

# Superovulation Of Beef Cows And Heifers By Injection Of Pregnant Mare Serum (PMS) Following Estrus Synchronization By Means Of An Oral Progestogen

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## Story in Brief

The effect on ovulation rate of injections of PMS and HCG timed from a synchronized estrus was studied. Estrus was synchronized by feeding CAP for 18 days (10 mg./head/day for 16 days then 5.0 mg./head/day for 2 days). All PMS injections were timed from the average day of estrus, which was the third day after the last feeding of CAP.

A total of 46 yearling beef heifers and 19 lactating beef cows received subcutaneous injections of 1500 IU PMS and 18 cows received 1000 IU PMS on day-5 with all receiving a second injection of 2000 IU PMS on day-17 of the cycle. All PMS injections were subcutaneous. All animals received 4000 IU HCG, intravenously, the third day after the second PMS injection and were artificially inseminated at this time and 24 hours later.

The injection of 1500 IU PMS on day-5 gave more desirable results than did the 1000 IU level. Of the 64 cows and heifers receiving the 1500 IU dose, on day-5 followed by 2000 IU on day-17, 48.4 percent had the desired 2 or 3 egg ovulation and only 21.9 percent ovulated four or more eggs.

Twenty-four of 46 heifers (52.2 percent) and 18 of 37 cows (48.6 percent) conceived to the inseminations. However, only 5 heifers and 4 cows were still pregnant after 90 days. The reason for the high rate of embryonic mortality could not be determined. However, all cows and heifers were laparotomized 11 days following inseminations.

## Introduction

A number of research studies have demonstrated that the incidence of multiple births in beef cattle can be increased by the injection of a

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variety of gonadotropins, including pregnant mare serum (PMS). The results of an earlier study involving PMS injections conducted at the Fort Reno Livestock Research Station were reported at the 1969 Feeder's Day (Miscellaneous Publication No. 82).

Pregnant mare serum is processed from the blood serum of pregnant mares. It is rich in a gonadotropic substance which has a similar physiological activity to that of follicle stimulating hormone (FSH). FSH is one of the gonadotropic hormones produced by the anterior pituitary gland, and its effect is to stimulate the growth of the follicle on the ovary and the maturation of the ovum (egg) which it contains. The gonadotropic substance of PMS is produced by structures in the uterus that are associated with the placenta and, for this reason, it is referred to as a placental gonadotropin.

A second type of placental gonadotropin is produced by pregnant women. It is formed in the chorion, one of the membranes of the placenta, and is excreted in the urine. This gonadotropic substance is extracted from the urine of pregnant women by pharmaceutical houses, processed and sold under the name human chorionic gonadotropin (HCG). This material has a physiological activity similar to that of the luteinizing hormone (LH) produced by the anterior pituitary. Its effect in the body is to cause rupture (ovulation) of the mature follicle in the ovary and the release of the egg contained in the follicle.

If a cow is exposed to above normal levels of FSH, either because of overproduction by the pituitary or as a result of it being injected, more than one follicle will develop. If these follicles are subjected to ample levels of LH all should ovulate. This is known as superovulation. PMS and HCG provide a means, therefore, for researchers to stimulate superovulation by hormone injections.

Almost without exception, researchers in this field have reported high death losses among triplet and larger litters, but a relatively low mortality rate among twins. Therefore, the determination of the proper dose level and sequence of treatments that will result in a high percentage of two egg ovulations is one of the requirements for developing a practical program for the induction of the multiple births in cattle.

In addition, it is important that the gonadotropin injections be timed rather precisely during the estrous cycle with reference to the occurrence of estrus. This means there is a high labor requirement in checking for estrus and then injecting the cows on an individual basis according to the day that estrus occurred. Thus, it appears that a practical program must also include some type of estrus synchronization which will cause most of the cows to be in estrus within a period of two or three days. If estrus can be synchronized to this extent, it should be possible to give the gonadotropin injection to all cows on the same day.

The objective of the research being reported here was to study the effect of a sequence of PMS and HCG injections, timed from a synchronized estrus, that would result in a high percentage of two egg ovulations in beef cows and heifers.

## Materials and Methods

This study included 37 non-lactating beef cows and 46 yearling beef heifers of Hereford, Angus, Hereford x Angus and Hereford x Angus x Holstein breeding maintained at the Fort Reno Livestock Research Station. The animals were randomly assigned to treatments on the basis of weight and breeding.

Estrus was synchronized by feeding 6-chloro- $\Delta^4$ -17 acetoxyprogesterone (CAP)<sup>1</sup>, individually, for 18 days, 10 mg per day the first 16 days and 5 mg per day at the last 2 days. CAP feeding was started in all cows at the same time without regard to stage of the estrous cycle. The PMS injections were given during the estrous cycle following CAP feeding.

Gonadotropin treatment consisted of two subcutaneous injections of PMS<sup>2</sup> and a single intravenous injection of HCG<sup>3</sup>. The PMS injections were given to all animals on days 5 and 17 of the estrous cycle as timed from the average date of synchronized estrus. The average date of synchronized estrus was day-3 post-CAP, therefore, the PMS injections were given on days 8 and 20 after last feeding of CAP. HCG was injected on the third day following the second PMS injection.

The occurrence of estrus was determined by the use of sterilized bulls running with the cows at all times. Ovulation rate was determined by counting the corpora lutea on the ovaries exposed by means of a laparotomy carried out 5 to 13 days after HCG administration. All breeding was by means of artificial insemination using frozen semen obtained from a commercial bull stud.

### Trial 1

Fifteen Hereford and 31 Angus heifers, weighing 580 to 800 lb. were employed. The heifers were on native pasture with milo fed at the rate of 2.0 lb. per day for the first phase of the trial, and milo increased to 10.0 lb. per day during the second phase.

In phase 1 a total of 30 heifers were synchronized by feeding CAP and treated with 1,500 IU PMS on day 5 and 2,000 IU PMS on day 17 of the post-CAP estrous cycle. All PMS treated heifers were injected with 4,000 IU HCG on day 3 post-PMS and were inseminated twice: at the

<sup>1</sup> Eli Lilly Company, Greenfield Laboratories, Greenfield, Indiana.

<sup>2</sup> Lyophilized powder containing 100 IU PMS/mg supplied by the Eli Lilly Company, Greenfield Laboratories, Greenfield, Indiana.

<sup>3</sup> Lyophilized powder containing 80 IU HCG/mg supplied by the Eli Lilly Company, Greenfield Laboratories, Greenfield, Indiana.

time of the HCG injections and 24 hours later. Sixteen heifers were also fed CAP, but were not treated with PMS and served as controls. The control heifers were not inseminated.

A high lumbar laparotomy was performed on all control heifers and on one-half of the PMS treated heifers 5 days following the first PMS injection to observe the effect of this injection on the ovaries. A second high lumbar laparotomy was performed on all heifers 11 days after the HCG injections and inseminations. Response to PMS was determined from numbers and location of corpora lutea and the location and size of follicles were recorded.

The second phase of the trial included all heifers of the control group and the 19 PMS treated heifers that had failed to conceive to inseminations following the PMS and HCG injections of phase 1. Each heifer received 1,500 and 2,000 IU PMS on days 5 and 17, respectively, timed from the estrus following the second laparotomy of the first phase. All heifers received 4,000 IU HCG on day 3 following the second PMS injection and were inseminated at the time of HCG administration and 24 hours later. A high lumbar laparotomy was performed 5 to 12 days following the HCG injections to determine response to PMS.

Heifers not returning to estrus were considered to have settled and this was confirmed by rectal palpation carried out 30, 60 and 90 days after insemination. All heifers, diagnosed pregnant at 30 days post-insemination, were slaughtered 90 days following insemination and the reproductive tracts were recovered.

## **Trial 2**

Twenty-one Hereford, three Angus and 13 Hereford x Angus cows, weighing from 870 to 1300 lb. were used. The cows were maintained on native pasture and were fed 5.0 lb. milo plus 2.0 lb. 43 percent cottonseed cake per day. During the CAP feeding period the daily feed was 2.0 lb. milo containing the daily dose of CAP.

All cows were fed CAP and the initial sequence of PMS injections were timed from the average date of post-CAP estrus. Eighteen cows received 1,500 IU PMS on day 5. All cows received 2,000 IU PMS on day 17 of the estrous cycle and 4,000 IU HCG on day 3 post-PMS. All cows were inseminated on the day of HCG injection and 24 hours later.

Cows that came back in estrus within 45 days post-insemination were retreated with PMS and inseminated in the same manner as described for the heifers that received a second sequence of injections in trial 1. Conception was determined as described for trial 1, except no palpations were made 30 days following inseminations after the second sequence of injections. A high lumbar laparotomy to determine effect of PMS on the ovaries was performed on all cows 5 to 12 days following the HCG injections.

## Results

### Trial 1

This trial, conducted from August, 1969 to February, 1970, was designed to study ovulation rates of heifers administered PMS following a synchronized estrus with HCG given on day 3 post-PMS.

Ovulation rates of the heifers following the initial (phase 1) and second sequence (phase 2) of PMS injections are presented in Table 1. The mean ovulation rate was 2.33 eggs with 40 percent of the heifers ovulating two eggs and 10 percent ovulating 3 eggs. Only 16.6 percent of the heifers ovulated more than 3 eggs but 33.4 percent failed to respond to PMS and had only a single ovulation (9 heifers) or no ovulation (1 heifer).

The heifers appeared to develop a refractoriness to PMS since only 51.6 percent of the heifers retreated with PMS gave a superovulatory response compared to 80.0 percent of the control group that were being treated for the first time (Table 1). The mean ovulation rates were 1.95 and 3.0 in the retreated and control groups, respectively.

Twenty-four of the 46 heifers (52.2 percent) in trial 1 conceived to insemination following PMS and HCG injections; 10/30 (33.3 percent) following the initial sequence of injections timed from post-PMS estrus, 7/19 (36.8 percent) of the heifers receiving the second sequence of PMS injections and 7/15 (46.7 percent) of the heifers which were used as controls during the initial phase of the trial.

Of the 24 heifers diagnosed to be pregnant 30 days after insemination, only 9 were pregnant at 60 days and only 5 were pregnant at 90 days post-insemination. Examination of the reproductive tracts from the 19 heifers, diagnosed pregnant 30 days post-insemination but which were

**Table 1. Ovulation Rates of Beef Heifers Treated with 1500 IU PMS on Day 5 and 2000 IU PMS on Day 17 of the Estrous Cycle and 4000 IU HCG on Day 3 Post-PMS (Trial 1)**

Treatment	No. heifers	No. of heifers with								
		This no. of corpora lutea/heifer								
		0	1	2	3	4	5	6	7	8
Received initial PMS sequence	30	1	9	12	3	1	0	4	0	0
Retreated with PMS— Received second PMS sequence	19	1	8	8	0	0	1	0	0	1
Controls—Received only second PMS sequence	15	1	2	4	1	5	1	0	1	0

not pregnant when slaughtered 90 days post-insemination confirmed that the heifers had been pregnant and had apparently resorbed the fetuses.

There was a higher conception rate in heifers with single ovulations, (47.3 percent) than in those with multiple ovulations (37.5 percent). However, there appeared to be no relationship between number of ovulations and maintenance of pregnancy since the five heifers pregnant at 90 days had each ovulated more than one ova. All fetuses appeared to be viable and there was no evidence of fetal resorption in the pregnant heifers. Three of the 5 pregnant heifers had multiple fetuses with the numbers of corpora lutea (CL) and numbers of fetuses as follows: 2 CL, 1 fetus; 3 CL, 1 fetus; 5 CL, 2 fetuses; 4 CL, 3 fetuses; 4 CL, 3 fetuses.

## Trial 2

This trial, conducted from October, 1969 to April, 1970, was designed to study the effects of two levels of PMS given at the first injection on the ovulation rates of cows.

The ovulation rates of the cows following the initial and second sequence of PMS injections are presented in table 2. Following the initial PMS and HCG treatment, mean ovulation rate was 2.22 and 2.37 for cows given 1,000 and 1,500 IU PMS, respectively, on day 5 of the estrous cycle. However, cows given 1,500 compared to 1,000 IU PMS on day 5 tended to have more two and three egg ovulations, 57.9 percent *vs.* 33.3 percent and fewer 4-plus egg ovulations, 10.5 percent *vs.* 22.2 percent.

Although a control group of cows was not available for comparison, the cows also appeared to be somewhat refractory to the second sequence of PMS and HCG injections (table 3). Only 37.0 percent of the cows gave a superovulatory response and the mean ovulation rate was 1.56 following the second sequence of injections, compared to 68.4 percent and

**Table 2. Ovulation Rates of Beef Cows Treated with 1000 or 1500 IU PMS on Day 5 and 2000 IU PMS on Day 17 of the Estrous Cycle and 4000 IU HCG on Day 3 Post-PMS (Trial 2).**

Treatment	Total treated	No. of heifers with									
		This no. of corpora lutea/cow									
		0	1	2	3	4	5	6	7	8	9
Initial PMS treatment <sup>1</sup>	18	1	7	4	2	3	0	0	1	0	0
Initial PMS treatment <sup>2</sup>	19	0	6	7	4	1	0	0	0	0	1
Retreated with PMS <sup>3</sup>	27	2	15	6	2	1	1	0	0	0	0

<sup>1</sup> Received 1,000 IU PMS on day 5, 2,000 IU on day 17.

<sup>2</sup> Received 1,500 IU PMS on day 5, 2,000 IU on day 17.

<sup>3</sup> Received 1,500 IU PMS on day 5, 2,000 IU on day 17.

2.37 following the initial treatment of PMS timed from post-CAP estrus.

Conception rate, based on non-return to estrus by 45 days post-insemination, was 48.6 percent. Nine of the 37 cows conceived following the first sequence of gonadotrophin injections and nine additional cows conceived following the second sequence of injections.

Rectal palpation 60 days post insemination of the nine cows that did not return to estrus following the initial sequence of gonadotrophin injections revealed that all cows had apparently been pregnant earlier but by 60 days the embryos had died and were being resorbed. A similar situation was found at 60 days post-insemination in five of the nine cows, which had not come back in heat within 60 days following the second sequence of injections. The four cows pregnant at 60 days post-insemination were also pregnant at 90 days post-insemination. All subsequently calved with one cow producing twins.

## Results and Discussion

The results obtained in this study suggests that PMS injections may be timed from a synchronized estrus. Of the 64 cows and heifers receiving 1,500 IU PMS on day 5 and 2,000 IU PMS on day 17, 48.4 percent had the desired 2 or 3 egg ovulation. Although 29.7 percent did not respond with more than one ovulation, it is encouraging that only 21.9 percent ovulated four or more eggs. From the standpoint of limiting births to no more than twins it is essential that the number of 4+ ovulations be reduced as much as possible.

The reduced superovulatory response to a second sequence of PMS injections was not unexpected. It has been reported by other researchers that cows develop a refractoriness to repeated PMS injections.

The reason for the low conception rate and high rate of embryonic mortality could not be determined in this study. It has been reported that rectal palpations early in pregnancy will cause abortion of as much as one-third of the pregnancies. Other researchers have also reported low conception and pregnancy rates in PMS treated heifers which were laparotomized following breeding. Both laparotomies and rectal palpations were carried out in this study. It will remain for future research to determine whether this low fertility is typical of PMS treatments following estrus synchronization, or whether it was the result of excessive handling of the tract as a result of the palpations and laparotomies.

Although there is no doubt that superovulation can be induced by hormone injections, this means of increasing multiple births in beef cattle is still strictly experimental. The research conducted to date has revealed a number of important problems which must be resolved by additional research if such treatments are to ever be made practical.