ENDOCRINE AND REPRODUCTIVE RESPONSE OF BEEF COWS TO PMSG

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Summary

Thirty-one 3-year-old Angus cows and 23 2-year-old Angus heifers were randomly allotted to two treatment groups to examine the influence of PMSG on endocrine and reproductive responses. Sixteen cows and 11 heifers received a single subcutaneous injection of 2,000 IU PMSG on day 17 of the estrous cycle (Trt 17); 15 cows and 12 heifers received 1,500 IU PMSG on day 5 and 2,000 IU on day 17 (Trt 5, 17). Plasma progesterone, estradiol and LH concentrations were quantified in jugular blood collected at regular intervals during the treatment cycle. Treatment with PMSG on days 5, 17 resulted in increased estrous cycle lengths in both cows (P<.05) and heifers (P<.10) compared to a single treatment on day 17. The number of corpora lutea observed at laparotomy 7 to 11 days after estrus was greater in cows on Trt 17 than in cows on Trt 5, 17 (P<.10), but was similar in both heifer groups (P>.25). Plasma progesterone concentrations were similar in both groups prior to treatment but increased after day 5 in Trt 5, 17 compared to Trt 17 (P<.005). Plasma progesterone at time of laparotomy was significantly correlated with the number of corpora lutea (r = .75, P<.01). Plasma estradiol also increased after day 5 in Trt 5, 17 (P<.025). Estradiol was similar in both groups on day 17; but on day 19, cows on Trt 17 had greater plasma estradiol (P<.025) than cows on Trt 5, 17. Similarly, plasma estradiol concentrations for heifers treated only once were greater on day 19 than for heifers treated on days 5 and 17 (P<.05). Plasma LH concentrations were not significantly different between the two treatments. These results indicate the PMSG alters ovarian function when given either late in the luteal phase or during both early and late luteal phases of the estrous cycle.

(Key Words: Superovulation, PMSG, Progesterone, Estradiol, Luteinizing Hormone.)

Introduction

Among the many problems associated with the use of PMSG to induce multiple births in beef cows, uncontrolled superovulatory response still ranks as one of the most important. Schilling and Holm (1963) reported that a series of two PMSG injections given both early and late in the estrous cycle resulted in 70% of the treated cows having the desired two or three egg ovulations. Turman et al. (1971), using a similar sequence of PMSG injections, reported 23 multiple births from 52 cows conceiving at the first estrus following the PMSG injections.
Ovarian endocrine function is altered when cattle are treated with a single injection of PMSG (Spilman et al., 1973; Henricks and Lamond, 1972; Lamond and Gaddy, 1972; Henricks et al., 1973; Henricks and Hill, 1978). However, the effects of injections of PMSG twice during the estrus cycle has not been determined.

The objective of this study was, therefore, to determine the endocrine and reproductive responses of cows and heifers to PMSG administered either late in the luteal phase or during both the early and late luteal phases of the estrous cycle.

Materials and Methods

Thirty-one 3-year-old lactating Angus cows and 23 2-year-old Angus heifers maintained under range conditions on native grass pastures were used in this study. Thirteen of the cows had been treated with PMSG 1 year earlier and were blocked across the two treatments. None of the heifers had received prior PMSG.

Sixteen cows and 11 heifers received a single subcutaneous injection of 2,000 IU PMSG on day 17 (day 0 = day of estrus) of the estrous cycle (Trt 17) while 15 cows and 12 heifers received 1,500 IU PMSG on day 5 followed by 2,000 IU PMSG on day 17 (Trt 5, 17). The potency of the PMSG was determined within 1 month of use by bioassay according to the method of Cole and Erway (1941), using the World Health Organization Second International Standard for Serum Gonadotropin, Equine.

The occurrence of estrus prior to the PMSG treatments was determined by use of vasectomized or penectomized bulls equipped with chinball markers. Subsequent to PMSG treatment on day 17 all animals were mated to fertile Angus bulls. Ovarian response was determined by a lumbar laparotomy 7 to 11 days after breeding. Rectal palpation was performed 60 days post-treatment and all pregnant females were retained in the herd until parturition.

Jugular blood was collected on days 1, 3, 5, 6, 7, 9, 11, 13 and 15 of the treatment estrous cycle, and daily from day 17 until estrus or until day 26. Blood was also collected on the day of laparotomy. Blood samples (40 ml) were collected in tubes containing 32 mg oxalic acid, cooled to 5C, centrifuged (5,000 × g for 10 min) within 30 min and the plasma decanted and stored at -10C.

Plasma progesterone was quantified by radioimmunoassay (Wettemann et al., 1978). Plasma estradiol was quantified by a radioimmunoassay similar to that described by Wettemann et al. (1972) as modified by Hafs et al. (1974) using sheep antisera to 17β-estradiol-6β-bovine serum albumin. To validate the assay for bovine blood plasma, plasma was extracted with benzene and values for the same samples were compared before and after estradiol was isolated from Sephadex LH-20 columns (Chenault et al., 1975). The amount of estradiol in benzene extracts (3.8 ± 1.0 pg/ml) did not differ significantly from that determined after chromatographic isolation (4.6 ± 1.0 pg/ml; r = .95). When 2, 4 or 6 pg estradiol were added to 1 ml plasma samples, 97 ± 7, 98 ± 7 and 106 ± 2%, respectively, were recovered. The between assay coefficient of variation was 10% and the within assay coefficient of variation was 5%.

Plasma LH was quantified by a radioimmunoassay similar to that described by Niswender et al. (1969) using an antiserum to bovine LH. The method for radioiodination of purified bovine LH (LER 1072-2) was similar to that of Niswender et al. (1969) except 125I was used. The assay detected .1 ng of bovine LH using an antiserum dilution of 1:80,000. Dose response curves for plasma from steers, cows and anterior pituitary homogenates were parallel to the LH standard (NIH-LH-B8) curve. When 100 ng of growth hormone (NIH-GH-B17) and 200 ng prolactin (NIH-P-B3) were tested in this assay, each had less than 1 ng equivalent LH, and 1 μg follicle stimulating hormone (NIH-FSH-S9) was equivalent to about 2 ng LH. Ten nanograms of thyroid stimulating hormone (NIH-TSH-B6) was, however, equivalent to about 3 ng LH, which may have been due to contamination of the TSH with immunologically active LH since the two hormones appear to be related immunologically (Selenkow et al., 1966; Guillemin, 1967).

Estrous cycle length and ovulation rate were analyzed by analyses of variance using completely randomized designs. Chi square techniques were employed to test conception rate data and percentage of animals having various numbers of ovulations. Endocrine data were subjected to split plot analyses of variance as described by Gill and Hafs (1971) with one
between block treatment (PMSG) and one within block treatment (days after estrus). When a significant treatment × day interaction was detected, an analysis of variance was utilized to evaluate treatment differences within a bleeding time.

Results and Discussion

Reproductive Response. The number of animals exhibiting estrus after treatment was not influenced by treatment. Between 64 and 100% of the animals within each treatment were in estrus within 10 days after the day 17 PMSG injection (table 1). Cows on Trt 5, 17 had significantly longer estrous cycles than those on Trt 17 (23.9 ± .5 vs 21.9 ± .7 days, respectively, P<.05). Similarly, heifers on Trt 5, 17 had a cycle length of 23.6 ± .5 days, compared to 21.6 ± 1.1 days in heifers on Trt 17 (P<.10).

Percentages of animals that conceived to the first post-PMSG estrus, as determined by palpation and subsequent calving dates, are shown in table 1. Conception rate among heifers was low and none had multiple births. Multiple births (all twin sets) occurred in three cows on Trt 17 (18.7%), and in one cow on Trt 5, 17 (6.7%). The conception rate and multiple birth response of cows on Trt 5, 17 were lower than those reported for similarly treated cows in earlier studies at this station (Turman et al., 1971; Johnson et al., 1975). The reduced rate of multiple births in this study could be related to laparotomy which was not done in the earlier studies.

The number of follicles less than 10 mm in diameter at 7 to 11 days after estrus was similar for both treatments in the cows and heifers (table 2). Cows on Trt 17 tended to have more follicles greater than 10 mm than those on Trt 5, 17 (2.8 ± .5 vs 1.5 ± .4, respectively, P<.10). Large follicles (greater than 20 mm) were observed in 12.5% of the cows and 27.3% of the heifers in Trt 17 and 20% of the cows and 16.7% of the heifers in Trt 5, 17. A similar incidence of abnormally large follicles has been reported by others (Laster et al., 1971; Henriks et al., 1973).

Ovulation rate of the heifers did not differ between the two PMSG treatments (P<.25). However, cows on Trt 17 had more (P<.10) ovulations (2.2 ± .4) than those on Trt 5, 17 (1.3 ± .3). There was a suggestion that treatment with PMSG 1 year previously may have influenced the response of the cows to PMSG. Within Trt 17, there were no significant differences in response related to the previous year treatment. The mean ovulation rate of cows not previously treated was 2.56 ± .5 compared to 1.86 ± .55 for those previously treated (P>.25). All of the Trt 5, 17 cows that had not been previously treated with PMSG ovulated at least one egg with a mean ovulation rate of 1.78 ± .46. However, three of six cows previously treated with PMSG failed to ovulate and none ovulated more than one egg for a mean ovulation rate of .50 ± .22 (P<.10). These data support the suggestion of Willett et al. (1953) that animals may become refractory to PMSG.

Endocrine Response. Cows given a single

<table>
<thead>
<tr>
<th>TABLE 1. NUMBERS OF ANIMALS EXHIBITING ESTRUS, ESTROUS CYCLE LENGTHS, CONCEPTION RATES AND MULTIPLE BIRTHS FOLLOWING TREATMENT WITH PMSG</th>
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</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Animals (no.)</td>
</tr>
<tr>
<td>Exhibiting estrus (no.)</td>
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<td>Cycle length (days)a</td>
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<tr>
<td>Animals that conceived at 1st post-PMSG estrus (no.)</td>
</tr>
<tr>
<td>Animals that produced a multiple birth (no.)</td>
</tr>
</tbody>
</table>

a Significant treatment effect for cows (P<.05) and heifers (P<.10).
b Mean ± SE.
injection of PMSG on day 17 had maximum plasma progesterone concentrations on day 15 (8.7 ± .5 ng/ml; figure 1). Progesterone concentrations prior to treatment were similar to those reported during normal estrous cycles (Plotka et al., 1967; Stabenfeldt et al., 1969; Sprague et al., 1971; Kazama and Hansel, 1970; Wettmann et al., 1972; Glencross et al., 1973). Cows on both treatments had similar (P > .25) plasma progesterone concentrations on day 5 (3.8 ± .3 vs 4.1 ± .3 ng/ml, for Trt 17 and Trt 5, 17, respectively). By 48 hr after cows on Trt 17 had received the initial PMSG injection on day 5, progesterone concentrations were greater (P < .025) than in cows on Trt 17 (12.5 ± .8 vs 7.6 ± .6 ng/ml, respectively). Plasma progesterone continued to increase to a maximum of 14.9 ± 2.0 ng/ml on day 17 in Trt 5, 17 cows compared to 7.9 ± .6 ng/ml for Trt 17 cows (P < .005). Plasma progesterone remained elevated for several days after the maximum in Trt 5, 17 cows and did not decline to low concentrations in all cows until day 25 (1.6 ± .6 ng/ml). Increased plasma progesterone in cows on Trt 5, 17 may be associated with the longer estrous cycles that were observed.

Plasma progesterone response to PMSG in heifers was similar to that observed in cows (figure 2). Progesterone concentrations increased after PMSG treatment on day 5 (P < .05). Mean progesterone concentrations of heifers in response to PMSG on day 17 were slightly greater and remained elevated for a longer period before the proestrus decline than those of cows. However, both cows and heifers attained the minimal progesterone concentration associated with estrus at about the same time after treatment. Similar increases in progesterone after PMSG injections have also been reported (Spilman et al., 1973; Henricks et al., 1973; Henricks and Hill, 1978).

Plasma progesterone at time of laparotomy in both cows and heifers was related to number of corpora lutea (r = .75, P < .01). Plasma

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**Table 2. Ovarian Response of Cows and Heifers Treated with PMSG**

<table>
<thead>
<tr>
<th>Item</th>
<th>Cows</th>
<th>Heifers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Trt 17</td>
<td>Trt 5, 17</td>
</tr>
<tr>
<td>Follicles &lt; 10 mm (no.)</td>
<td>2.7 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>Follicles &gt; 10 mm (no.)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.8 ± .5</td>
<td>1.5 ± .4</td>
</tr>
<tr>
<td>Ovulations (no.)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.2 ± .4</td>
<td>1.3 ± .3</td>
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<td>Distribution of animals</td>
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<td></td>
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<tr>
<td>with various number</td>
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<tr>
<td>of ovulations (%)</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>6.2</td>
<td>20.0</td>
</tr>
<tr>
<td>1</td>
<td>37.5</td>
<td>60.0</td>
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<tr>
<td>2 to 4</td>
<td>50.0</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>6.2</td>
<td>6.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Estimates determined at laparotomy 7 to 11 days post-estrus.

<sup>b</sup> Mean ± SE.

<sup>c</sup> Significant treatment effect for cows (P < .10).

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*Figure 1. Plasma progesterone in cows treated with PMSG on either day 17 (n = 16) or on days 5 and 17 (n = 15) after estrus.*
progesterone increased \(9.6 \pm 1.2\) ng/ml with each additional corpus luteum, which agrees with the results of Lamond and Gaddy (1972) and Spilman et al. (1973).

Plasma estradiol concentrations of cows and heifers treated with PMSG are shown in figures 3 and 4, respectively. Similar to previous reports (Wettemann et al., 1972; Echternkamp and Hansel, 1973; Chenault et al., 1975), average plasma estradiol concentrations of animals on Trt 17 were less than 5 pg/ml from day 1 through day 17 of the estrous cycle. Plasma estradiol increased within 2 days after PMSG injection on day 5 in cows (\(P<.005\)) and by 1 day after treatment in heifers (\(P<.025\)). On day 7, estradiol in cows on Trt 5, 17 was 5.4 \(\pm\) 1.2 pg/ml compared to 2.4 \(\pm\) 5 pg/ml in cows on Trt 17 (\(P<.025\)). Maximum estradiol after PMSG treatment on day 5 occurred on day 9 in cows and day 11 in heifers. These increases in estradiol could have resulted from increased follicular growth stimulated by the PMSG, but this could not be verified since laparotomies were not performed until 7 to 11 days after the subsequent estrus. No animals were detected in estrus within 14 days after the day 5 PMSG treatment, suggesting that even though estradiol was evaluated, the concurrent increase in progesterone inhibited estrus.

Plasma estradiol concentrations increased in both cows and heifers on both treatments after PMSG on day 17. Cows on Trt 17 and Trt 5, 17 had similar plasma estradiol on day 17 (2.8 \(\pm\) 4 pg/ml \(vs\) 2.8 \(\pm\) .8 pg/ml, respectively). By 2 days after treatment, cows on Trt 17 had greater (\(P<.025\)) estradiol than did those on Trt 5, 17 (7.2 \(\pm\) .9 \(vs\) 4.2 \(\pm\) .5 pg/ml, respectively). Similarly, heifers on Trt 17 had greater estradiol concentrations compared to Trt 5, 17 on day 19 (17.2 \(\pm\) 3.2 \(vs\) 8.3 \(\pm\) 2.3 pg/ml, respectively; \(P<.05\)). These data suggest that follicular growth in both cows and heifers as indicated by increased plasma estradiol can be stimulated by PMSG either early or late in the estrous cycle, but the estradiol response to PMSG given on day 17 is reduced if animals were treated with PMSG on day 5. Similar estrogen increases have been reported after treatment of cattle with PMSG late in the cycle (Henricks et al., 1973; Henricks and Hill, 1978) and after treatment of prepubertal heifers (Testart et al., 1977). Maximum estradiol after PMSG on day 5 or 17 was not correlated with corpora lutea numbers observed at laparotomy after estrus. There was some evidence in cows on Trt 17, however, of a
relationship between maximum estradiol and numbers of corpora lutea plus numbers of follicles greater than 10 mm in diameter present at time of laparotomy ($r = .58; P<.05$). A similar relationship in heifers on Trt 17 only approached significance ($P<.10$). Henricks and Hill (1978) found that the estrogen concentration at estrus was correlated with the number of ovulations. Similarly, Testart et al. (1978) observed a quantitative relationship between plasma estradiol and number of ovulating follicles in prepubertal heifers treated with fluorogestone acetate and PMSG.

Plasma LH was not influenced by treatment within age group, and values throughout the estrous cycle averaged approximately 1.0 ng/ml. LH concentrations greater than 2 ng/ml were not evident in any of the treatment groups between days 5 and 7 of the estrous cycle (figures 5 and 6). However, 55% and 67% of the heifers on Trt 17 and Trt 5, 17, respectively, had at least one sample with greater than 2 ng/ml LH between day 17 and estrus. Fifty-six percent of the cows on Trt 17 had similar elevations; while only 13% of the cows on Trt 5, 17 showed increased LH near the expected times of estrus. Since the ovulatory surge of LH in cattle persists for 6 to 8 hr (Schams and Karg, 1969; Henricks et al., 1970; Swanson and Hafs, 1971) estimates of LH concentration near the time of estrus vary with time of sampling during the ovulatory surge.

Spilman et al. (1973) demonstrated that plasma LH concentrations increased on the day after PMSG injection and declined thereafter in prepuberal and mature heifers. Henricks et al. (1973) observed increased plasma LH earlier prior to estrus in PMSG treated heifers than in controls. These previous results would indicate that plasma LH may be influenced by PMSG treatment, but daily sampling is not sufficient to detect these changes. Plasma LH was not significantly related to either estradiol or ovulation rate. Similarly, Henricks et al. (1973) failed to detect a relationship between plasma LH and reproductive criteria after PMSG treatment.

These data demonstrate that PMSG alters both the ovarian and endocrine function when given either on day 5 or day 17 of the estrous cycle. Treatment with PMSG on day 5 may influence the ovulatory and endocrine response observed when PMSG is given again on day 17.

**Literature Cited**


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