FACTORS ASSOCIATED WITH SHORTENED ESTROUS CYCLES AFTER ABORTION IN BEEF HEIFERS

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ABSTRACT

Forty-six crossbred Hereford heifers were allotted into five experimental groups. Fenprostalene, a prostaglandin analogue, was administered to induce abortion and (or) to regress the corpus luteum (CL) in four groups of heifers about 75 d after conception. The four groups were 1) pregnant heifers, 2) pregnant heifers with the ovary contralateral to the gravid uterine horn removed 24 h after fenprostalene injection, 3) same as Group 2 but with the ipsilateral, rather than the contralateral, ovary removed and 4) heifers with uterus removed (ovaries intact) about 35 d after conception. A fifth group of nonpregnant heifers received implants containing norgestomet from 10 to 76 d after estrus and were given fenprostalene 24 h before removal of the implant. Average length of the first estrous cycle after the fenprostalene injection was 11.2, 8.3, 9.7 and 19.1 for Groups 1, 2, 3 and 5, respectively. Estrous cycles were longer (P < .05) in norgestomet-treated heifers. Hysterectomized heifers (Group 4) did not exhibit a second estrus by 50 d after fenprostalene treatment; otherwise, all first estrous cycles after the fenprostalene injection were either "short" (7 to 13 d) or "normal" (17 to 23 d). Twenty-two of 24 heifers that aborted had short estrous cycles and two had normal estrous cycles. Short estrous cycles occurred after abortion regardless of whether the ovary ipsilateral or contralateral to the previously gravid uterine horn ovulated. Early regression of the CL (short luteal phase) did not occur in nonpregnant heifers after long-term progestogen stimulation or after hysterectomy, but it did occur in heifers with a previously gravid uterus. The previously gravid uterus apparently plays an essential role in early regression of the first CL formed after abortion.

(Key Words: Progestogens, Abortion, Hysterectomy, Ovariectomy, Estrous Cycles, Heifers.)

Introduction

When pregnant cows and heifers are aborted with prostaglandin F₂ alpha or its analogues during the first half of gestation, the estrous cycle that follows usually is of short duration (Ingrahm et al., 1978; Lindell et al., 1981; Copelin et al., 1987; Wright and Kiracofe, 1988). Serum progesterone concentrations indicate that ovulation accompanies the estrus after abortion and that the subsequent corpus luteum (CL) regresses prematurely, resulting in estrous cycles of only 7 to 13 d (Wright and Kiracofe, 1988). The mechanism(s) inducing early luteal regression have not been determined but could be similar to those causing early luteal regression in postpartum cows after early weaning (Odde et al., 1980) and could involve the recently gravid uterus. In fact, it is possible that a previously gravid uterus is required for expression of shortened luteal phases.

The percentage of heifers exhibiting short estrous cycles after abortion increases as stage of gestation increased from 50 to 100 d (Wright and Kiracofe, 1988). Length of time the animal is exposed to a progestogen, uterine changes associated with pregnancy and ovarian vascular...
dynamics associated with the gravid uterine horn are factors that change during gestation and could contribute to early CL regression.

The present study was designed to determine if heifers exposed to 75 d of endogenous or exogenous progestogen stimulation would have short estrous cycles when they had either no uterus, a nongravid uterus or a recently gravid uterus. A second objective was to determine if the side of ovulation, relative to the previously gravid uterine horn, influenced the length of estrous cycles after abortion.

Materials and Methods

Experimental Plan. Crossbred Hereford heifers were kept in drylots and fed daily a ration of sorghum silage and protein supplement that was formulated to meet NRC (1984) requirements for growing, medium-frame heifers. Mineral blocks and water were supplied continuously.

Forty-six heifers were used in the experiment. Thirty-six were selected from a group of heifers that had been artificially inseminated 32 to 38 d previously and were pregnant as diagnosed by rectal palpation. Those 36 heifers were randomly allotted to four treatment groups: 1) injected s.c. with 2 mg fenprostalene between d 72 and 78 of gestation to induce abortion (n = 10), 2) same as Group 1 plus the ovary contralateral to the gravid uterine horn was removed 20 to 28 h after the injection (n = 8), 3) same as Group 2 except the ipsilateral rather than the contralateral, ovary was removed (n = 8) and 4) same as Group 1 but the uterus was removed 32 to 38 d of gestation (ovaries were not removed; n = 10). Heifers in this group were palpated per rectum 1 d before hysterectomy to verify pregnancy. Blood samples were collected daily from the time of palpation until 10 d after hysterectomy and concentration of progesterone was measured. If progesterone was less than 1 ng/ml of serum for 4 d or more after hysterectomy, the heifer was removed from the experiment. The remaining 10 heifers (Group 5) had at least one 18 to 22 d estrous cycle (heifers were not inseminated) then were implanted with norgestomet from 10 to 76 d after estrus. Each heifer in Group 5 received a hydron ear implant containing 6 mg norgestomet 10 d after estrus and a new implant every 10 d through d 70. To ensure a constant supply of norgestomet, each implant was left in the ear for 20 d. Therefore, each heifer had two implants from 20 to 76 d after estrus. Both remaining implants were removed at d 76. Group 5 heifers also were injected s.c. with 2 mg fenprostalene 24 h before the end of norgestomet treatment.

Rationale. The rationale for giving the fenprostalene injection at about d 75 of gestation was that all CL should be regressed by the injection, thus causing abortion in all pregnant heifers (Schultz and Copeland, 1981). The injection also should regress CL retained after hysterectomy (Copelin et al., 1987). Norgestomet-treated heifers should not have a CL, because it regresses when estrus is inhibited with exogenous progestogens (Hendricks et al., 1973). Based on results of Wright and Kiracofe (1988), about 80% of the heifers were expected to have short estrous cycles after abortion. Unilateral ovariectomy would force the subsequent ovulation to occur either on the ovary ipsilateral or contralateral to the previously gravid uterine horn, thus providing a test of the effects of proximity to a previously gravid uterine horn. The norgestomet group provided heifers that had been under exogenous progestogen stimulation and had an intact, nongravid uterus. Hysterectomized heifers were exposed to endogenous progesterone stimulation but no uterus or conceptus, whereas the heifers that aborted had a similar duration of progesterone exposure plus a conceptus and uterus. Consequently, if all CL regressed after the fenprostalene injection in Groups 1 through 4 and no CL were present when norgestomet was removed in Group 5, the heifers would be expected to return to estrus and to ovulate. The life span of the subsequent CL, therefore, would provide a response criterion for evaluating the effect of 75-d progestogen stimulation with a recently gravid uterus, a nongravid uterus and no uterus.

Data Collection. Beginning at the fenprostalene injection, all heifers were observed at least twice daily for signs of estrus. Observations continued until one estrous cycle of at least 16 d was exhibited or for 50 d after injection, if fewer than two estrous periods were observed. A heifer was considered in estrus if she stood for mounting by another heifer. Periods of hyperactivity, such as riding other heifers and nervousness, also were recorded.

Blood samples were collected from all heifers

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5 Bovilene, Syntex Animal Health Inc., West Des Moines, IA.
6 Syncro-Mate B Implants, Ceva Laboratories Inc., Overland Park, KS.
by jugular venipuncture between 0600 and 0900, beginning 3 d before the fenprostalene injection and continuing daily for at least 23 d. If a heifer exhibited a short (<16 d) estrous cycle during the sampling period, daily bleedings continued until 11 d after the second estrus or until 50 d after injection if a second estrus was not observed.

Blood samples were collected in evacuated tubes, refrigerated, allowed to clot and centrifuged within 12 h after collection. Serum was harvested and stored in plastic vials at -20°C until progesterone was determined by RIA (Skaggs et al., 1986). Intraassay and interassay CV were 8.7 and 7.9%, respectively. In 11 test assays, 88.7% of the progesterone in bovine serum was recovered during extraction; corrections were made for that procedural loss.

Abortion and luteal regression were verified by rectal palpation, estrus detection and a progesterone concentration (<1.0 ng/ml by 48 h after injection. Estrus and progesterone concentration were used as a positive indication of luteal regression in hysterectomized heifers.

Data concerning estrous cycles were analyzed by one-way ANOVA, and data concerning progesterone concentration were analyzed by a two-way ANOVA that included time and treatment effects. The SAS GLM procedure (1982) was used. Frequency of short estrous cycles in heifers was analyzed by chi-square procedures.

Surgical Procedures. Hysterectomies were performed at 32 to 38 d of gestation. Heifers were tranquilized with 10 to 15 mg of acepromazine maleate given i.m. and then injected with approximately 20 ml of 2% lidocaine hydrochloride in the body wall of the left paralumbar fossa. The uterus was removed through an incision in the left paralumbar fossa, using the following technique. The cervix was severed between the internal and external os. The broad ligament then was cut along both sides of the uterus, and the uterus was retracted toward the incision. The oviducts were left intact but were severed from the uterus at the uterotubal junction. The right ovary was palpated and severed from the uterine connection, taking care not to interrupt the ovarian blood supply. The left ovary could be observed through the incision and was separated from the uterus. After the uterus was removed, the incision was closed internally with absorbable sutures, and the skin was closed with nonabsorbable sutures that were removed 10 to 14 d later.

Unilateral ovariectomies were performed 20 to 28 h after the fenprostalene injection to induce abortion. Heifers were anesthetized as described above. Palpation per rectum revealed that 12 heifers had a conceptus in their right uterine horn and four heifers had a conceptus in their left uterine horn. Equal numbers of each group had the ovary removed ipsilateral or contralateral to the pregnant uterine horn. Ovaries were removed through an incision in the adjacent paralumbar fossa by crusing and cutting the ovarian hilus. After the ovary was removed, the incision was closed as described above.

Results and Discussion

Progesterone concentrations <1 ng/ml of serum were detected for at least 4 d for two hysterectomized heifers beginning 5 to 7 d after surgery, indicating that the CL regressed because of surgery. Data from those two heifers were not included in the results. None of the other 44 heifers exhibited estrus before conception or start of norgestomet treatment and fenprostalene injection. Twenty-four of 26 pregnant heifers aborted and exhibited signs of estrus within 15 d after the fenprostalene injection. Although the other two also had aborted, they did not exhibit any signs of estrus after fenprostalene, and their data were not included. Thirty-seven of the 42 heifers included in the subsequent data exhibited standing estrus and five had periods of hyperactivity by 12 d after the fenprostalene injection. Concentrations of progesterone in serum of heifers exhibiting hyperactivity were not different (P < .5) from those of heifers exhibiting estrus, indicating that all 42 had ovulated in association with hyperactivity or estrus. Hyperactivity or standing estrus were used in the analysis of data as d 0 of the estrous cycle, and hyperactivity was considered as an estrus in calculating the interval to estrus and length of estrous cycles. Groups 1, 2, 3 and 4 contained 1, 1, 2 and 1 heifers showing hyperactivity, respectively.

The average interval from fenprostalene injection to estrus was longer in both groups of unilaterally ovariectomized heifers than in the other three groups (P < .05; Table 1). That
TABLE 1. ESTROUS CYCLES AFTER CORPUS LUTEUM REGRESSION OR NORGESTOMET WITHDRAWAL IN INTACT, PREGNANT OR HYSTERECTOMIZED HEIFERS

<table>
<thead>
<tr>
<th>Item</th>
<th>Abort</th>
<th>Abort + C-ovx</th>
<th>Abort + I-ovx</th>
<th>Utx</th>
<th>Nor</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of heifers</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Interval from injection to estrus, d</td>
<td>3.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Length of first estrous cycle, d</td>
<td>11.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&gt; 50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.1&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Length of second estrous cycle, d</td>
<td>17.9</td>
<td>24.1</td>
<td>19.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of heifers with a short first estrous cycle</td>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>All heifers were injected with fenprostalene 72 to 78 d after their last estrus. Abort = heifers 72 to 78 d pregnant were aborted with the fenprostalene; Abort + C-ovx = same as Abort except the ovary contralateral to the gravid uterine horn was removed 24 h after injection; Abort + I-ovx = ipsilateral rather than contralateral ovary was removed; Utx = hysterectomized 32 to 38 d of gestation; Nor = cycling heifers implanted with norgestomet from 10 d after estrus to 24 h after injection.

<sup>b</sup>One heifer in Abort and one in Abort + C-ovx did not exhibit estrus after fenprostalene injection and two Utx heifers had low progesterone concentration in serum after surgery. Those four heifers are not included.

<sup>c</sup>,<sup>d</sup>,<sup>e</sup>Means in the same row without common superscripts differ (P < .05).

The average first estrous cycle was shorter (P < .05) for the three groups (1, 2 and 3) of heifers aborted between d 72 and 78 of gestation than for the norgestomet-treated or hysterectomized groups (Table 1). Twenty-two of 24 heifers that aborted had first estrous cycles of 7 to 13 d. The most frequent estrous cycle length was 8 d. There was no difference in frequency of short estrous cycles after abortion among heifers with the ovary removed contralateral or ipsilateral to the previously gravid uterine horn or with both ovaries intact. Therefore, the anatomical relationship of the ovary bearing the CL with the previously gravid uterus was not associated with premature regression of the CL. None of the norgestomet-treated or hysterectomized heifers had first estrous cycles of less than 17 d. The previously gravid uterus appeared to be involved in the mechanism producing short estrous cycles because none of the heifers without a uterus or with a nongravid uterus had short estrous cycles.

Progesterone before and at the fenprostalene injection was > 2.0 ng/ml in all groups except those treated with norgestomet, indicating that all but the latter heifers had a functional CL (Table 2). The serum progesterone concentration declined by 24 h after injection of fenprostalene in all pregnant and hysterectomized heifers. The longer interval from injection to estrus for unilaterally ovarioctomized heifers apparently was not a result of higher serum progesterone concentrations. Concentrations were not greater at 48 h after injection (Table 2) than those of ovary-intact heifers that aborted. Because heifers that aborted and had intact ovaries did not receive anesthesia or sham surgery, the reason for the prolonged interval cannot be determined.

Progesterone concentrations in serum remained low in all heifers for at least 72 h after fenprostalene injection (Table 2). Progesterone concentrations increased by 6 d after estrus in all heifers in the hysterectomized and norgestomet-implant groups and remained elevated though d 16 (Figure 1), indicating that those heifers ovulated at the first estrus after injection and maintained a CL capable of producing normal amounts of progesterone. All heifers that aborted and returned to estrus showed increases in serum progesterone concentrations by 5 or 6 d after estrus, indicating that ovulation occurred, but progesterone concentrations declined by d 7 to 10 in those with short cycles. The short luteal phases appear similar to those observed in postpartum cows after weaning (Odde et al., 1980) in which ovulation occurred and the CL regressed prematurely (Ramirez-Godinez et al., 1982). Long-term exposure to progestogens did not result in short luteal phases after abortion unless a previously gravid uterus was
Table 2. Mean Progesterone in Serum (ng/ml) After Fenprostalone Injection

<table>
<thead>
<tr>
<th>Days pre- or post injection</th>
<th>Abort</th>
<th>Abort + C-ovx</th>
<th>Abort + I-ovx</th>
<th>UTX</th>
<th>Nor</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2</td>
<td>2.6&lt;sup&gt;def&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>3.4&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;bcede&lt;/sup&gt;</td>
<td>.2&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>-1</td>
<td>2.7&lt;sup&gt;cdef&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;bcdef&lt;/sup&gt;</td>
<td>.2&lt;sup&gt;1h&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>3.0&lt;sup&gt;bcdde&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;bcdde&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>.2&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>.8&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;g&lt;/sup&gt;</td>
<td>.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>.2&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>.46&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>.23&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.36&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>.36&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>.2&lt;sup&gt;2h&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>.26&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.16&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.25&lt;sup&gt;h&lt;/sup&gt;</td>
<td>.19&lt;sup&gt;gh&lt;/sup&gt;</td>
<td>.18&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*All heifers were injected with fenprostalone 72 to 78 d after their last estrus. Abort = heifers 72 to 78 d pregnant were aborted with the fenprostalone; Abort + C-ovx = same as Abort except the ovary contralateral to the gravid uterine horn was removed 24 h after injection; Abort + I-ovx = ipsilateral rather than contralateral ovary was removed; Utx = hysterectomized 32 to 38 d of gestation; Nor = cycling heifers implanted with norgestomet from 10 d after estrus to 24 h after injection.

b,c,d,e,f,g,h Means with no common superscripts differ (P < .05).

Figure 1. Mean progesterone concentrations in serum after estrus in aborted heifers (AB; Groups 1, 2 and 3), hysterectomized heifers (UTX; Group 4) and norgestomet-treated heifers (NOR; Group 5).

Present. No short luteal phases occurred in hysterectomized or nonpregnant uterine-intact heifers exposed to either endogenous progesterone or an exogenous progestogen for 75 d. However, heifers with a recently gravid uterus had a high percentage of short luteal phases after abortion. The previously gravid uterus apparently plays an essential role in the early regression of the first CL formed after abortion.

**Literature Cited**


