

# The Experimental Production of Multiple Births in Beef Cows by Hormone Injections

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## Story in Brief

The injection of beef cows with a sequence of two subcutaneous doses of PMS (pregnant mare serum), 1500 i.u. on day 4, 5 or 6 and 2000 i.u. on day 16, 17 or 18 resulted in a marked increase in the incidence of multiple births. Of 81 cows so treated, 10 failed to conceive during a 3 months breeding season, while 52 conceived at the first heat following the second PMS injection. These 52 cows produced 29 single and 23 multiple births (12 sets of twins, 8 sets of triplets, 2 sets of quadruplets and 1 set of quintuplets).

Average birth weights were: singles, 83.3 lb.; twins, 63.0 lb.; triplets, 46.2 lb.; quadruplets, 37.5 lb.; and quintuplets, 30.0 lb. Numbers of calves surviving until weaning and adjusted 205 day weaning weights were, respectively: singles, 28 and 462 lb.; twins, 23 and 398 lb.; triplets, 12 and 349 lb.; quadruplets, 3 and 348 lb.; quintuplets, 2 and 316 lb.

Multiple births were not associated with an increased incidence of calving difficulty. Eleven of the 23 cows producing multiples had retained placentas, however, this did not appear to be a factor related to delayed rebreeding. Twenty-five percent of the cows nursing twins were delayed in rebreeding. Five cows nursing twins had failed to settle during the normal 3 months breeding season, but all subsequently settled. Calf crop percentage weaned was 109 percent based on the 81 cows placed in the breeding herd, or 124 percent based on the 71 cows that were wintered.

## Introduction

The beef cow is one of the least efficient of all of the meat animals. She is maintained year long to produce only one useful product, her weaner calf, and her production efficiency is measured by the weight and grade of this calf. If she fails to wean a calf, her contribution for that year is nil, and her maintenance costs must be borne by the productive members of the cow herd.

The percent calf crop in the United States is variously estimated to be from 65 to 90 percent annually, with 85 percent suggested as the average for some of the more productive areas of the country. Thus, the average cow herd owner must maintain one hundred cows for every eighty-five calves he weans. However, if he is to maintain this rate of

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production, he normally must add replacement heifers for approximately 20 percent of the cow herd each year. Therefore, he has only sixty-five marketable calves per one hundred cows weaning an 85 percent calf crop.

The primary means available for improving productive efficiency in the beef cow is to wean a greater number of calves each year. This would mean that with no increase in cow numbers, and only a limited rise in production costs, a substantial increase in net income could be achieved. One way in which this could be accomplished would be to increase the rate of twinning in the beef cow.

Unfortunately, the incidence of natural twin births is low. It occurs much more frequently in the dairy breeds, averaging about one set in every 55 births. However, it is a much less frequent occurrence in the beef breed with twins being born in only about 4.4 percent of all births, or one time in every 250 births. Also, most studies have indicated that the heritability of the tendency to produce twins is low. Thus, selection for increased twinning rate should have little or no effect on twinning rate. Therefore, some other means for increasing twinning rate must be developed.

Means for experimentally increasing twinning rate was provided by the basic research that was conducted in the early 1930's on the relationship of the gonadotropic hormones of the anterior pituitary gland to ovulation. It was determined that a specific hormone, follicle stimulating hormone (usually referred to as FSH) was released by the pituitary and carried by the bloodstream to the ovary where it stimulated the growth and development of a structure called the follicle within which the egg matured. When the egg was ready to be fertilized, the follicle ruptured and the egg was expelled (a process called ovulation). Ovulation was brought about by another gonadotropic hormone of the anterior pituitary gland, leuteinizing hormone or LH.

These early research studies also demonstrated that the injection of gonadotropic hormones would result in stimulation of the ovary to develop and ovulate more than the normal number of follicles (superovulation). In the early 1940's, superovulation was reported in a large number of research studies involving cows, sows and ewes as a result of the injection of gonadotropic hormones. The usual source of the gonadotropic hormone used in these early studies was extracts of pituitary glands obtained from slaughterhouses. This was not a reliable source, it was costly and quantities were restricted. In the late 1930's, researchers at the University of California reported the presence of a potent gonadotropic substance in the blood of pregnant mares during part of the gestation period. This substance, called pregnant mare serum or PMS, readily induced superovulation in females when injected. Thus, one of the most

practical methods for the experimental production of superovulation was developed.

Pregnant mare serum, as the name implies, is the blood serum from pregnant mares. It is rich in a gonadotropic substance that is similar in physiological activity to that of FSH. This substance appears in the blood of mares about the 40th day of pregnancy, reaches a high point between the 70th and 120th days, then declines to nearly zero by the 180th day. It is produced by the structures in the uterus that are associated with the placenta or fetal membranes. Thus, it is one of a group of substances referred to as placental gonadotropins.

A second type of placental gonadotropin is produced by the pregnant woman (and other primates). It is produced by the chorion, one of the membranes of the placenta, and is called HCG or human chorionic gonadotropin. It has a physiological activity similar to that of the leuteinizing hormone of the anterior pituitary; thus, when injected, should stimulate ovulation of mature follicles. HCG reaches a peak in the urine of pregnant women by about the 50th day and declines to a low level by the 140th day. It is the basis of several tests for pregnancy in women.

Pharmaceutical supply houses process human pregnancy urine and blood from pregnant mares. Thus, commercial preparations of PMS and HCG are readily available to veterinarians, and have been routinely used by them for many years in treating reproductive problems in farm animals. These hormone preparations are restricted by federal law to be sold only on the order of a licensed veterinarian. Therefore, any livestock producer interested in using them for any purpose will find it necessary to work with his veterinarian.

Many research studies have been reported concerning the effectiveness of PMS in inducing superovulation. However, very few have been directed toward determining whether not only superovulation but also superfetation (increased number of embryos) could be established and multiple births obtained. The few studies that have been reported were not highly successful and pointed up many problems that were involved. In 1963, research workers in Germany reported very promising results from a limited study involving a sequence of two PMS injections on the 5th and the 16th to 18th days after the last heat. This report, and others, stimulated the initiation of a large scale project at the Fort Reno Station to determine whether it was possible to develop a practical means for increasing the occurrence of multiple births by the use of PMS. This paper is a report of the progress of this project to date.

## Materials and Methods

The cows used in this study were in a herd at the Fort Reno Research Station and were maintained on native grass range. The PMS treatments were given in June and July of 1967. The cows were lactating, of mixed ages and breeding as shown in Table 1. They were assigned as equally as possible on the basis of age, weight and date of calving to one of three groups to receive their first subcutaneous injection of 1500 i.u. of PMS on either day 4, 5 or 6 of the cycle, counting the day of estrus as day 0. The cows were further sub-divided within each of these three groups to receive a second subcutaneous injection of 2000 i.u. of PMS on either day 16, 17 or 18. The PMS used was "gonadin" prepared by the Cutter Laboratories and obtained through the OSU Veterinary Clinic.

Sterilized bulls were placed with the cows to detect their estrus period prior to the first PMS injection and to determine whether estrus occurred between the first and second PMS injections. Immediately following her second PMS injection, each cow was placed with a fertile bull. On day of first estrus following PMS (first post-PMS estrus) when a cow was observed to have mated, she was immediately removed from the pen, given an intravenous injection of 2500 i.u. of chorionic gonadotropin and hand-mated to a different bull. Following the first post-PMS mating, all cows were pasture exposed to fertile bulls for 2 to 3 months. The pastures were checked twice daily for signs of estrual activity. The cows were examined for pregnancy by rectal palpation in early November. No attempt was made to determine the number of embryos in the uterus because such would require excessive handling that might damage the developing embryos.

The cows were wintered on native grass pastures with a moderate level of protein supplement. At time of calving, the surplus multiple

**Table 1. Summary of 1968 Calving Performance of All Cows Treated with PMS in 1967.**

| Breeding of Cows   | Age When Treated (years) | Body Wt. (lbs.) | Total Treated | No. Conceived at 1st Post-PMS Estrus | No. Open | No. of Cows Producing |       |       |       |        |
|--------------------|--------------------------|-----------------|---------------|--------------------------------------|----------|-----------------------|-------|-------|-------|--------|
|                    |                          |                 |               |                                      |          | Singles               | Twins | Trips | Quads | Quints |
| Crossbred          | 2                        | 917             | 26            | 16                                   | 2        | 5                     | 6     | 4     | 1     | 0      |
| Hereford           | 4                        | 1188            | 43            | 29                                   | 6        | 19                    | 11    |       |       | 0      |
| Angus              | 5+                       | 1110            | 12            | 7                                    | 2        | 5                     | 1     | 0     | 0     | 1      |
| Total for All Cows |                          |                 | 81            | 52                                   | 10       | 29                    | 2     |       |       | 1      |
| Live Calves        |                          |                 |               |                                      |          | 28                    | 23    |       |       | 2      |

calves from triplet and quadruplet sets were transferred to cows with singles or to cows that had lost their calves so that no cow reared more than twins. The cows rearing twins were maintained in a separate pasture with creep feed provided for the calves. Single calves were not creep fed. The calves were weaned in early November at an average age of 7 months.

## Results and Discussion

Table 1 presents the results obtained in the 1968 spring calving season for all cows in the study. The data given under the heading "No. of Cows Producing Singles, Twins, Triplets, Quadruplets and Quintuplets" refers only to the cows that conceived at the first estrus following the second PMS injection. This is the only group of cows that would be expected to be stimulated to produce multiple births as a result of the PMS treatments.

A total of 81 cows were treated, with 52 (or 64.2 percent) conceiving at the first estrus following PMS. Of these 52 conceptions, 23 (or 44.2 percent) resulted in a multiple birth with the type of multiple births distributed as shown in Table 1. The results are not broken down on the basis of time of receiving the PMS injections. It was found that there were no differences in the response of cows, in terms of numbers of multiple births, associated with whether the first PMS injection was given on day 4, 5 or 6. Likewise, there was no difference in the occurrence of multiple births in cows receiving the second PMS injection on either day 16 or 17. However, there was good evidence that day 18 was too late, since fewer cows receiving their second PMS injection on day 18 produced multiples.

The figures given in Table 1 for live calves are for calves surviving at least one month. The survival rate of twins in this study was 100 percent, but fairly heavy death losses were suffered among triplets, quadruplets and the set of quintuplets. However, despite these losses, there were still more live calves than cows in every multiple birth group.

There were three age groups of cows. However, for all practical purposes, the results on the Hereford and Angus groups can be combined. Over  $\frac{2}{3}$  of the conceptions (68.8 percent) in the Angus-Hereford crossbred heifers resulted in multiples; whereas, 34.5 percent of the conceptions in the Hereford and 28.6 percent of the conceptions in the Angus resulted in multiple births. Just what caused this greater response in the two-year old crossbred heifers cannot be determined from the results obtained in this study. It could be due to the age and body weight difference, or it could be a heterotic effect because she was a crossbred.

The 12.3 percent incidence of open cows is somewhat high when compared to other cow herds maintained at Fort Reno. The open cows

were marketed with no attempt being made to examine the reproductive tracts for possible abnormalities as a result of PMS treatment. However, there was no indication of any abnormalities of the reproductive tracts as a result of the PMS treatments. All cows were observed in estrus following the removal of the bulls, and their ovaries appeared normal when checked by palpation. One factor that may have contributed to the greater number of open cows was the fact that the breeding season for this particular group of cows did not start until mid-June and this meant that most of the matings occurred during hot weather. In contrast, in the other herds at Fort Reno, the breeding season begins May 1, and most of the cows are pregnant prior to the onset of hot weather. There seems to be little doubt that breeding efficiency is reduced during periods of high temperature.

As might be expected, there were differences in the birth weight of single calves as compared to calves from multiple births. The average birth weights were: singles, 83.3 lb.; twins, 63.0 lb.; triplets, 46.2 lb.; quadruplets, 37.5 lb.; and quintuplets, 30.0 lb. It is very likely that this reduced birth weight is one of the important reasons why only two cows producing multiple births required assistance at time of calving.

Multiple births were also associated with a shorter gestation period. The average gestation lengths were: singles, 280.8 days; twins, 277.4 days; triplets, 269.2 days; quadruplets, 262.5 days; and quintuplets, 258.0 days. This reduction in gestation length of nearly three weeks in the case of quadruplets and quintuplets and of nearly two weeks for triplets has very important implications. These calves are physiologically much less mature than are calves born after a gestation of normal length. It is likely that these calves would be much more susceptible to many forms of stress such as exposure to cold weather. Therefore, one might expect higher death losses among these calves unless they receive special attention and protection not required by calves born after a normal gestation.

One problem that was anticipated was that of retained placentas (afterbirth). Eleven of 23 cows producing multiple births suffered retained placentas compared to only one cow of the 29 producing singles. However, the occurrence of retained placentas at time of calving did not appear to be associated with failure of the cows to rebreed.

Table 2 presents the weaning data on the calves (both singles and multiples) dropped by the cows that conceived at the first estrus following PMS. With a few exceptions, all calves from multiple births were raised as twins and had access to creep feed. Single born calves were reared as singles and were not creep fed.

In terms of total number of calves, the increased incidence of multiple births had a decided effect on calf crop percentage weaned. The 71 pregnant cows that were wintered weaned a total of 88 calves, or a calf

**Table 2. Pre-Weaning Performance of Calves Conceived at the First Post-PMS Estrus.**

| Item                 | Type of Birth |       |       |       |         |
|----------------------|---------------|-------|-------|-------|---------|
|                      | Singles       | Twins | Trips | Quads | Quints. |
| Total Sets           | 29            | 12    | 8     | 2     | 1       |
| Total Calves Born    | 29            | 24    | 24    | 8     | 5       |
| Total Calves Weaned  | 28            | 23    | 12    | 3     | 2       |
| Avg. Wean. Wt. (lb.) | 462           | 398   | 349   | 348   | 316     |
| Gain-Birth to Wean.  | 379           | 335   | 303   | 310   | 286     |
| Lb. Calf/Cow         | 462           | 796   | 698   | 696   | 632     |
| Lb. Creep Feed/Calf  | 0             | 550   | 550   | 550   | 550     |

crop percentage of 109 percent. It is evident that this technique has great potential for increasing cow productivity, particularly if additional research will enable reducing the number of open cows and death loss among multiples and increasing the number of cows producing multiples.

It is apparent that the calves reared as twins did not grow as rapidly from birth to weaning as did the singles even with the help of creep feed. There are two possible reasons for this: (1) the beef cows used in this study did not produce enough milk to allow two calves to grow as rapidly as a single calf. Free access to creep feed could not overcome the early retardation that occurred at the time the calf was dependent entirely on milk; (2) calves from multiple births are born after a shorter gestation and at a lighter birth weight. As has been pointed out, they are physiologically less mature than are singles; and, as a result, they are actually younger than their chronological age as counted from birth. It is likely that neither of these fully explains the results, and the real reason for the lighter weaning weights is a combination of these two factors. However, despite the reduced weaning weight per calf, it is apparent that total production per cow is increased. In the case of twins, an additional 334 lbs. of calf was obtained. The very good record in liveability and growth rate of twins suggests the need for additional research to not only increase the incidence of multiple births but to limit it to twins.

This report covers only one year's results, and it must be understood that this project is continuing and additional results will be obtained and reported. Therefore, it should be emphasized that many questions still remain to be answered; and, as a result, there are many limitations associated with the use of PMS to obtain multiple births. A listing and discussion of the more important of these limitations follows.

- (1) Retained placentas appear to be a problem associated with multiple births. A total of 11 out of the 23 cows were so affected. This could affect the rebreeding of the cows, although in this study it did not appear to be a factor causing cows to not rebreed.
- (2) A greater than normal number of cows dropping multiples may be

delayed in rebreeding. In this study approximately 25 percent of the cows raising twins failed to rebreed during a normal 3 months breeding season while they were nursing calves. This may have been a result of their having produced a multiple birth. However, it is more likely the result of the stress of the additional lactation required to nurse twins, since all of the cows conceived in a subsequent breeding season begun soon after the calves were weaned. This delayed rebreeding is definitely a problem that will have high priority in the additional research that will be conducted.

- (3) Under present conditions, these treatments are costly. The hormone cost was approximately \$7.00 per cow treated. This meant a total of \$567 for the 81 cows to produce the 23 sets of multiples. It is hoped that additional research will show ways to reduce the cost per extra calf by: (a) increasing number of cows producing multiples; (b) saving more of the calves from multiple births; and (c) reducing the cost of the hormone by pointing to new and cheaper sources of gonadotropins reducing levels required, and by the increased demand for these hormones, encouraging pharmaceutical houses to go into quantity production, and thus reduce production costs.
- (4) There are large labor requirements under the present system of treatments. Research is now underway to combine these treatments with estrus synchronization. This will permit giving all injections on the same day and reduce the need for heat detection and injections of a few heifers every day.
- (5) These procedures are not adapted to extensive systems of production such as are typical of most of our beef cattle herds. Careful and frequent observations are required, not only during the breeding season but during the calving season. It appears best adapted to some type of confinement system.
- (6) The restricted milking ability of most beef cows greatly limits the usefulness of this technique in beef herds. It would appear to be most useful if the cows were dairy-beef crossbreds or if the surplus calves were reared artificially.
- (7) Approximately 90 percent of the heifers born twin to a bull are "freemartins" and are incurably infertile. Since at least one-half of the twin pairs would be expected to be unlike sexed, increased rate of twinning would not be expected to increase the number of breeding heifers. At best, one would just break even. Since higher death losses would probably result, the number of breeding heifers would probably be reduced somewhat. For this reason, until methods for controlling sex of calf are developed, an increase in occurrence of multiple births would probably appeal most to the commercial breeder who intends to send all calves to market for slaughter.



## Summary

The results reported in this paper definitely indicate that the incidence of multiple births in beef cows can be greatly increased by injections of PMS. It should be emphasized, however, that many questions remain to be answered by further research. It, therefore, should be considered to be still in the research stage and not ready for routine use in cattle production.

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# Evaluation of the K<sup>40</sup> Counter as A Predictor of Lean in Beef Cattle

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## Story in Brief

Sixteen Angus heifers and fifteen Angus steers were counted in the potassium-40 (K-40) whole body counter at the OSU live animal evaluation center during the spring of 1968. The 31 head were divided into four groups and each group was counted after shrinking periods of 24 and 72 hours. The animals were slaughtered and the carcasses were counted after chilling about 40 hours. The right side was separated into lean, fat, and bone; and chemical determinations were made to obtain an estimate of the amount of fat-free lean (FFL).

The data was first analyzed to see how well the two counts on the same animal during the same shrink period agreed. The results suggested that shrinking 72 hours improved the extent to which the two counts agreed. The agreement between carcass counts was better than the 24-hour counts, but was not as good as the agreement between 72-hour counts.

Each live and carcass count and the average of the two counts for each counting period was examined to observe the relationship between count and pounds of FFL. Significant positive relationships were observed between all live and carcass counts and pounds of fat-free lean. The 72-hour count did not, however, predict fat-free lean any better than the 24-hour count. Other relationships such as count to percent FFL, count to live weight, and live weight to pounds of FFL were also studied.

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