MULTIPLE BIRTHS IN BEEF COWS TREATED WITH EQUINE GONADOTROPIN (PMS) AND CHORIONIC GONADOTROPIN (HCG) 1

E. J. Turman,2 D. B. Laster,3 R. E. Renbarger4 AND D. F. Stephens4
Oklahoma Agricultural Experiment Station, Stillwater 74074 and U. S. Department of Agriculture, El Reno, Oklahoma 73036

IT is well established that the injection of a wide variety of gonadotropic hormone preparations, including pregnant mare serum (PMS), will result in superovulation. There are many reports of the effectiveness of PMS in inducing superovulation in the cow. However, few studies have been concerned with its effectiveness in inducing superfetation and subsequent multiple births. In general, these studies were not highly successful but did identify many of the associated problems (Hammond and Bhattacharya, 1944; Hammond, 1949; Gordon, Williams and Edwards, 1962; Hafez, Jainudeen and Lindsay, 1965).

Promising results were reported by Schilling and Holm (1963) from a limited study involving a sequence of two PMS injections. Their report stimulated the initiation of a study at the Fort Reno Research Station to determine the effectiveness of a sequence of two PMS injections in inducing superovulation and multiple births in beef cows.

Materials and Methods

This study was initiated in May, 1967. It involved 81 lactating beef cows of Angus, Hereford and Angus X Hereford breeding ranging in age from 3 to 8 years. The cows had calved in February and March and were maintained under range conditions on native grass pastures at the Fort Reno Livestock Research Station.

Seventy-four cows were assigned as equally as possible on the basis of breeding, age, weight and date of previous calving to one of three treatment groups to receive a subcutaneous injection of 1,500 IU of PMS (first PMS) on either day 4, 5 or 6 of the cycle, counting the day of estrus as day 0. The groups were further subdivided to receive a second subcutaneous injection of 2,000 IU of PMS (second PMS) on day 16, 17 or 18. The remaining seven cows were given their first PMS injection on day 3 and their second PMS injection on day 17.

Vasectomized bulls were used to detect the occurrence of estrus prior to the first PMS injection and to determine whether estrus occurred between the first and the second PMS injections. Each cow was placed in a lot with a fertile bull immediately following administration of her second PMS injection. On the day of her first post-PMS estrus, when a cow was observed to have mated, she was given an intravenous injection of 2,500 IU of chorionic gonadotropin 6 and mated to a second bull. On the day following her first post-PMS estrus, each cow was placed in a pasture with a fertile bull for 3 months and checked twice daily for signs of estrus. The cows were examined for pregnancy by rectal palpation 3 to 5 months after the post-PMS breeding.

The cows were wintered on native grass pastures with 0.91 kg of 43% cottonseed cake per day. In addition ground milo was fed at a rate sufficient to maintain weight gains of approximately 0.23 kg per day from December 1 to the time of calving.

At birth the surplus calves from triplet and quadruplet sets were transferred to cows with singles so that no cow reared more than twins. Efforts were made to insure that as many as possible of the cows that calved multiples reared twins. Cows rearing twins were maintained in a separate pasture with a high energy creep feed provided for the calves. Single calves were not creep fed. The cows nursing calves were in pastures with fertile bulls for 90 days beginning June 1, 1968, when the cows were 30 to 85 days post-partum. All calves were weaned in mid-October and fed in dry lot on a 65% concentrate ration for 150 days. At time of slaughter the reproduc-

1 Journal Article 2065 of the Agricultural Experiment Station, Oklahoma State University, Stillwater, Oklahoma. Research conducted by the Department of Animal Sciences and Industry in cooperation with the Animal Science Research Division, Agricultural Research Service, U.S.D.A.
2 Oklahoma State University, Stillwater.
3 Present address: Department of Animal Science, Iowa State University, Ames.
4 Fort Reno Livestock Research Station, A.R.S.
5 Gonadin, Cutter Laboratories, Berkeley, California.
6 Burns Pharmaceuticals, Oakland, California.
Multiple Births in Beef Cows

963

... tracts were recovered from all heifers from multiple births.

The data were analyzed by analysis of variance (Steel and Torrie, 1960) and the adjusted treatment sum of squares were obtained by the least squares procedure of Harvey (1960). Duncan’s Multiple Range Test was used to determine differences among group means (Steel and Torrie, 1960). Calves from triplet, quadruplet and quintuplet births were included in one group for statistical comparisons with calves from single and calves from twin births. A chi-square analysis was used to determine if there were differences in numbers of multiple births among the treatment groups and ages of cows.

Results and Discussion

A total of 81 cows were treated and 52 (64.2%) conceived at the first post-PMS estrus and subsequently calved (table 1). Nineteen cows conceived at a subsequent estrus; 12 cows at the second, six cows at the third and one cow at the fifth post-PMS estrus. Ten cows were not pregnant when palpated in November. Seventy of the 71 pregnant cows calved.

Although untreated control cows were not available for comparison, the conception rate at the post-PMS estrus in this study does not appear to be depressed. Other workers have reported that conception rates do not appear to be adversely affected by PMS (Hammond, 1949; Gordon et al., 1962; Hafez et al., 1964). In contrast, however, Schwartz and Shelby (1969) reported that only 11% of 18 cows receiving PMS treatments similar to those used in this study were pregnant at slaughter 90 to 120 days post-insemination. Hafez et al. (1965) reported studies in which the conception rate of PMS treated heifers was low.

The figures given for live calves (table 1) are for those living at 2 weeks of age. Most death losses occurred at time of parturition, and none later than 3 days post-parturition. No calving losses were suffered in twin births, however, fairly heavy death losses occurred among triplets, quadruplets and quintuplets. Gordon et al. (1962) reported similar observations. In their study, 87.5% of 35 sets of twins compared to 29.2% of 8 sets of triplets survived to 2 weeks of age.

There were no significant differences in the conception rates at the first post-PMS estrus among the various breeding groups of cows. The differences in the response of the breeding groups in terms of multiple births approached significance (P<.10). Of the conceptions at the first post-PMS estrus, 68.8% in the Angus-Herford crossbreds, 34.5% in the Herefords, and 28.6% in the Angus resulted in multiple births. Whether this greater response in the 2-year-old crossbred heifers was due to the age or body weight difference or was a heterotic effect cannot be determined from the data obtained in this study. The heifers were approximately 100 kg lighter than the Hereford and Angus cows in body weight. Gordon et al. (1962) reported that small cows responded to PMS injections more readily than did large cattle. However, in this study, within age groups there was no association observed between body weight and response to PMS as measured by numbers of multiple births.

There were no differences among the groups of cows receiving their first PMS injection on day 3, 4, 5 or 6 in terms of subsequent multiple births (table 2). There were no interactions between times of first and second PMS

---

**Table 1. Summary of the Breeding Performance of All Cows Treated with PMS** and HCG and the Calving Performance of Cows Conceiving to Natural Service at the First Post-PMS Estrus

<table>
<thead>
<tr>
<th>Breeding of cows</th>
<th>Age when treated (years)</th>
<th>Body wt. (kg)</th>
<th>No. conceived at 1st post-PMS estrus</th>
<th>No. open</th>
<th>No. of cows conceiving at 1st post-PMS estrus producing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Singles Twins Trips Quads Quints</td>
</tr>
<tr>
<td>Angus X Hereford</td>
<td>2</td>
<td>417</td>
<td>26 16 2</td>
<td>5</td>
<td>6 4 1 0</td>
</tr>
<tr>
<td>Hereford</td>
<td>4</td>
<td>540</td>
<td>43 29 6</td>
<td>19</td>
<td>5 4 1 0</td>
</tr>
<tr>
<td>Angus</td>
<td>5+</td>
<td>505</td>
<td>12 7 2</td>
<td>5</td>
<td>1 0 0 1</td>
</tr>
<tr>
<td>Total for all</td>
<td></td>
<td></td>
<td>81 52 10</td>
<td>29</td>
<td>12 8 2 1</td>
</tr>
<tr>
<td>Live calves</td>
<td></td>
<td></td>
<td></td>
<td>28</td>
<td>24 12 3 2</td>
</tr>
</tbody>
</table>

---

* PMS injections subcutaneous, 1,500 IU on day 3, 4, 5 or 6 and 2,000 IU on day 16, 17 or 18 of estrual cycle.
* HCG injections intravenous, 2,500 IU on day of first post-PMS estrus.
* Calves alive at 2 weeks of age.
TABLE 2. SUMMARY OF THE BREEDING PERFORMANCE OF ALL COWS AND THE CALVING PERFORMANCE OF COWS CONCEIVING AT THE FIRST POST-PMS ESTRUS FOLLOWING TREATMENT WITH 1500 IU PMS ON DAYS 3, 4, 5 OR 6, 2000 IU PMS ON DAYS 16, 17 OR 18, AND 2500 IU HCG ON DAY OF POST-PMS ESTRUS

<table>
<thead>
<tr>
<th>Item</th>
<th>Total treated</th>
<th>No. conceived at 1st post-PMS estrus</th>
<th>No. open</th>
<th>No. of cows conceiving at 1st post-PMS estrus producing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Singles</td>
</tr>
<tr>
<td>Time of first PMS injection (1,500 IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Day 4</td>
<td>25</td>
<td>16</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Day 5</td>
<td>25</td>
<td>16</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Day 6</td>
<td>24</td>
<td>15</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Time of second PMS injection (2,000 IU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 16</td>
<td>27</td>
<td>19</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Day 17</td>
<td>32</td>
<td>19</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Day 18</td>
<td>22</td>
<td>14</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

* Cows receiving first PMS on day 3 received a second PMS injection on day 17.
* Cows receiving first PMS on days 4, 5 or 6 received a second PMS injection on either day 16, 17 or 18.
* All cows had received a first PMS injection on either day 3, 4, 5 or 6.

injections. Schilling and Holm (1963) proposed that the injection of a subovulatory level of PMS on day 5 of the cycle was essential for desirable superovulatory response following a second PMS injection given on day 16 to 18 of the same cycle. The data obtained in this study does not test the necessity of an injection given early in the cycle. However, it does suggest that, if necessary, this injection may be given anytime between day 3 and day 6.

Although the differences were not significant, the data in table 2 suggests that the PMS injection given in the follicular phase of the cycle can be given too late for maximum response in terms of subsequent multiple births. Only 28.6% of the conceptions following the second PMS injection given on day 18 resulted in multiple births, compared to 50% of the conceptions following PMS on days 16 or 17 (table 2). Gordon et al. (1962) reported that a single injection of 800 to 2,000 IU PMS resulted in fewer ovulations when the injection was given on day 18 or 19 than on day 16 or 17.

Figure 1 presents data that suggests a reason for the reduced response of cows given their second PMS on day 18. Estrus was observed in 93.8% of the cows by day 8 post-PMS. Cows conceiving at an estrus on either day 3, 4 or 5 post-PMS produced 87.0% of the multiple births. Estrus was observed on either day 3, 4 or 5 post-PMS in 75.0% and 74.2% of the cows receiving second PMS on day-16 and day-17, respectively, compared to 42.9% of those injected on day-18. Not only were fewer of the cows that received their second PMS on day 18 in estrus during the period when most multiples were conceived, but this group also contained 63.2% of all cows in the study that were in estrus on day 1 or 2 post-PMS.

The data reported in figure 1 suggests that the most effective treatment would be to induce estrus 3 to 5 days post-PMS. This supports the observation of Hafez et al. (1965) that an interval of at least 3 days was desir-
MULTIPLE BIRTHS IN BEEF COWS

able for normal estrous behavior and satisfactory ovulatory response. They reported that more follicles were produced after a 4-day interval than after 2, 3 or 5 days. Hammond (1949) and Gordon et al. (1962) also reported that the most satisfactory ovulatory response to PMS followed an interval from injection to estrus of at least 3 days.

There were significant differences in birth weights and gestation lengths (table 3). Single calves were heavier than calves from multiple births (P<.01). Twins were heavier than triplets, quadruplets and quintuplets (P<.01). Gestation length of singles was longer than that of twins (P<.05) or that of triplets, quadruplets and quintuplets (P<.01). Twins were carried for a longer gestation (P<.01) than were triplets, quadruplets and quintuplets. These gestation lengths are very similar to those reported by Bellows et al. (1970) for singles and twins following FSH injections, but longer than those reported by Gordon et al. (1962) for twins and triplets.

Retained placentas were a problem, primarily among the triplet and larger litters. They occurred in 11 of 23 cows producing multiple births compared to two of 29 cows producing single births. The distribution according to type of multiple birth was: twins, 3/12; triplets, 5/8; quadruplets, 2/2; and quintuplets, 1/1. Gordon et al. (1962) also reported a higher incidence of retained placentas associated with triplets (87.5%) than with twins (42.4%). Dystocia was not a problem associated with multiple births. There were two cows producing multiples and two producing singles that required assistance at time of calving.

This study evaluated the response to PMS only in terms of incidence of multiple births. No attempt was made to determine ovulation rate following second PMS, or to determine whether superfetation had occurred. Gordon et al. (1962) observed that palpations carried out at the optimum time to determine superfetation, approximately 6 weeks post-breeding, induced fetal death in 32.5% of the cows examined. Since the primary objective of this study was to determine whether multiple births could be induced, it was desired to give the cows every opportunity to conceive multiples and carry them to term. Consequently, palpations were delayed until 3 to 5 months post-breeding and were made only to determine pregnancy.

Both pre- and post-weaning survival of all calves was good (table 3). One twin and one single born calf died at 3 to 4 months of age, and one twin died in the feedlot. The increased incidence of multiple births had a decided effect on calf crop percentages. A total of 88 calves were weaned, or a calf crop percentage weaned of 124% based on the 71 pregnant cows wintered, or 109% based on the 81 cows originally treated.

The weaning and feedlot data in table 3 are biased since only the multiple birth calves were creep fed. Despite this preferential treatment accorded multiple born and reared calves, single born calves were significantly heavier (P<.05) than multiples at 205 days of age. The adjusted 205 day weight of twins was significantly heavier (P<.05) than that of triplets, quadruplets and quintuplets. However, despite the reduced weaning weight per calf, the total production per cow was increased. In the case of twins, an additional 151 kg of calf was obtained.

There were no significant differences in feedlot gains of calves of different birth types. Steers gained significantly faster (P<.05) than heifers. No carcass data were obtained. A total of 17 heifers that had at least one male littermate were slaughtered. All were freemartins and are described by Laster et al. (1970).

All cows raising singles and 15 or 20 cows nursing twin calves rebred during a 3-month exposure to bulls beginning 60 days post-partum. The five open cows were again exposed to fertile bulls following weaning, and all conceived within 6 weeks. The cause of this delayed rebreeding cannot be determined from the data obtained in this study. It did not appear to be associated with retained placentas since only two of the 11 affected cows were in the group delayed in rebreeding.

The results obtained in this study suggests that the sequence of two PMS injections proposed by Schilling and Holm (1963) will increase the incidence of multiple births in beef cattle. There are many problems yet to be solved before the hormonal induction of multiple births can be recommended for use by beef producers. However, the liveability and growth rate of twins observed in this study encourages additional research on multiple births.

Summary

The effect of the injection of beef cows with a sequence of two subcutaneous doses of PMS, 1,500 IU on day 3, 4, 5 or 6 and 2,000 IU
TABLE 3. GESTATION LENGTHS AND AVERAGE BIRTH WEIGHTS OF ALL CALVES CALVED AS SINGLES OR IN MULTIPLE SETS BY COWS TREATED WITH PMS¹ AND HCG² AND THE ADJUSTED 205 DAY WEIGHT AND FEEDLOT GAINS OF THE SURVIVING CALVES

<table>
<thead>
<tr>
<th>Type of birth²</th>
<th>No. of sets</th>
<th>Gestation length (days)</th>
<th>Birth weight (kg)</th>
<th>No. calves weaned</th>
<th>Adj 205 day wt.</th>
<th>A.D.G. in feedlot³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male SD</td>
<td>Female SD</td>
<td>kg/day SD</td>
<td>kg/day SD</td>
</tr>
<tr>
<td>Singles</td>
<td>29</td>
<td>280.8 ± 4.7</td>
<td>37.7 ± 3.2</td>
<td>37.8 ± 4.2</td>
<td>28 209.6 ± 24.1</td>
<td>1.25 ± 0.16</td>
</tr>
<tr>
<td>Twins</td>
<td>12</td>
<td>277.4 ± 4.9</td>
<td>27.1 ± 3.1</td>
<td>30.0 ± 2.8</td>
<td>23 180.5 ± 25.1</td>
<td>1.19 ± 0.15</td>
</tr>
<tr>
<td>Triplets</td>
<td>8</td>
<td>269.2 ± 3.8</td>
<td>20.9 ± 2.5</td>
<td>21.0 ± 2.9</td>
<td>12 158.3 ± 33.9</td>
<td>1.25 ± 0.10</td>
</tr>
<tr>
<td>Quadruplets</td>
<td>2</td>
<td>262.5 ± 4.0</td>
<td>17.5 ± 1.5</td>
<td>16.0 ± 2.4</td>
<td>3 158.3 ± 25.1</td>
<td>1.19 ± 0.11</td>
</tr>
<tr>
<td>Quintuplets</td>
<td>1</td>
<td>258.0 ± ...</td>
<td>13.8 ± 2.0</td>
<td>13.2 ± ...</td>
<td>2 143.3 ± 24.1</td>
<td>0.94 ± ...</td>
</tr>
</tbody>
</table>

¹ PMS injections subcutaneous, 1,500 IU on day 3, 4, 5 or 6 and 2,000 IU on day 16, 17 or 18 of estrual cycle.
² HCG injections intravenous, 2,500 IU on day of first post-PMS estrus.
³ Calves from triplet, quadruplet and quintuplet birth combined and differences between them not tested for significance.
⁴ Number in parentheses indicate number of animals in each group.
⁵ Values within columns with different superscripts are significantly different (P<0.05).
on day 16, 17 or 18, followed by an intravenous injection of 2,500 IU HCG on day of estrus was studied. Of 81 cows so treated, 52 conceived at the first estrus following the second PMS injection and produced 29 singles and 23 multiple births (12 sets of twins, eight sets of triplets, two sets of quadruplets and one set of quintuplets).

At birth single calves were heavier than multiples \((P<.01)\) and twins were heavier than triplets, quadruplets and quintuplets \((P<.01)\). The gestation length of singles was longer than twins \((P<.05)\), and of twins was longer than triplets, quadruplets and quintuplets \((P<.01)\). No twins were lost at calving, but death loss of triplets, quadruplets and quintuplets was 54%.

The adjusted 205 day weights of single birth calves was significantly heavier than that of multiples \((P<.05)\), and that of twins significantly heavier than that of triplets, quadruplets and quintuplets \((P<.05)\). Preweaning survival of all calves was good. The calf crop percentage weaned was 124% based on the 71 pregnant cows wintered, or 109% based on the 81 cows originally treated.

There was no significant difference in postweaning feedlot performance of calves of different birth types. All of the 17 surviving heifers from multiple births with at least one male littermate were freemartins.

Multiple births were not associated with an increased incidence of calving difficulty. Eleven of the 23 cows producing multiples had retained placentas. This did not appear to be associated with delayed rebreeding. Twenty-five percent of the cows nursing twins did not rebreed while lactating, but did conceive within 6 weeks after the calves were weaned.

**Literature Cited**


