

## Estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant

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### Abstract

**Aim:** The study was undertaken to find out the estrus induction and fertility response in delayed pubertal Kankrej heifers treated with norgestomet ear implant.

**Materials and methods:** Total eighteen anoestrus Kankrej heifers of delayed puberty weighed above 250 kg and attained between 30 to 36 months of age were selected and divided in to three groups of six animals each at random to conduct the experiment. Animals in group 1 were implanted Crestar ear implant for 9 days. In addition to this, group 2 received 500 IU of PMSG on the day of implant removal. In group 3, treatment protocol remained same as in group 2, but Inj. Receptal @ 2 ml was given additionally at the time of breeding.

**Results:** All the animals exhibited estrus with average duration of  $25.41 \pm 0.94$ ,  $21.95 \pm 0.20$  and  $22.68 \pm 1.46$  hours between implant withdrawal and estrus induction in group 1, 2 and 3, respectively. The duration of estrus was significantly ( $P < 0.05$ ) longer ( $25.61 \pm 2.95$  hours) in group 2, followed by group 1 ( $18.88 \pm 1.45$  hours) and group 3 ( $13.48 \pm 1.92$  hours). The pregnancy rate at induced estrus was 33.33 percent in group 2. In group 1 and group 3 none of the heifers found pregnant at induced estrus. The overall conception rate was maximum in group 2 (66.67 percent) followed by group 3 (50 percent) and group 1 (33.33 percent) after the 3<sup>rd</sup> service.

**Conclusion:** Although the conception rate at induced estrus was lower, norgestomet ear implant could be utilized to induced estrus in delayed pubertal cow heifers.

**Key words:** Conception rate, Delayed puberty, Estrus induction, Heifers, Norgestomet

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### Introduction

Puberty in the female is defined as the attainment of a developmental state that supports normal ovarian cyclicity (follicular development and ovulation) and the ability to become pregnant. The event is of prime importance among the post - natal phenomenon of reproductive origin. High life time reproductive efficiency requires early attainment of puberty. Delayed puberty is considered to be the most important challenging problem associated with dairy heifer reproduction particularly in countries like India. It is the main cause of lowered fertility in heifers and is responsible for huge economic losses to the dairy farmers by decreasing life time milk yield and the number of calves produced by a cow in her lifetime. In spite of having attained pubertal age and body weight, a large percentage of Indian zebu heifers fail to commence cyclicity [1].

Delayed onset of puberty lowers the reproductive rate of females as it delays their entry into breeding herds [2]. This necessitates exogenous intervention to induce ovarian activity in delayed pubertal animals. In addition to herbal heat inducer drugs [3-7] biostimulation [8,9] vitamin and mineral mixture supplementation [10,11] several hormonal preparations also available to induce estrus [12-16] in cyclic as well as acyclic animals. Hormonal preparations for induction of estrus include administration of prostaglandin, progesterone, gonadotrophins and Gonadotrophin Releasing Hormone (GnRH) or their synthetic analogues, either alone or in various combinations. Progesterone treatment successfully induces ovarian cyclicity in bovines [12,15,17,18,19]. Equine Chorionic Gonadotrophin (eCG) has been frequently used with progesterone to induce fertile estrus and also to stimulate final follicular maturation and ovulation

**Table - 1. Estrus induction response in different groups of Kankrej heifers treated with Crestar ear implant**

Parameter	Group 1 (NOR)	Group 2 (NOR + PMSG)	Group 3 (NOR + PMSG + GnRH)
Number of heifer treated	n = 6	n = 6	n = 6
Induction of estrus (Heifers responded)	6 (100)	6 (100)	6 (100)
Onset of estrus after withdrawal of implant (hr), Mean $\pm$ SEM	25.41 $\pm$ 0.94	21.95 $\pm$ 0.20	22.68 $\pm$ 1.46
Duration of estrus (hr), Mean $\pm$ SEM	18.88 <sup>ab</sup> $\pm$ 1.45	25.61 <sup>a</sup> $\pm$ 2.95	13.48 <sup>b</sup> $\pm$ 1.92

a, b: Means bearing different superscript within row differ significantly ( $P < 0.05$ ), Figures in parenthesis indicate percentage.

in anoestrus cattle [1]. Crestar implant alone and in combination with Folligon was tried by Nayak *et al.* [20] for estrus induction in true anoestrus buffaloes. Since GnRH has the property of inducing follicular growth and ovulation, incorporation of GnRH in the norgestomet regimen may improve fertility in anoestrus cows [21]. The mechanism of action of both human Chorionic Gonadotrophin (hCG) and GnRH is via the induction of accessory corpus luteum (CL) by ovulating the dominant follicle of the first follicular wave as well as having a stimulatory effect on the production of progesterone by the primary CL[22]. Keeping all these facts in view, the present study was undertaken on delayed pubertal Kankrej heifers to evaluate the efficacy of Crestar ear implant alone or in combination with PMSG and GnRH in inducing estrus and subsequent fertility response.

### Materials and Methods

**Animals:** The study was approved by the committee framed for the research by the university authority. The present investigation was carried out during the period from December 2003 to June 2004 on anoestrus Kankrej heifers of delayed puberty weighed more than 250 kg body weight and attained over 30 months of age, because at least at this stage of age and body weight the heifers should reach their puberty. Such 18 heifers of Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar were selected and randomly divided in to three groups i.e., group 1 (NOR); group 2 (NOR + PMSG) and group 3 (NOR + PMSG + GnRH). Each group included 6 heifers. All the experimental animals were maintained under uniform managerial conditions.

**Treatment Protocols:** Animals of all the three groups were treated with 3 mg norgestomet ear implant (Crestar®; Intervet International B.V.–Boxmeer, Holland) subcutaneously in the middle of the outer face of the ear pinnae with the help of special applicator. At the time of implant insertion 2 ml Crestar injection (3 mg norgestomet and 5 mg oestradiol valerate) was administered intramuscularly.

The implants were kept *in situ* for the period of 9 days. In the NOR group, no drug was administered at the implant removal. In NOR + PMSG group, 500 IU Folligon (Intervet International B.V.–Boxmeer, Holland) injected intramuscularly on day 9 immediately after implant removal. In addition to the treatment regimen of NOR + PMSG group, the heifers of the third group received Injection Receptal (Intervet International B.V.–Boxmeer, Holland) 2 ml intramuscularly at the time of breeding. After removal of the implant on day 9, all the heifers were subjected to detection of estrus by parading the bull of good sexual drive.

**Examination of animals post-treatment and analysis of data:** Percentage of heifers exhibiting estrus, time interval between implant removal and onset of estrus and duration of estrus for each heifer were recorded (Table 1). The first acceptance of male by the female was considered as the onset of estrus and non-acceptance of male by the female was considered as cessation of estrus. The duration of estrus was recorded from onset of estrus to cessation of estrus. Heifers identified in estrus were bred by artificial insemination with good quality semen. All the heifers were closely observed for re-occurrence of estrus. The heifers which failed to return in estrus following breeding were examined per rectally on 60th day post breeding for confirmation of pregnancy. Percentages of heifers conceived at induced estrus were considered as first service conception rate. The heifers failed to conceive at the induced estrus and returned to estrus were rebred at subsequent (2nd or 3rd) estrus. Heifers conceived at 2nd and 3rd estrus following induced estrus plus heifers conceived at induced estrus were considered as overall conception rate (Table 2). The data collected were suitably tabulated and analyzed following standard statistical method shown by Steel and Torrie [23]. The tests of significance between the treatment groups for onset of estrus after withdrawal of implant and duration of estrus were made by simple completely randomized block design and Duncan's new multiple range test. The Duncan's new multiple

**Table - 2. Conception rate in different groups of Kankrej heifers treated with Crestar ear implant**

No. of Services	Group 1(n = 6)	Group 2 (n = 6)	Group 3 (n = 6)
First service	0/6 (0.0)	2/6 (33.33)	0/6 (0.0)
Second service	1/5 (20.0)	2/4 (50.0)	3/5 (60.0)
Third Service	1/4 (25.0)	0/2 (0.0)	0/1 (0.0)
Overall Conception	2/6(33.33)	4/6 (66.67)	3/6 (50.0)

Figures in parenthesis indicate percentage.

range test was performed at 5 percent level of significance.

## Results and Discussion

**Induction of estrus:** 100 percent (18/18) heifers of all the three groups in the present study responded to the treatment and found in estrus following removal of Crestar ear implant (Table-1). The estrus can be successfully induced in delayed pubertal anoestrus Kankrej heifers with the use of Crestar ear implant alone or in combination with PMSG. The findings obtained by Balasubramanian and Quayam[24], Luthra *et al.* [25], Sarmah *et al.* [26], Markendeya and Bharkad[27] and Luthra *et al.* [28] were in agreement with the present study. However, MacMillan *et al.* [29] observed only 64 percent Friesian heifers in estrus. 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[30] 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. The use of PMSG along with Crestar may be useful as PMSG is known to increase blood estrogen and in turn leads to induction of behavioral estrus [12].

**Onset of estrus after withdrawal of implant:** The interval of onset of estrus following the removal of Crestar ear implant was non - significantly longer in group 1 ( $25.41 \pm 0.94$  hours) then group 2 ( $21.95 \pm 0.20$  hours) and group 3 ( $22.68 \pm 1.46$  hours) (Table 1). The interval of the induction of estrus from the day of removal of implant did not differ with the inclusions of PMSG. The mean interval between Crestar implant withdrawal and onset of induced estrus reported in group 1 was in agreement with King *et al.* [31]. Whereas, Rentfrow *et al.* [32] and Pinheiro *et al.* [33] reported comparatively longer estrus induction interval than the present study after withdrawal of implant. The mean interval reported in group 2 and

group 3 was in agreement with Luthra *et al.* [25] and Sarmah *et al.* [26]. However, Rao *et al.* [34] and Singh *et al.* [1] reported comparatively higher interval for onset of estrus following norgestomet plus PMSG treatment.

**Duration of estrus:** In the present study, significant ( $P < 0.05$ ) difference in duration of estrus was observed between group 2 ( $25.61 \pm 2.95$  hours) and group 3 ( $13.48 \pm 1.92$  hours). Whereas, group 1 ( $18.88 \pm 1.45$  hours) did not differ significantly from any of the group (Table 1). The duration of induced estrus was significantly longer when PMSG was injected on the day of removal of implant. However, the duration of estrus was at par when Crestar alone or Crestar plus PMSG plus GnRH were used. The mean duration of estrus reported in group 1 was in agreement with Agarwal *et al.* [35]. While, Tregaskes *et al.* [36] and Pinheiro *et al.* [33] recorded comparatively shorter duration of estrus. The mean duration of estrus recorded in group 2 was comparatively shorter than those reported by Sarmah *et al.* [26] in animals treated with norgestomet plus PMSG, and, it was longer than those noticed by Singh *et al.* [1] and Nath *et al.* [37]. The observed differences among the various workers may be due to the method adopted by them for the calculation of duration of estrus.

**Fertility Response:** 33.33 percent (2/6) first service conception rate was obtained in group 2. However, none of the heifers found pregnant at first service in group 1 and group 3. The reduced fertility at norgestomet induced estrus may be owing to the luteal dysfunction [31], which may be due to insufficient LH production following implant withdrawal [38]. The pregnancy rate in heifers at induced estrus was quit low in present investigation. But the injection of PMSG may help to improve the conception rate. Similarly, Luthra *et al.* [28] obtained better conception rate in group of non-cyclic post-pubertal Sahiwal heifers treated with CIDR along with PMSG at the time of CIDR withdrawal in comparison to the group of heifers treated with CIDR alone. The first service conception rate in group 1 and 3 of the present study was in agreement with Hixon *et al.* [38]. They also

reported zero percent first service conception rate in anoestrus heifers following induced estrus with Syncro-Mate-B. Khurana [39] reported a single pregnancy out of 105 poor conditioned cows with the same treatment. Selvaraju and Rajasundaram [40] obtained 12.5 percent conception rate in anoestrus cows after norgestomet alone treatment. While, better conception rate was obtained by Rentfrow *et al.* [32] in Syncro-Mate-B treated Brahman heifers (18.2 percent), Singh *et al.* [41] in anoestrus heifers and cows (40 percent). The first service conception rate obtained in group 2 was better than those reported by Sarmah *et al.* [26]. They reported zero percent conception rate at induced estrus with Crestar plus PMSG in prepubertal indigenous heifers. Similarly, low first service conception rate (25 percent) was reported by Rao *et al.* [34] after norgestomet plus PMSG treatment. Dodamani *et al.* [42] also gained no extra beneficial effect of PMSG either on estrus response or conception rate in buffaloes.

The overall conception rate obtained in group 1 and group 3 was better than those reported by Selvaraju and Rajasundaram [40]. They obtained 25 percent conception rate in norgestomet treated anoestrus cows. Again, Selvaraju and Rajasundaram [21] achieved 25 percent conception rate with Crestar alone and 50 percent conception rate with Crestar plus GnRH treatment. The result of the present study in group 2 was in close agreement with Singh *et al.* [1] and Selvaraju and Rajasundaram [40]. They reported 73.7 percent and 62.5 percent overall conception rate after norgestomet plus PMSG treatment, respectively. However, conception rate of group 2 was lower than those obtained by Luthra *et al.* [25]. They obtained 86 percent overall conception rate after norgestomet plus PMSG treatment.

### Conclusion

Although the conception rate at induced estrus was lower, norgestomet ear implant could be utilized to induced estrus in delayed pubertal cow heifers. Compared to norgestomet alone, good fertility response could be achieved when PMSG was injected at norgestomet implant removal.

### Authors' Contribution

CF Chaudhari, BN Suthar, VK Sharma, VS Dabas and HH Panchasara implemented the study design and carried out the experiment. CF Chaudhari, BN Suthar, VK Sharma, VS Dabas analysed the data. CF Chaudhari, BN Suthar, VK Sharma, VS Dabas, HH Panchasara and NF Chaudhari drafted and revised the

manuscript. All authors read and approved the final manuscript.

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### Competing interests

Authors declare that they have no Competing interests.

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