table 4. Effect of different treatments on Reproductive performance of postpartum silent oestrus Surti buffaloes**

Parameters	Group-I (n=6)	Group-II (n=6)	Group-III (n=6)	F value	P value
Overall Conception rate	4/6 (66.67)	5/6 (83.33)	3/6 (50.0)	--	--
Number of services per conception (Mean ± SEM)	1.50 ± 0.29 <sup>ab</sup>	1.20 ± 0.20 <sup>a</sup>	2.33 ± 0.33 <sup>b</sup>	4.470*	0.045
Service period (days) (Mean ± SEM)	75.75 ± 7.26 <sup>a</sup>	73.00 ± 4.36 <sup>a</sup>	94.33 ± 4.06 <sup>a</sup>	3.753	0.065

Means bearing different superscripts within a row (between the groups) differ significantly (p < 0.05).  
 Figures in the parenthesis indicate percentage.  
 Group-I = T1 (Norgestomet), Group-II = T2 (Norgestomet + PGF<sub>2</sub>α), Group-III = T3 (Silent oestrus Control)

#### Overall conception rate

The overall conception rate was calculated as some animals became pregnant at first, second & third services and it was maximum in treatment Group-II, 5/6 (83.33 per cent) followed by treatment Group-I, 4/6 (66.67 per cent) and control Group-III, 3/6 (50.00 per cent). (Tab3 & 4) Higher conception rate in treated groups (T1 & T2) as compared to control group (T3) might be attributed to effect of early onset of induced estrus due to hormonal treatment up to 120 days as compared to normal estrus followed by conception during that period.

The findings were in agreement with overall conception rate in Group-I (Norgestomet) as 64.24 per cent by Gopikrishnan *et al.* [38] in the crossbreed heifers following Norgestomet ear implant treatment. While, lower per cent overall conception rate as 33.33 per cent; 41.66 per cent; 43.0 per cent; 43.75 per cent and 50.0 per cent reported by Chaudhari *et al.* [37] in delayed pubertal Kankrej heifers; Shukla [45]. in postpartum anoestrus (HF X Shahiwal) crossbred cows; Tada *et al.* [46] postpartum cyclic beef cows; Selvaraju and Veerapandian [47] in repeat breeder crossbreed cows and Kiser *et al.* [48] in lactating Beef cows, respectively following Norgestomet ear implant treatment. However, higher per cent of overall conception rate reported as 70 per cent and 80 per cent by Markendeya and Bharkad [49] in postpartum anoestrus Deoni cows and Agarwal *et al.* [50] in postpartum anoestrus cattle, respectively following Norgestomet ear implant treatment.

These findings were in close agreement with overall conception rate in Group-II (Norgestomet + PGF<sub>2</sub>α) as 83.33 per cent reported by Yadav *et al.* [51]. in anoestrus buffaloes following Norgestomet ear implant plus PGF<sub>2</sub>α and GnRH treatment. Whereas, little bit higher overall conception rate reported as 88.00 per cent reported by Beal *et al.* [21]. in postpartum cyclic beef cows treated with Norgestomet ear implant plus PGF<sub>2</sub>α. However, lower per cent of overall conception rate as 66.00 per cent; 66.66 per cent and 75.00 per cent reported by Beal *et al.* [21]. in postpartum non-cyclic cows; Yadav *et al.* [51]. in anoestrus buffaloes treated with Norgestomet plus PGF<sub>2</sub>α and FSH and Agarwal *et al.* [50]. in postpartum anoestrus cows following Norgestomet ear implant plus PGF<sub>2</sub>α treatment. Whereas, very low rate of overall conception rate following Norgestomet ear implant plus PGF<sub>2</sub>α treatment as 37.50 per cent reported by Selvaraju and Veerapandian [47]. crossbreed cows and 40.0 per cent reported by Utage *et al.* [30]. in anoestrus buffaloes following Norgestomet ear implant plus PGF<sub>2</sub>α treatment.

Higher overall conception rate in treatment Group-I and Group-II as compare to control Group-III might be attributed to Norgestomet and Norgestomet plus PGF<sub>2</sub>α treatment as against placebo treatment in control group. Though, without any inference 50.0 per cent overall conception rate was observed in the present findings as usually follows in natural condition.

Moreover, higher and lower overall conception rate observed by various workers as compared to Group-I and Group-II in the present study could be due to breed, species, parity as well as different drug, dose and regimen used by them.

#### **Number of services per conception**

The mean of number of services per conception was calculated for treatment group (T1), treatment group (T2) and control group (T3) of Surti buffaloes were  $1.50 \pm 0.29$ ,  $1.20 \pm 0.20$  and  $2.33 \pm 0.33$ , respectively. The number of services per conception reported in Group-I (Norgestomet) and Group-II (Norgestomet + PGF<sub>2</sub>α) was in agreement with average 1.33 numbers of services per conception reported by Rede [52]. in postpartum suboestrus Surti buffaloes treated with PGF<sub>2</sub>α. Whereas, higher average of number of services per conception recorded as 1.85 by Markendeya and Bharkad [49]. in Deoni cows following Crestar ear implant treatment. The mean of number of services per conception in control (T3) group was found significantly higher as compare to treatment (T2) group might be due to more number of animals conceived at second service and one more at third service. Since, gestation length is fixed parameter, the number of services required for each conception (conception rate) influence the age at first calving, service period and calving interval. The goal should be 1.5 services per conception. However, two services per conception are acceptable.

#### **Service period**

The mean intervals (days) from calving to conception (service period / open days) was calculated from above parameters for treatment Group-I (T1), treatment Group-II (T2) and control Group-III (T3) and observed to be  $75.75 \pm 7.26$  days,  $73.00 \pm 4.36$  days and  $94.33 \pm 4.06$  days, respectively. The mean intervals (days) from calving to conception (service period) varied non- significantly ( $p > 0.05$ ) between treatment groups (T1 & T2) as well as between each treatment (T1, T2) and control Group (T3). Although, the service period was found to be shorter i.e. up to one cycle in the treatment Group-I and treatment Group-II as compared to control Group-III. The calculated data is tabulated in Tab.2. The late induction of natural & irregular estrus in control Group-III (T3) as compare to induced estrus in treated Group-I (T1) and treated Group-II (T2), followed by settling of animals at different number of services per conception in control Group-III (T3) might be the known reason that turned apparently higher ( $p < 0.05$ ) mean intervals (days) from calving to conception (service period) between control and each treatment group. However, no significant difference ( $p > 0.05$ ) was observed between in the mean intervals (days) from calving to conception (open days) among the treatment groups. The mean of service period reported in Group-I (Norgestomet) and Group-II (Norgestomet + PGF<sub>2</sub>α) was in agreement with  $80.42 \pm 4.98$  days (ranging from  $66.83 \pm 4.07$  to  $96.00 \pm 8.50$  days) reported by

([52].Rede, 2014) in postpartum suboestrus Surti buffaloes treated with PGF<sub>2</sub>α. However, Khsatiya *et al.* [53]. reported  $59.73 \pm 3.76$  days mean service period in suboestrus postpartum Surti buffaloes treated with PGF<sub>2</sub>α, which was comparable with the result of the study.

The fertility response of suboestrus buffaloes to the hormonal therapy (Norgestomet alone and in combination with PGF<sub>2</sub>α combination) was comparable to several previous reports in buffaloes. It might be due to subluteal levels of progesterone by way of injections or implants increases LH pulse frequency leading to maturation of the dominant follicle ([54].Yavas and Walton, 2000). The combination of progesterone with PGF<sub>2</sub>α causes increased estradiol production leading to behavioural estrus followed by ovulation and thus favors conception. Administration of PGF<sub>2</sub>α probably helps complete the recovery of the hypothalamus-pituitary-gonadal axis function already stimulated by the progesterone treatment. Prostaglandin (PGF<sub>2</sub>α) was given before the time of progesterone withdrawal, improved the ovulation rate. PGF<sub>2</sub>α has been frequently used with progesterone to induce fertile estrus and also to stimulate final follicular maturation and ovulation in suboestrus cattle. The addition of PGF<sub>2</sub>α to a progesterone based protocol might improve the ovulation and pregnancy rates of suboestrus buffaloes.

The study held in breeding season might be provided better environment for conception rate in buffaloes. Better synchrony and conception rates have been documented after synchronization during the breeding season than the non-breeding season [55]. The breeding frequency in buffaloes was highest during the winter, decreased in autumn and spring, and was lowest in the summer [56]. The buffaloes display a distinct seasonal variation in reproductive pattern. Breeding season provided a boosting effect in improving the fertility in Surti buffaloes when hormone protocol was used Prajapati, [57].

It could be concluded that Norgestomet ear implant alone and in combination with PGF<sub>2</sub>α can be successfully used to induce the estrus in the postpartum silent oestrus Surti buffaloes. All the postpartum silent oestrus Surti buffaloes responded to treatment and the estrus can be successfully induced in silent oestrus buffaloes with the use of Norgestomet ear implant alone or in combination with PGF<sub>2</sub>α. The interval of the induction of estrus from the day of implant removal in Norgestomet ear implant alone or in combination of PGF<sub>2</sub>α was significantly shorter in terms of days as compared to silent oestrus control Surti buffaloes. The duration of induced estrus was significantly longer when PGF<sub>2</sub>α was injected one day before (on the 8<sup>th</sup> day) removal of implant. Additional exogenous injection of PGF<sub>2</sub>α along with Norgestomet ear implant could be helped to resolve the problem of silent oestrus in Surti buffaloes. So, Norgestomet ear implant alone

and in combination with PGF<sub>2</sub>α therapy might have been used as an initial package of practices in the treatment of silent oestrus (suboestrus) in the field conditions to augment fertility in those animals. Use of Norgestomet ear implant alone or in combination with PGF<sub>2</sub>α in silent oestrus (suboestrus) condition in Surti buffaloes could be minimized the service period about 18 to 21 days.

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