INDUCTION OF OESTRUS WITH NORGESTOMET IN ACYCLIC POST-PUBERTAL HOLSTEIN HEIFERS

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Abstract

The study was carried out during April and May 2006, on thirty-eight 15 – 23-month-old Holstein heifers with inactive ovaries, which were selected from a private dairy herd with 300 heifers. The heifers were randomly distributed into two groups: norgestomet group (n=29), and a control group (n=9). In the norgestomet group, silicone ear implants containing 6 mg of norgestomet were implanted, and a solution containing 3 mg of norgestomet and 5 mg of oestradiol valerate was injected intramuscularly. The silicone implants were removed 11 d later, and the heifers were continuously observed for signs of oestrus for three days. The heifers were inseminated 48 and 78 h after the removal of the implants, and after the first insemination they were treated with 50 µg of GnRH analogue. The rate of the induced oestrus was 86.2% (25/29) and 0% (0/9) in norgestomet and control groups, respectively. The pregnancy rate was 48.2% (14/29) and 0% (0/9) in norgestomet and control groups, respectively. Our results showed that fertile oestrus can be stimulated successfully in post-pubertal heifers with inactive ovaries by a norgestomet treatment, and successful pregnancy rate can be obtained by a fixed-time insemination during this oestrus.

Key words: heifer, oestrus synchronisation, inactive ovary, norgestomet.

Inactive ovaries are associated with a lack of functional structures related to the sexual cycles on the ovaries leading to true anoestrus. It can be observed that ovaries are flat, small, and soft, and sometimes round during rectal palpation. It has been reported that a case which is commonly seen in postpartum cows can also be seen in heifers (19). In the case of inactive ovaries, as the time to reach puberty and the time between birth and next gestation is prolonged, reproductive performance decreases, which causes economic losses.

In various livestock farms, incidence of inactive ovaries can be different, depending on environmental factors and nutritional conditions. In heifers, delayed puberty and inactive ovaries can be seen depending on nutritional deficiencies, diseases causing weight loss, various infectious agents, and environmental and climatic conditions (14, 15, 19).

Pituitary and placenta originated gonadotropins, oestrogens, progestagens, GnRH, and vitamin and mineral combinations have been utilised for the treatment of inactive ovaries. The aim of progesterone and progestagens used in order to stimulate oestrus in non-cyclic heifers is to artificially induce the luteal phase (3). Such a luteal phase enables an accumulation of gonadotropins. When the progesterone source is restricted, gonadotropins are released. This is followed by an LH peak and ovulation. Progestagens can be administered as daily injections, oral formulations, intravaginal devices, and subcutaneous implants (3). Progesterone also primes the brain for exhibiting behavioural oestrus (32). In addition, norgestomet, a synthetic progestagen, increases follicular concentrations of 17β-oestradiol, corresponding to an increased follicular maturation in non-cycling cows, and thereby reducing the occurrence of short cycles following ovulation induced by GnRH (30).

The existence of a relationship between the reproduction and nutritional status in cattle has been established (22). The nutritional status of an animal evaluated through a body condition score (BCS), reflects the body reserves available for basic metabolism, growth, lactation, and activity (18). Regular BCS has been recommended, as a means to evaluate the relative degree of negative energy balance (13).

It has been reported in the previous studies that progestagens can stimulate oestrus in acyclic cows, and can be used successfully for the treatment of cows with inactive ovaries (32, 20). However, studies on inactive ovaries have mostly focused on the postpartum period in cows (2) and pre-puberty period in heifers (1). There is limited number of studies on inactive ovaries in post-pubertal heifers. In addition, there is still a need to investigate the therapeutic effects of norgestomet on inactive ovaries. Therefore, in the present study, we aimed to investigate the effects of 11 d norgestomet
administration on the induction of oestrus and pregnancy rates in non-cyclic post-pubertal heifers.

**Material and Methods**

This study was carried out during April and May 2006, on thirty-eight 15–23-month-old (mean 18.8±2.1 months) Holstein heifers with inactive ovaries, which were selected from a private dairy herd with 300 heifers. All the heifers used, reached puberty and showed regular oestrus cycles previously. The owner of the herd reported that the heifers had a low level of nutritional intake for two month due to managerial problems. After this time, anoestrous cases developed and despite elimination of the nutritional problem, anoestrus continued in some heifers. During the treatment, in addition to pasture, the heifers’ diet was reinforced with dried clover, corn silage, and barley.

The selection of heifers with inactive ovaries was based on the findings of rectal palpation and sera progesterone levels. The heifers not showing any sign of oestrus were examined by rectal palpations at ten day intervals for the total herd. The heifers with small ovaries, which on rectal palpation were flat, smooth or rounded, and with progesterone levels lower than 1 ng/mL in blood samples, taken on rectal palpation days, were diagnosed as having inactive ovaries.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Experimental procedure applied in norgestomet treatment and control group heifers with inactive ovaries</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>Day 11</td>
</tr>
<tr>
<td>Norgestomet (n=29)</td>
<td>Norgestomet ear implant + Norgestomet injection</td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>Placebo injection</td>
</tr>
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</table>

A summary of the experimental procedure is shown in Table 1. The heifers were randomly distributed into two groups: norgestomet group (n=29) and control group (n=9). The norgestomet group heifers were implanted subdermally with a silicone ear implant, containing 6 mg of norgestomet (Crestar, Intervet, Boxmeer - Holland), and injected intramuscularly with a solution containing 3 mg of norgestomet and 5 mg of oestradiol valerate. The control group was observed daily, in the morning and in the afternoon, with respect to the possible signs of oestrus occurring spontaneously. The silicone implants were removed after 11 d, and the heifers were continuously observed for any signs of oestrus for 3 d. The implants were not accidentally lost by any of the heifers. The heifers were fix-timed inseminated 48 h and 78 h after the removal of the implants, and after the first insemination, they were treated with 50 µg of GnRH analogue (Lesirelin acetate, Dalmarelin®, Fatro, Italy). The aim of GnRH administration was to synchronise ovulation for a fixed-time of artificial insemination (AI). The pregnancy diagnosis was carried out 60 days after AI by palpation per rectum.

The average BCS was measured in all heifers (study animals and rest of herd) according to 1-10 system as described by Roche et al. (24).

**Blood samples.** Two blood samples were collected (day 20 and day 10) in order to determine the concentrations of progesterone. The blood samples were centrifuged (1 000 x g, 15 min), and the sera were stored at –20°C until progesterone (P4) analyses. 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 the two samples were <1ng/mL, then the heifers were considered to be anoestru ones.

**Statistical analyses.** The rates of oestrus expression and pregnancy were calculated. The oestrus detection rate was defined as the proportion of total heifers detected in oestrus during 72 h after removal of the implant. The pregnancy rate was defined as the proportion of all treated heifers that became pregnant. A “t” test was used to compare body condition scores. A chi-square test was used to compare the rate of oestrus expression and pregnancy rate between norgestomet and the control group.

**Results**

Average BCS interval to oestrus, oestrus and pregnancy rates of norgestomet and control groups are shown in the Table 2. The rate of induced oestrus was 86.2% (25/29) and 0% (0/9) in the treatment and control groups, respectively. In the treatment group, the time from the removal of implants to the beginning of oestrus was 47.2±9.7 h. No heifer of the control group showed oestrus throughout the study. In the norgestomet group, pregnancy was diagnosed by rectal palpation in 14 heifers (14/29, 48.2%). A significant difference (P<0.05) between control and norgestomet group, with respect to oestrus and pregnancy rates was observed. In heifers with inactive ovaries (n=38) and in the remaining heifers in the herd (n=262), the average BCS was 4.2±0.9 and 4.7±0.9, respectively (P<0.01). The average BCS was 4.5±0.9 and 3.9±0.8 in pregnant and non pregnant heifers among the norgestomet treated heifers. Although the determined difference in BCS was in favour of pregnant heifers, it was not considered significant (P>0.05).
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Interval to oestrus</th>
<th>Oestrus rate</th>
<th>Pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCS</td>
<td>(h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norgestomet (n=29)</td>
<td>47.2±9.7</td>
<td>25/29</td>
<td>14/29</td>
</tr>
<tr>
<td></td>
<td>%86.2a</td>
<td>%48.2ad</td>
<td></td>
</tr>
<tr>
<td>Control (n=9)</td>
<td>-</td>
<td>0/9</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>%0c</td>
<td>%0f</td>
<td></td>
</tr>
</tbody>
</table>

Values within same columns are different (P<0.05); ±SD

Discussion

In comparison to the synchronisation programmes, in which luteolytic agents are used, the greatest advantage of norgestomet treatment is that it can initiate oestrus in heifers and cows with inactive ovaries (28). If we considering that the frequency of inactive ovary cases, due either to negative energy balance or other reasons are commonplace, the synchronisation treatment that is researched in this paper, provides an important advantage for the stimulation of fertile ovulations in non-cycling heifers and cows (28).

Accurate determination of the oestrus following the removal of implants carries a great importance, because the inseminations will be carried out in a fixed time. Cavalieri and Fitzpatrick (4) reported that the time from the end of the norgestomet treatment to the beginning of oestrus is shorter in acyclic heifers, compared to cyclic ones. A similar situation in acyclic cows was also reported by Stevenson et al. (30). Cavalieri et al. (5) found that in heifers, oestrus begins 41.4 h after the removal of the implant. In another study, this time has been reported to be 46.8 h in cycling heifers (6). Singh et al. (27) have previously demonstrated that non-cycling zebu heifers had significantly longer interval to oestrus compared with the zebu cows. According to the data given in the prospectus of the manufacturer company, it is reported that the oestrus will begin within approximately 44±12 h after norgestomet treatment. In our study, the initiation of the oestrus (average 47.2±9.7 h) took longer than observed by other authors. As the oestrus’s beginning time is related to follicular development in the ovaries and also to factors such as climate, nutrition, and breed, further studies are needed to elucidate the factors that affect interval to oestrus after norgestomet removal among heifers assigned to the synchronisation protocol.

Doses of progestins, typically administered in oestrus-synchronisation programmes, are not sufficient to prevent an increase in the LH pulse frequency or the development of persistent follicles (26). Although these persistent follicles eventually ovulate after the removal of the exogenous progestin, fertility of the oocyte is compromised, leading to a decreased fertilisation rates and increased embryonic death within a few days (29). Therefore, minimisation of the occurrence of persistent follicle is important in oestrus synchronisation protocols (7). DoValle et al. (9) previously demonstrated that the administration of GnRH 30 h after norgestomet implant removal enhanced timed AI pregnancy rates. According to these reports, GnRH injection at the first AI may positively influence pregnancy rates in our study.

It has been reported that (8) the resumption of oestrus in a rate varying from 0% to 66% in anoestrous cows, following a short term (5-9 d) progesterone treatment. In addition, when norgestomet was implanted in anoestrous nursing beef cows, the occurrence of short oestrus cycles was reduced dramatically (30). Treatment time, type of progestin used, route of treatment, animal breed, breeding type, anoestrous duration, and many other factors cause variance in the results. Wishart et al. (31) have detected oestrus rates of 95% in five days in cyclic heifers synchronised with norgestomet and oestradiol valerate. Cavalieri et al. (5) reported that heifers showed an oestrus rate of 86.7% following norgestomet treatment. Favero et al. (10) have found that heifers treated with norgestomet showed an oestrus rate of 82%. Singh and Khurana (27) have reported that zebu heifers showed an oestrus rate of 92.3% following the removal of implant. As it can be understood from these studies, norgestomet can synchronise oestrus with a high efficiency. Similarly to the above studies, we synchronised oestrus using norgestomet at a high rate of 86.2% in our study.

Previous reports indicate that the actions of norgestomet on follicular development may be mediated by an increase in frequency of LH pulses (25). In addition, when norgestomet was administered in the presence of the CL, a greater percentage of heifers and cows were pregnant as a result of the insemination at the synchronised oestrus, compared to that of norgestomet-treated animals that did not have a CL. Norgestomet-treated animals that did not have a CL had greater concentrations of E2 in the circulation during the treatment period, and had a higher incidence of luteal abnormalities during the subsequent oestrous cycle than did norgestomet-treated animals that had a CL during the treatment period (25). This unfavourable circumstance only affects cycling heifers or cows, and it was not reported for non-cycling heifers.

Whishart et al. (31) have demonstrated that most successful results have been obtained from inseminations 48 h after oestrus, in cycling heifers synchronised by norgestomet and oestradiol valerate. They obtained a pregnancy rate of 55% following the inseminations of treated animals. A pregnancy rate of 50% was found in Bos taurus cattle by the inseminations 48 h following the removal of the norgestomet implants (21). Geary et al. (12) obtained a pregnancy rate of 43% in postpartum anoestrous cows by fix-time inseminations at 48 h following the removal of the norgestomet implants. In our study, we have found the pregnancy rate at 48.2% following fixed-time AI at 48h and 72 h. This pregnancy rate is similar to previous reports. It has been found that norgestomet treatment can stimulate oestrus behaviour independent from follicular development in
ovaries (23). This fact can be an explanation for moderate pregnancy rates despite higher oestrus rates. It has been described that the duration of oestrus and oestrus expression rates declined in heifers with low BCS scores (17). Stevenson et al. (30) have reported that oestrus synchronisation and pregnancy rates decreased in animals with BCS scores lower than 5, and even a single unit increase of BCS score can increase pregnancy rates by 27%. In our study, it can be seen that, in norgestomet group, the average BCS scores (4.5±0.9) of pregnant heifers was higher (P<0.05) compared to non-pregnant heifers (3.9±0.8). However, because of a limited number of materials, the difference was not considered statistically significant. In addition, heifers with inactive ovary had lower BCS scores (4.2±0.9) than the rest of the herd (4.9±0.9) in this study (P<0.01). These results showed that ovarian activity is closely associated with BCS in post-pubertal heifers.

In conclusion, our results showed that fertile oestrus can be stimulated successfully in post-pubertal heifers with inactive ovaries, by the use of norgestomet treatment, and successful pregnancy rates can be obtained by fixed-time insemination performed during such an oestrus.

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