Norgestomet Implants Synchronize Estrus and Enhance Fertility in Beef Heifers Subsequent to a Timed Artificial Insemination\textsuperscript{1,2}

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**ABSTRACT:** Three trials involving 128 heifers were conducted to determine whether norgestomet implants administered during the mid- and late luteal phases after breeding could be used to synchronize a second estrus in nonpregnant, inseminated heifers without adversely affecting pregnancy in pregnant heifers. All heifers were initially synchronized with Syncro-Mate B and artificially inseminated 47 h after implant removal. On d 9 (Trial 1) or d 12 (Trial 2) after the timed AI, the heifers were randomly assigned to treated or control groups. Treated heifers received two silicone implants containing 10.0 mg of norgestomet each (Trial 1) or one silicone implant containing 3.6 mg of norgestomet (Trial 2). Silicone implants were removed on d 21 after the initial AI. In Trial 1, the calving rate to the initial AI of the control heifers was 35 vs 55\% for the norgestomet-implanted heifers ($P > .05$). In Trial 2 the calving rate to the initial AI of the control heifers was 9 vs 45\% in the treated heifers ($P < .01$). At the return estrus 52\% of the control heifers returned to estrus within a 3-d period, whereas 93\% of the norgestomet-treated heifers returned to estrus within a 3-d period ($P < .01$). Norgestomet treatment had no effect on serum progesterone concentrations of the pregnant heifers on d 21 after the initial AI. In Trial 3, both control and treated heifers were administered silicone implants containing 3.6 mg of norgestomet on d 12; additionally, the treated heifers received an injection containing 3.0 mg of norgestomet and 5.0 mg of estradiol valerate. Norgestomet implants were removed on d 21. The calving rate of the control heifers was 10 of 19 (53\%), compared with a calving rate of 1 of 21 (5\%) of the heifers that were implanted with norgestomet and injected with norgestomet and estradiol valerate on d 12. In summary, treatment with norgestomet implants on d 9 or 12, through d 21, increased calving rates of treated beef heifers and synchronized the estrus of nonpregnant, treated heifers.

Key Words: Beef Heifers, Estrus Synchronization, Fertility, Norgestomet, Progesterone

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**Introduction**

New technologies are rapidly entering the cattle industry. Artificial insemination and embryo transfer have had major effects on the livestock industry. Embryo cloning technologies are developing rapidly and will affect the industry shortly. The critical limiting factor for any of these technologies is accurate identification or control of the stage of the estrous cycle.

Estrus synchronization programs have been developed using a combination of norgestomet implants and estradiol valerate (Wishart and Young, 1974; Spitzer et al., 1978). This treatment is effective in inducing estrus and ovulation in both anestrous and estrous cyclic beef heifers and cows (Hixon et al., 1981; Gallab et al., 1984) and provides the degree of synchrony required for a single timed insemination (Miksch et al., 1978; Anderson et al., 1982).

During the 1950s several studies were conducted in attempts to improve fertility of repeat breeder dairy cows by administration of progesterone shortly after breeding (Herrick, 1953) or on d 3 or 4 after breeding (Dawson, 1954; Wiltbank et al., 1956). Administration of exogenous progesterone resulted in an improvement in pregnancy rate from 11 to 30\%.

Based on more recent studies in dairy cows, the potential exists for exogenous progestin administration to enhance conception rate of heifers and cows artificially inseminated (Robinson et al., 1989) or implanted with embryos (Salgado and Donaldson, 1984) before progestin treatment. Sreenan and Diskin (1986) administered exogenous progesterone to heifers on d 5 to 35 or 10 to 20 after breeding. If the

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results of the trials were pooled then the treatment resulted in a 29 and 17% increase in pregnancy rate.

Current estrus synchronization procedures are used to synchronize only one estrus and ovulation. The purposes of this study were 1) to determine whether methods using norgestomet treatment can be developed for use on both pregnant and nonpregnant animals, thus allowing a second synchronized opportunity for nonpregnant beef heifers and cows to be artificially inseminated and 2) to investigate the effect of this treatment on fertility of beef heifers.

**Materials and Methods**

**Trials 1 and 2**

In the spring of 1987 (Trial 1, n = 43) and 1988 (Trial 2, n = 44) a total of 87 beef heifers (Angus or Angus-Hereford × Simmental) were synchronized with Syncro-Mate B (SMB). The SMB treatment consists of an intramuscular injection of norgestomet (3.0 mg) and estradiol valerate (5.0 mg) in a sesame oil and benzyl alcohol (10%) carrier and a hydron ear implant that contains 6.0 mg of norgestomet. The implant was subcutaneously inserted into the convex surface of the middle one-third of the ear. After 9 d the norgestomet implants were removed. Approximately 47 h after removal of the norgestomet implants all heifers were artificially inseminated by a single inseminator. The semen that was used was commercially frozen Angus semen. Service sire selection was made before the timed AI and before allotment to the treatment groups outlined below.

All heifers were observed for signs of behavioral estrus at least twice daily, morning and evening, beginning on the day after the SMB-timed AI until d 30 of the breeding season. Standing to be mounted was the criterion used to determine estrus. Heifers that returned to estrus were rebred by natural service (Trial 1) or AI approximately 12 h after they were detected in estrus (Trial 2). After d 30, natural service was used for the remainder of the breeding season in Trial 2. In both trials the total breeding season was 63 d.

On d 9 (Trial 1) or d 12 (Trial 2) after the SMB-timed AI, the heifers were randomly assigned to treated or control groups. During both trials, treated and control heifers were housed and managed together. Treated heifers were administered norgestomet implants that were subcutaneously inserted into the convex surface of the ear. In Trial 1 treated heifers were administered two norgestomet implants (10.0 mg of norgestomet each), whereas in Trial 2 the treated heifers received one norgestomet implant that contained 3.6 mg of norgestomet. The polymer used in the implants that were administered to the treated heifers was silicone (polydimethylsiloxane); however, a different polymer hydron (polyethylene glycomethacrylate) was used in the norgestomet implants that were administered at the initial SMB estrus synchronization. The silicone implants were 3.45 mm in diameter and 20.0 mm in length.

On d 21 after the SMB-timed AI, the norgestomet implants were removed from the treated heifers. At the time of implant removal serum samples were collected (via jugular venipuncture) from all heifers and the samples were immediately placed in ice until centrifugation to prevent progesterone metabolism (Wiseman et al., 1982). Serum samples were stored at -20°C until they were assayed for progesterone concentrations. Progesterone concentrations were determined by a validated enzyme immunoassay as described by Kesler et al. (1990). Intra- and interassay CV for the progesterone enzyme immunoassay were 2.38 and 4.69%, respectively. Parallelism of the assay was tested by assaying 50, 100, and 150 µL of a sample; the resulting progesterone levels were 2.59, 5.08, and 7.74 ng/tube, respectively (r = .9998). Specificity of the progesterone enzyme immunoassay tube was high. Crossreactivity of 11-α-hydroxyprogesterone was 12.8%. Crossreactivities of all the other steroids that were tested were < 1%, and the crossreactivities of norgestomet, estradiol-17-β, and estrone were < .01%.

**Trial 3**

In the spring of 1990, 41 beef heifers (Angus or Angus-Hereford × Simmental) were initially synchronized with SMB as described above. The purpose of this study was to investigate the effect of administration of an injection of estradiol valerate and norgestomet, at the time of administering norgestomet implants on d 12 after AI, on the first-service fertility of beef heifers. This treatment was investigated to determine whether it was detrimental to first-service calving rates. If adverse effects on fertility are encountered then the complete SMB procedure should not be used on previously inseminated animals. Approximately 47 h after removal of norgestomet implants all heifers were artificially inseminated. On d 12 after the timed AI all heifers were administered one silicone ear implant containing 3.6 mg of norgestomet. At the time of administration of implants heifers were assigned to control (n = 19) and treated (n = 22) groups. In addition to the implant, treated heifers were administered an injection that contained 5.0 mg of estradiol valerate and 3.0 mg of norgestomet. Control heifers in Trial 3 received no treatment other than the norgestomet ear implant. Norgestomet implants were removed on d 21 after the initial AI. In Trial 3 the only measurement was calving rate to the initial AI.

Figure 1 illustrates the timing of administration of estrus synchronization products and treatments to the control and treated heifers in Trials 1, 2, and 3.

Over the 3-yr period heifers weighted 250 to 425 kg and were from the same genetic pool. Heifers were 12
to 14 mo old and all were in good body condition. Treatments were imposed at the same time of the year during each of the 3 yr.

Calving rates were determined by calving dates. Comparisons of calving rates were made using chi-square analysis (Snedecor and Cochran, 1980). Progesterone concentrations on d 21 were compared for both pregnant and nonpregnant and control and treated heifers using analysis of variance (Huntsberger and Billingsley, 1981). Estrous cycle lengths were compared for control and treated heifers by analysis of variance.

**Results and Discussion**

**Trials 1 and 2**

During the two trials, five heifers were removed because they exhibited estrus before d 9 (Trial 1; n = 3) or d 12 (Trial 2; n = 2). The remaining 82 beef heifers were included in the data set. The norgestomet implants that were administered on d 9 (Trial 1) or on d 12 (Trial 2) through d 21 were effective in suppressing estrus in the nonpregnant heifers. Only one of the 20 nonpregnant, treated heifers displayed estrus during the implantation period, whereas 16 of 33 nonpregnant, control heifers displayed estrus before d 21 (P < .01; Figure 2). The one heifer that displayed estrus during the implantation period was in Trial 1, and in this trial two 10.0-mg norgestomet implants were used. The heifer that exhibited estrus during the implantation period was not pregnant at the end of the breeding season. The period of norgestomet implantation was 12 d in Trial 1 and 9 d in Trial 2. The single implant containing 3.6 mg of norgestomet that was used in Trial 2 was as effective in suppressing estrus for 9 d as were the two 10.0-mg, 12-d implants used in Trial 1. The norgestomet implants were not removed until d 21 after the initial timed AI to ensure luteal regression in the nonpregnant animals, and this significantly (P < .01) increased the estrous cycle length (Table 1). After implant removal, the nonpregnant, treated heifers displayed estrus 1 to 3 d after implant removal. In both trials, the treatment increased (P < .01) the percentage of heifers that displayed estrus during the peak 3-d period of estrous activity (93.3 vs 52.2%, treated vs control respectively). The percentages of the nonpregnant heifers that were detected in estrus within 7 d of norgestomet implant removal are reported in Table 1. Of the heifers that were not pregnant to the initial insemination in Trial 1, 77% of the control heifers and 67% of the implanted heifers were detected in estrus between d 11 and 24 after the initial timed AI. During Trial 2, 65 and 82% of the nonpregnant heifers were detected in estrus between d 16 and 24 after the initial timed AI (control and norgestomet-treated, respectively). This is in contrast to a recent study using progesterone-releasing intravaginal devices (Stevenson and Mee, 1991). They found that administration of progesterone on d 5 to 13 or 13 to 21 after AI increased the percentage of nonpregnant heifers that did not return to estrus for an extended period. In the present experiment the treatment synchronized the return to estrus of the nonpregnant heifers and, therefore, has the potential of decreasing the time required for estrus detection.

Most control heifers returned to estrus 16 to 22 d after the initial SMB-timed AI (Table 1, Figure 2). Control heifers that returned to estrus had an average estrous cycle length of 18.7 d. The return estrus in these heifers was grouped, but was not synchronized,
Table 1. Effect of norgestomet treatment on estrus detection efficiency and return estrus date of beef heifers

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment group</th>
<th>Total</th>
<th>No. detected in estrus</th>
<th>No. returning to estrus in peak 3-d period*</th>
<th>Average length of estrous cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>13</td>
<td>10 (77)</td>
<td>6 (60)</td>
<td>18.8 d</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>9</td>
<td>6 (67)</td>
<td>5 (83)</td>
<td>22.0 d*</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>20</td>
<td>13 (65)</td>
<td>6 (46)</td>
<td>18.7 d</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11</td>
<td>9 (82)</td>
<td>9* (100)</td>
<td>22.8 d**</td>
</tr>
</tbody>
</table>

*Based on the number of heifers that returned to estrus before d 28 of the breeding season.

**Treatment groups significantly differ (P < .01).

Van Cleeff et al. (1991) were not able to detect a difference in the pregnancy rate of control heifers and heifers treated with progesterone-releasing intravaginal devices on d 7 to 13 after AI. Similarly, Stevenson and Mee (1991) treated cows with progesterone-releasing intravaginal devices on d 5 to 13 or 13 to 21 after AI. The treatment did not improve pregnancy rate to the AI and seemed to inhibit interval to and/or detection of the return estrus of the nonpregnant animals.

The effect of norgestomet treatment on pregnancy rate at the return estrus varied between the two trials. In Trial 1, norgestomet-treated heifers tended (P > .05) to have a lower pregnancy rate at the return estrus. This may have been caused by the treatment or natural service rebreeding. Treated heifers returned to estrus in a synchronized manner and may have exceeded the serving capacity of one bull. In Trial 2, AI was used and the pregnancy rate at the return estrus was 66.6 and 23.1% for the treated and control heifers, respectively (P < .05).

Cumulative pregnancy rate for the first 28 d of the breeding season was improved by norgestomet implant treatment (Table 2). In Trial 1, norgestomet treatment did not affect the percentage of heifers that became pregnant during the first 28 d of the breeding season. In Trial 2, more (P < .01) of the treated heifers became pregnant during the first 28 d of the breeding season (Trial 2, 22.7 vs 75.0%; control and treated respectively). If the results of the two trials are combined, more (P < .05) of the norgestomet-treated heifers became pregnant during the first 28 d of the breeding season (72.5 vs 47.6%; control vs treated, respectively).

Overall, the treatment did not alter the number of heifers that did not become pregnant during the breeding season (Table 2). The length of the breeding season for both trials was 63 d. If the nonpregnant heifers exhibited estrous cycles of average length, then four estrous cycles could be exhibited during the breeding season. Norgestomet treatment increased the length of the first estrous cycle by approximately 3.8...
Table 2. Effect of administration of norgestomet implants on fertility of beef heifers

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment group</th>
<th>First timed AI CR (%)a</th>
<th>Return estrus CR (%)b</th>
<th>Cumulative end of season CR (%)c</th>
<th>Nonpregnant at end of seasond</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>7/20 (35)</td>
<td>8/10 (80)</td>
<td>15/20 (75)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11/20 (55)</td>
<td>3/7 (43)</td>
<td>14/20 (70)</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>2/22 (9)</td>
<td>3/13 (23)</td>
<td>5/22 (23)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>9/20** (45)</td>
<td>6/9* (67)</td>
<td>15/20** (75)</td>
<td>3</td>
</tr>
<tr>
<td>Overall</td>
<td>Control</td>
<td>9/42 (21)</td>
<td>11/23 (48)</td>
<td>20/42 (48)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>20/40** (50)</td>
<td>9/16 (56)</td>
<td>29/40** (73)</td>
<td>6</td>
</tr>
</tbody>
</table>

aCalving rate to the initial timed AI was based on calving data for all trials.
bCalving rate to the return estrus in the first 28 d of the breeding season was based on calving data.
cTotal number of heifers calving to both the first and second services (bred the first 28 d of the breeding season).
dTotal number of heifers that did not become pregnant during the breeding season.

*Treatment groups significantly differ (P < .05).
**Treatment groups significantly differ (P < .01).

Because of the lengthened estrous cycle during the treatment period, some of the nonpregnant treated heifers possibly had one fewer opportunity to become pregnant during the breeding season. This could be corrected in a production setting by extending the breeding season by 2 or 3 d.

Progesterone concentrations on d 21 are reported in Table 3. Progesterone concentrations were not significantly lower in the treated heifers during both trials. This suggests that norgestomet implants did not have a negative feedback effect on the hypothalamus and(or) anterior pituitary gland of the pregnant heifers that was sufficient to impair luteal function via decreased gonadotropins. The levels of progesterone differed between the two trials.

The relationship between progesterone levels on d 21 and pregnancy status is shown in Table 4. Overall, progesterone concentrations were 87.7% accurate in determining pregnancy status based on calving. All the heifers with progesterone concentrations on d 21 that were <1.5 ng/mL of serum were pregnant (87.5 and 84.6%, control and treated, respectively). The number of pregnant heifers with high progesterone concentrations was lower in Trial 2; only two of six control heifers and 9 of 12 treated heifers with high progesterone calved to the initial AI the following spring. The accuracy of pregnancy determination by progesterone concentration on d 21 was not different between trials or between treatment groups. The accuracy of progesterone concentration on d 21 in these trials is similar to results of other studies (Elmore, 1986; Meyers et al., 1988).

Studies have been conducted that concluded that the stage of the estrous cycle at the initiation of a progestin-based estrus synchronization procedure influences the conception rate at the synchronized estrus (Brink and Kiracofe, 1988). The studies have shown that if the progestin treatment is initiated during the later stages of the estrous cycle such that the interval between ovulations is increased then the conception rate will be lowered. Long-term norgestomet administration effectively suppresses estrus but is accompanied by lowered fertility (Wishart and Young, 1974;

Table 3. Effect of norgestomet treatment on serum progesterone [P_4] concentrations on day 21

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment group</th>
<th>High P_4^a</th>
<th>Low P_4^b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pregnant^c</td>
<td>Nonpregnant^c</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>4.61 ± .79</td>
<td>7.53 ± .90</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>3.54 ± .27</td>
<td>2.22 ± .47</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>11.74 ± 3.57</td>
<td>7.89 ± 3.94</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>8.73 ± .26</td>
<td>6.81 ± 1.66</td>
</tr>
</tbody>
</table>

^aProgesterone concentrations ≥ 1.5 ng/mL.
^bProgesterone concentrations < 1.5 ng/mL.
^cPregnant or nonpregnant to the initial timed AI based on calving data.
^dNone of the heifers with low progesterone concentrations was pregnant to the initial AI.
Table 4. Relationship between progesterone \(P_4\) concentration on day 21 and pregnancy status in beef heifers

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment group</th>
<th>High (P_4^a)</th>
<th>Low (P_4^b)</th>
<th>(P_4) accuracy(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pregnant(c)</td>
<td>Nonpregnant(c)</td>
<td>Pregnant</td>
</tr>
<tr>
<td>Overall</td>
<td>Control</td>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>20</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>7</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>11</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>9</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a\)Progesterone concentrations \(\geq 1.5\) ng/mL.
\(b\)Progesterone concentrations < 1.5 ng/mL.
\(c\)Pregnant or nonpregnant to the initial timed AI based on calving data.
\(d\)Accuracy of progesterone determination to estimate pregnancy status.

Spitzer et al., 1978). It seems that norgestomet in the absence of a corpus luteum is capable of suppressing estrus but does not suppress the frequency of LH pulses to the extent that exogenous progesterone or a corpus luteum does (Taylor and Rajamahendran, 1991). This inability to suppress LH release may be the reason that no decreases in progesterone concentrations on d 21 of the pregnant, norgestomet-treated heifers were observed. In the present trial, the norgestomet treatment was initiated on d 9 or 12 after a timed AI and was continued until d 21. This did not greatly increase the interval between ovulations and did not allow the nonpregnant heifers to be solely under the control of norgestomet for an extended period of time. Calving rate to the return estrus was consistently (although not significantly) higher than the calving rate to the initial synchronized estrus (Table 2). This may have been because only heifers exhibiting estrus were artificially inseminated at the return estrus, whereas all heifers were artificially inseminated at the initial synchronization.

In previous studies we demonstrated that norgestomet implants were capable of maintaining pregnancy in heifers that were ovariectomized on d 10 after breeding (Favero et al., 1990). In that study and in the present trial, norgestomet treatment did not result in any defects or malformations of the resulting offspring. In the present study, some of the resulting female offspring have been bred and they have produced normal offspring and lactated normally.

**Trial 3**

One of the norgestomet-treated heifers lost the norgestomet implant that was administered on d 12 and the heifer was removed from the study. Administration of an injection of estradiol valerate and norgestomet on d 12 was pregnant to the initial AI. Because both the control and treated heifers were administered norgestomet implants on d 12 after AI and the injection of norgestomet and estradiol valerate was the only additional treatment to the treated heifers, it seems that the injection of norgestomet and estradiol valerate caused the significant \(P < .01\) decrease in first-service fertility.

The purpose of Trial 3 was to determine the effect of an injection of norgestomet and estradiol valerate on early pregnancy. In previous studies (Favero et al., 1988) we found that 13.6% of synchronized animals that were studied were not synchronized (either a delayed estrus [1 to 4 d after AI]) or had low (< 1.5 ng/mL) progesterone concentrations on d 6 or 7 after AI. Furthermore, 10.5% of the animals that were considered to have a synchronized estrus/ovulation had luteal dysfunction (estrus 8 to 10 d after AI or elevated progesterone concentrations on d 6 or 7 and low progesterone concentrations on d 10 to 14). The corpora lutea may not have had fully regressed on d 21 after the timed AI for these animals. We hypothesized that administration of the norgestomet and estradiol valerate injection at the time of norgestomet implantation would improve synchrony of nonpregnant animals. The results of Trial 3 show that the injection of estradiol valerate and norgestomet at the time of administration of norgestomet implants caused a severe decrease in calving rate. In the other trials it was determined that norgestomet implants had beneficial effects on fertility. This suggests that the administration of estradiol valerate on d 12 after breeding causes early embryonic mortality, possibly by modifying luteal function or interfering with the maternal recognition of pregnancy.

**Implications**

Treatment of inseminated beef heifers with norgestomet implants during the middle and late luteal
phase was effective in synchronizing subsequent estrus in the nonpregnant, inseminated heifers. Norgestomet treatment had no detrimental effects on the developing zygote of the pregnant heifers and increased the calving rate to the initial artificial insemination. Fertility at the estrus after norgestomet implant removal was normal, as were progesterone levels on d 21. The administration of an injection containing estradiol valerate and norgestomet at the time of norgestomet implant administration on d 12 severely decreased calving rates and should not be used with this proposed procedure.

Literature Cited


Brink, J. T. and G. H. Kiracofe. 1988. Effect of estrous cycle stage at levels on d 21. The administration of an injection phase was effective in synchronizing subsequent gestomet treatment had no detrimental effects on the implant removal was normal, as were progesterone insemination. Fertility at the estrus after norgestomet time of norgestomet implant administration on d 12 containing estradiol valerate and norgestomet at the proposed procedure.


