

## Induction of Oestrus with Norgestomet Ear Implant and PRID in Acyclic Holstein Heifers

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**Abstract:** The aim of this study, was to compare the efficacy of treatment with norgestomet implant and Progesterone Releasing Intravaginal Device (PRID) in combination with GnRH on estrus response and pregnancy rates in acyclic holstein heifers. A total of 64 heifers (14-24 months age) were randomly divided into 3 groups. Group Norgestomet (n = 25), Group PRID (n = 26) and Control group (n = 13). Group Norgestomet; ear implant implanted subcutaneous and solution containing 3 mg norgestomet and 5 mg oestradiol valerate were injected intramuscularly at the day of 0. The silicone implant removed after 11 days. Group PRID; PRID (1.55 g progesterone, 10 mg estradiol benzoate) was inserted into vagina and left in place for 11 days. Control group; saved as control group. GnRH (0.0042 mg buserelin acetate) was administered intramuscularly (2.5 mL) at implant removal or withdrawal of PRID in implant and PRID groups. The heifers were inseminated fix timed at 48 and 72 h after the removal of implant and PRID. The rate of induced estrus was 80 and 73.08% between norgestomet implant and PRID groups, respectively. No heifer of the control group showed estrus. The pregnancy rates were 44 and 53.85% in norgestomet and PRID groups, respectively. There was no significant difference in terms of estrus response and pregnancy rates between norgestomet and PRID groups. However, a significant difference between treatment groups and control group, with respect to estrus ( $p < 0.001$ ) and pregnancy rates ( $p < 0.01$ ) were observed. The average BCS were not different statistically between all groups. Results of the study showed that fertile estrus can be stimulated in acyclic heifers by norgestomet implant and PRID treatments combined with GnRH. Also, we concluded that norgestomet implant and PRID are equally effective for estrus induction and pregnancy rate in acyclic heifers.

**Key words:** Acyclic heifers, estrus induction, norgestomet, PRID

### INTRODUCTION

Inactive ovary (true anoestrus) is a condition, in which the ovaries are quiescent without signs of cyclicity or cycle related ovarian structures. The cow would not have shown any sign of estrus and rectal palpation reveals small ovaries, which are either flat and smooth or sometimes rounded (Zulu *et al.*, 2000). Anoestrus can occur as a herd problem or as a problem in individual cows in an otherwise normal herd (Ferry, 1997). This condition is most frequently diagnosed in high-yielding dairy cows and first calf heifers (Zulu *et al.*, 2000) but can also be seen in post-pubertal heifers (Cetin *et al.*, 2007). The reason for the failure of normal ovarian activity may be insufficient release or production of gonadotropins to cause folliculogenesis, or it may reflect the failure of the ovaries to respond to gonadotropins. Reproductive

performance is adversely affected by true anoestrus and it is one of the major causes of economic losses in both the dairy and beef industries.

Energy deficiency interacted with other factors, is the major cause of anoestrus. There is a very important interrelationship between nutrition and production performance of dairy cows and this interrelationship has far reaching effects on the physiological functioning of the reproductive system, which is constantly under the influence of the endocrine (Mwaanga and Janowski, 2000). The nutritional status of an animal evaluated through a Body Condition Score (BCS), reflects the body reserves available for basic metabolism (Montiel and Ahuja, 2005).

Therapy for the induction of cyclicity in the anoestrus animal has been attempted with a variety of exogenous hormones and management practices (Hopkins, 1986).

Many hormone treatments have been utilised for the treatment of inactive ovaries. These include estradiol, Gonadotropins Releasing Hormone (GnRH), Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and progesterone (Hopkins, 1986; Zulu *et al.*, 2000). Recent studies indicate that progesterone may be treatment of choice for inactive ovaries. Attempts to induce cyclicity and ovulation in acyclic cows (Belloso *et al.*, 2002) and heifers (Cetin *et al.*, 2007) have involved the use of exogenous progesterone or synthetic progestagens to establish an artificial luteal phase. Such a luteal phase enables an accumulation of gonadotropins. This results in an LH surge and ovulation on termination of progesterone therapy. Progesterone Releasing Intravaginal Device (PRID) and norgestomet implant has been successfully used to induce estrus with ovulation in anestrus animals (Mwaanga *et al.*, 2003; Zulu *et al.*, 2000). However, studies on inactive ovaries have mostly focused on the postpartum period in cows. There are only few reports about progesterone or norgestomet treatment on inactive ovaries in heifers. The aim of the present study, was to determine the effect of norgestomet and PRID administration and compare effect of PRID and norgestomet implant on the induction of estrus and pregnancy rates in non-cyclic heifers.

**MATERIALS AND METHODS**

The study was conducted in south-east Anatolia covering a total of eight dairy farms. A total of 64 Holstein heifers with inactive ovaries, used for the study were aged 14-24 months age (mean 18.2±2.2). The heifers were healthy and had no history of other reproductive problems. The BCS was measured in all heifers according to 1-5 systems as described by Edmonson *et al.* (1989).

The selection of heifers with inactive ovaries was based on the findings of rectal palpation and sera of progesterone levels. The heifers were examined by rectal palpation at ten day intervals for determining of activities of ovaries. The heifers with small and flat ovaries and with progesterone levels <1 ng mL<sup>-1</sup> in blood samples, taken on rectal palpation days, were diagnosed as having inactive ovaries.

The protocol for induction of estrus with PRID and implants is summarized in Table 1. A total of 64 heifers were randomly divided into 3 groups: group

norgestomet (n = 25), group PRID (n = 26) and group control (n = 13). The norgestomet treatment consists of an intramuscular injection of norgestomet (3 mg) and estradiol valerate (5 mg) and silicone ear implant that contains 6 mg of norgestomet (Crestar, Intervet, Boxmeer-Netherland). The implant was subcutaneously inserted into external surface of the ear and injection performed at day 0. The silicone implant was removed after 11 days. Progesterone releasing intravaginal device (1.55 g progesterone, 10 mg estradiol benzoate; Ceva Sante Animale, France) was inserted into vagina and left in place for 11 days. All untreated control group animals were observed by visually for oestrus signs twice daily, in the morning and in the afternoon, for 14 days from the placebo (normal saline solution) injection day (day 0). The visual signs of oestrus observed, included vulvar swelling, hyperaemia of vaginal mucosa, vaginal mucus discharge, bellowing, restlessness and response to vulvar massage. Norgestomet implants and PRIDs were removed after 11 days. At the removal of implant and PRID, GnRH (0.0042 mg buserelin acetate; Receptal 10 mL, Intervet®) in a dose of 2.5 mL was administered intramuscularly. After removal of norgestomet implants and PRIDs, the heifers were observed at 12 h observation interval for 4 days period. The heifers were inseminated fix timed at 48 and 72 h after the removal of implant and PRID. The pregnancy diagnosis was carried out 60 days after AI by rectal palpation.

**Blood samples:** Two blood samples were collected (-20 and -10 days) in order to determine the levels of progesterone. The samples were centrifuged at 3000 rpm for 15 min at 4°C and the sera were stored at -20°C until assay for concentration of Progesterone (P4). Concentrations of P4 in sera were determined in a single assay using radioimmunoassay kits with highly specific progesterone antiserum having very little cross-reactivity against other steroids (Coat-A-Count, Progesterone, DPC, USA). The intra and inter-assay coefficients of variation were 7.14 and 6.18%, respectively. If sera P4 concentrations of 2 samples were <1 ng mL<sup>-1</sup>, then the heifers were considered to be anoestrus ones.

**Statistical analyses:** The body condition score was analysed with t-test. The rate of estrus response and pregnancy rate between PRID, norgestomet and control group were compared by means of a chi-square test.

Table 1: Experimental design of the study

Variables	Day 0	Days 11	Days 13	Days 14
Norgestomet	Ear implant + Norgestomet injection	Removal of implant+GnRH	AI	AI
PRID	Insertion of PRID	Removal of PRID+GnRH	AI	AI
Control	---	Placebo injection	AI	AI

AI: Artificial Insemination

Table 2: Average age, BCS, oestrus and pregnancy rate in norgestomet, PRID and control groups

Variables	Age (month) (min-max)	BCS (min-max)	Oestrus rate	Pregnancy rate
Norgestomet	18.32±2.34 <sup>a</sup> (14-24)	2.66±0.57 <sup>b</sup> (2-4)	20/25 80% <sup>d</sup>	11/25 44% <sup>f</sup>
PRID	18.31±2.28 <sup>a</sup> (15-23)	2.56±0.49 <sup>b</sup> (2-3.5)	19/26 73.08% <sup>d</sup>	14/26 53.85% <sup>f</sup>
Control	18.08±1.98 <sup>a</sup> (15-22)	2.54±0.43 <sup>b</sup> (2-3.5)	0/13 0% <sup>e</sup>	0/13 0% <sup>e</sup>

Values within same columns are different (<sup>a</sup>p<0.001; <sup>f</sup>p<0.01); ±SD

## RESULTS

There were no significant differences in average BCS and age of the groups. Average age and BCS, oestrus and pregnancy rates in treatment and control groups are shown in the Table 2. The rate of induced oestrus was 80% (20/25) and 73.08% (19/26) in the norgestomet and PRID groups, respectively. No heifer of the control group showed oestrus throughout the study. A significant differences between treatments groups and control group, with respect to oestrus (p<0.001) and pregnancy rates (p<0.01) were observed.

## DISCUSSION

Progestagens suppress estrus in cattle and have been used widely to alter the estrous cycle. The efficacy of estrus synchronization is limited primarily by the proportion of cows that have initiated estrous cycles at the start of the breeding season (Wiltbank, 1970). As the proportion of cows that are anestrus at the start of the breeding season increases, the efficacy of luteolytic agents to synchronize estrus declines (Odde, 1990). One distinct advantage to progestin-based estrus synchronization systems is that progestin administration during the postpartum period may induce estrous cycles in anestrus cows (Anderson and Day, 1998).

Wiltbank and Gonzalez-Padilla (1975) firstly reported that a 9-days implant containing 6 mg norgestomet plus an injection of 5 mg of estradiol valerate and 3 mg of norgestomet given at the time of implant insertion successfully synchronized estrus and also induced estrus in non-cycling beef heifers. We previously reported that (Ozyurtlu *et al.*, 2008) oestrus induction rate following PRID removal was 75% in heifers with inactive ovaries. Cetin *et al.* (2007) have found that Holstein heifers treated with norgestomet showed an oestrus rate of 86%. Nak *et al.* (2005) reported a similar recovery rate of 72% after PRID treatment in heifers. Ghallab *et al.* (1986) found 61% oestrus rate in anoestrous beef cows treated with norgestomet ear implant devices. Mwaanga *et al.* (2003)

reported that 85% oestrus rate in acyclic dairy cows treated with norgestomet. Oestrus induction rate can be improved, if gonadotropin is given at the time of PRID removal (Zulu *et al.*, 2000).

An oestrus induction response was observed 73.08 and 80% of heifers after PRID and norgestomet treatment combined with GnRH in this study, respectively. The oestrus induction rates observed in this study agrees with the results of Mwaanga *et al.* (2003), Nak *et al.* (2005), Cetin *et al.* (2007) and Ozyurtlu *et al.* (2008). A high proportion of the treated animals were induced to oestrus, while none of the controls exhibited estrus, indicating the success of the norgestomet regimen and PRID application in Holstein heifers with inactive ovary.

The range of females showing estrus after norgestomet or PRID treatment was higher than 60% in most trials. The fertility of this estrus, however, was variable. The differences in conception rate across trials may be due in part to level of cyclicity. Brown *et al.* (1988) reported a 30% conception rate in non-cycling heifers treated with norgestomet and a 48% conception rate in cycling heifers. Ozyurtlu *et al.* (2008) reported a 33.3% conception rate in non-cycling heifers treated with PRID and a 45.4% conception rate in cycling heifers. The poor conception rates 28.6 and 30% were reported by Zulu *et al.* (2000) and Unal *et al.* (1992), in anestrus cows after PRID treatment, respectively. This poor conception rates after progesterone treatment may be related with inactive ovary condition in heifers or cows. However, acceptable pregnancy rates were achieved in this study (44 and 53.8% in norgestomet and PRID treated heifers, respectively). Similar pregnancy rates were obtained by Cetin *et al.* (2007) and Mwaanga *et al.* (2003) after norgestomet treatment in acyclic Holstein heifers (48.2%) and dairy cows (50%), respectively. DoValle *et al.* (1997) demonstrated that the administration of GnRH after progestagen treatment enhanced timed AI pregnancy rates. These differences in pregnancy rates may be generated by many factors such as fix-time or oestrus based artificial insemination, management factors, inseminators, sperm-related factors, breed, parity, season and gonadotropin treatment combined with progestagens.

Most animal and dairy scientists acknowledge successfully manipulating BCS as an important management factor, influencing animal health, milk production and reproduction in the modern dairy cow (Roche *et al.*, 2004). Body condition of beef cattle influences conception rate after Syncro-Mate B treatment

(Odde, 1990). Thin cattle have longer postpartum intervals to estrus and therefore are more likely to be non-cycling at the time of treatment, resulting in reduced conception rates. The efficacy of progestin treatment to induce onset of estrus cycles is affected by cow body condition.

The duration of oestrus declined in heifers with fat body condition (Villa-Godoy *et al.*, 1990). In the current study, there was no statistical difference in average BCS among the groups. Therefore, we think that the results of study not be affected from BCS.

### CONCLUSION

The results of this study, indicates that oestrus can be stimulated successfully by norgestomet or PRID treatment combined with GnRH in heifers with inactive ovaries and acceptable pregnancy rates can be achieved by fix-time inseminations.

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