OVULATION RATE OF COWS AND HEIFERS TREATED WITH EQUINE GONADOTROPHIN (PMS) AND CHORIONIC GONADOTROPHIN (HCG)

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CHAPTER I

INTRODUCTION

Increasing the incidence of multiple births has the potential of greatly increasing the efficiency of commercial beef cattle production. It has been repeatedly demonstrated that the injection of gonadotrophic hormones at various levels and stages in the estrual cycle will stimulate multiple ovulations in cattle. Only limited success has generally followed attempts to obtain multiple births by hormone treatments. However, PMS treatments have been followed by multiple births in up to 28 percent of the cows treated and it has been demonstrated that the induction of multiple births can be accomplished, to a limited extent, under practical conditions.

There are numerous problems which must be solved before induced multiple births will be suitable for practical application. Although it has been demonstrated that exogenous gonadotrophins can be used to induce superovulation in cattle, most attempts to induce predominantly two and three egg ovulations have given poor or unpredictable results. The prenatal and postnatal fetal mortality is less in twin than in higher multiple pregnancies and births. Thus, the determination of a sequence of injections that will result in a high percent of two egg ovulations is one of the first requirements for developing a practical program for the induction of multiple births. In addition, the need to time the PMS injections rather precisely during the follicular phase of

the estrual cycle suggests that any practical program must involve the use of estrus synchronization prior to gonadotrophin treatment.

The objective of this study was to determine a practical sequence of gonadotrophin injections that would give a high percent of two and three egg ovulations in beef cows and heifers.

CHAPTER II

REVIEW OF LITERATURE

Frequency of Natural Twinning

The incidence of natural twin births is very low in all types of cattle. The average frequency for all breeds of dairy cattle is 1.88 percent compared to 0.44 percent for the beef breeds (Lush, 1945).

Variations in the tendency for twinning are inherited to some extent, but most studies have indicated that the heritability of natural twinning in cattle is too low to increase the frequency through selection (Lush, 1925; Lush, 1945; Erb et al., 1960). Analysis of 30 years calving data from 1905 Holstein cows revealed that parents which were from twin births transmitted no more twinning tendency than those singly born from the same ancestors (Erb et al., 1960). Meckling and Carter (1964) reported on an Angus herd which had been subjected to intense selection for twinning for almost 30 years. The 75 cow foundation herd consisted of twins, daughters of twins and cows that otherwise had twinning in their background. After almost 30 years of selection, the frequency of twinning in the selected herd was only 1.71 percent compared to 1.64 and 0.81 percent in the two Virginia beef cattle research herds which had not been selected for twinning.

Early Gonadotrophin Research

Smith and Engle (1927) and Zondek and Aschheim (1927) working

independently of each other first demonstrated the adenohypophysial-gonadal relationship. However, it was the classic work of Casida (1934), demonstrating that anterior pituitary extracts would induce ovulation in immature female rats and rabbits, which led to the widespread use of exogenous hormones for altering reproductive processes in mammals.

Casida et al. (1943) were the first to use exogenous gonadotrophic hormones to induce superovulation in the bovine. Ovulation was induced in calves and cows by subcutaneous injections of unfractionated beef or sheep pituitary extracts or by subcutaneous injections of follicle stimulating extracts followed by an intravenous injection of a luteinizing extract. Ovulation was more consistently induced when the unfractionated or follicle stimulating extracts were followed by an intravenous injection of luteinizing extract. Similar procedures, using various gonadotrophin preparations have been employed many times with varying degrees of success.

Equine and Chorionic Gonadotrophin

Equine Gonadotrophin

The most commonly used source of exogenous gonadotrophin for farm animals has been pregnant mare's serum (PMS). Gonadotrophic activity in the serum of pregnant mares was first described by Cole and Hart (1930). These workers assayed the gonadotrophic activity of the blood serum from mares during progressive stages of pregnancy by determining the effects on sexual maturity of immature rats.

PMS is secreted by the endometrial cups of the mare's uterus (Butt, 1967) and appears in the blood between the 40th and 180th days

of pregnancy (Rowlands, 1963). High concentrations are found in the blood between the 40th and 100th days of pregnancy with maximum concentrations between the 50th and 70th days (Schmidt-Elmerdorff, 1962; Cole and Hart, 1930).

PMS can be obtained by extraction from the blood or from the endometrial cups of the uterus (Cole, 1936; Butt, 1967). Different procedures have been used for extraction and the activity of the extract varies from 100 IU per milligram for freeze-dried preparations to 15,000 IU per milligram for extracts subjected to cellulose chromatography and gel filtration (Butt, 1967).

PMS is a high molecular weight, carbohydrate containing protein molecule. Its biologic properties are similar to a mixture of FSH and luteinizing hormone (LH), but it usually elicits predominantly FSH activity (Rowlands, 1964; Turner, 1966). The hormone has not been separated into its constituents; therefore, only bioassay procedures can be used for standardization of its biologic activity (Butt, 1967). The ovarian weight assay is used most often because of its simplicity, precision and sensitivity (Cartland and Nelson, 1938; Butt, 1967).

Human Chorionic Gonadotrophin

Aschheim and Zondek (1927) first described the presence of a gonadotrophin in the urine of pregnant women. This gonadotrophin, which has a biologic activity very similar to that of the luteinizing hormone, is commonly referred to as human chorionic gonadotrophin (HCG) (Turner, 1966).

HCG is a low molecular weight, glycoprotein hormone produced by the Langhans cells of the fetal chorion in women (Diczfalusy and Troen,

1961) and to a lesser extent by the placenta of other primates (Tullner and Hertz, 1966). The hormone reaches a peak concentration in the urine of pregnant women by about the 50th day and declines to a low level by the 90th day (Turner, 1966). HCG has been widely used as the criteria for diagnosing pregnancy in women in past years and has only recently been replaced by immunological assays (Butt, 1967).

The reason the placental gonadotrophin produced by women is found in the urine and that by mares in the blood is due to the differences in the size of the molecules. The intact PMS molecule is too large to pass through the renal corpuscle and cannot be filtered through the nephron while the HCG molecule is small enough to enter the renal tubules. Due to its larger molecular size and slower rate of metabolism, PMS stays in the blood and lymph for longer periods of time following injection of the hormone into recipient animals. Thus, PMS exerts a more prolonged gonadotrophic effect than HCG and purified extracts of FSH and LH (Cole, 1936; Turner, 1966). Cole, Guilbert and Goss (1932) discovered that PMS exerts a prolonged gonadotrophic effect when they demonstrated that the ovarian response in the rat to a single injection of PMS was similar to the response to a series of divided doses of the same quantity, provided the length from the first injection to necropsy was the same.

Superovulation for Ova Transplantation

Much of the interest in superovulation has been related to its importance for ova transplantation. Studies for this purpose have been conducted with the objective of obtaining a maximum number of mature ova capable of being fertilized and transplanted to a host animal.

Although the objectives were to obtain maximum superovulation, a consideration of the treatments and responses in these studies may be important in designing experiments to study procedures for inducing two and three egg ovulations.

Prepuberal Females

Superovulation of the prepuberal calf for ova transfer has the potential of decreasing the generation interval by allowing early progeny tests of potentially superior genetic females and producing a maximum number of offspring from the genetic superior individuals (Onuma et al., 1969).

Exogenous gonadotrophins have been used successfully to obtain high ovulation rates in calves, but fertilization of these ova have been very low. Jainudeen, Hafez and Lineweaver (1966) used 2000 IU of PMS intramuscularly followed by an intravenous injection of LH or HCG 5 days later to superovulate calves 4 to 24 weeks of age. The calves were inseminated 12 to 24 hours after the LH or HCG injection. Ovulation rates ranged from 1 to 50, but fertilized ova were recovered from only two of the 13 calves.

Black et al. (1953) injected sheep FSH or unfractionated anterior pituitary extracts subcutaneously and HCG intravenously to induce superovulation in calves weighing 90 to 180 pounds. Follicular stimulation was induced in 31 of the 32 calves, but only 50 percent of the calves ovulated. Fifty-five ova were recovered from 10 calves, but only seven ova, representing two individuals, were cleaved.

Onuma et al. (1969) found that a single injection of 2000 IU of PMS induced a significantly higher ($P < .05$) mean ovulation rate in

prepuberal calves than 50 mg of FSH, divided into five equal doses, 15.4 compared to 3.8, respectively.

A difference in ovarian response to exogenous gonadotrophins might be expected between calves and postpuberal females because of the variable endogenous steroid and gonadotrophin levels during the estrual cycle of postpuberal females. Avery, Fahning and Graham (1962) compared ovulation rates of cows and calves treated with purified swine FSH and LH. Mean ovulation rates for both groups were 28.13 with the calves producing an average of 12.46 more ovulations than the cows.

Postpuberal Females

The ovarian response to exogenous gonadotrophins and subsequent fertilization in the cow may be modified by the presence of a functional corpus luteum (Rowson, 1951; Willett, McShan and Meyer, 1952a; Hafez, Suzie and Gordon, 1963), by previous treatment with progesterone (McDonald, 1961; Avery et al., 1962; Lamond, 1964), or by simultaneous treatment with estrogen (Hafez, Suzie and Hung, 1963).

Rowson (1951) reported that 4500 IU of PMS failed to induce ovulation in the presence of a large corpus luteum, while a mean ovulation rate of 3.1 was obtained when the existing corpus luteum was expressed. Enucleation of the corpus luteum at the time of injecting 3000 IU of PMS on days 12 to 18 of the cycle increased the ovulation rate, but decreased conception (Hafez et al., 1963). However, Lamond (1964) reported that enucleation of the corpus luteum, prior to six daily injections of progesterone for synchronization of estrus, increased fertility following injection of 1000 or 2000 IU of PMS.

Cows synchronized with progesterone prior to receiving swine

FSH produced 7.89 more ovulations than those in the unsynchronized group (Avery et al., 1962).

Administration of 17 B-estradiol following injection of PMS increased ovulation rate while estradiol treatment prior to PMS decreased ovulation rate, compared to cows receiving PMS and no estradiol. The estradiol had no effect on fertility (Hafez, et al., 1963).

Most of the studies in superovulating the cow for maximum number of ova have been directed toward determining the stage of the cycle and the levels and sources of gonadotrophins which will give the most desirable results.

Dowling (1949) used 55 cows to study different methods for obtaining multiple ovulations for ova transplantation. A single injection of 3000 IU of PMS given during the follicular phase of the cycle gave higher ovulation and fertilization rates than when administered during the luteal phase. Similar findings were reported by Casida et al. (1943) and Willett, McShan and Meyer (1952b). Cows treated with PMS 4 or 5 days before the expected onset of estrus had an average of 12 corpora lutea and 44 percent of the recovered ova were fertilized. Cows receiving three consecutive daily doses of 100 mg of horse anterior pituitary extracts, with the initial injection on the 15th day of the cycle, had a similar ovulation rate but 92 percent of the recovered ova were cleaved (Dowling, 1949).

Avery et al. (1962) reported a mean of 21.36 ova produced by cows treated with a simultaneous injection of swine FSH and LH subcutaneously followed by an intravenous injection of 100 mg of LH. Twenty milligrams of FSH and 5 mg of LH were given on day 16 of the cycle followed by 10 mg of FSH and 5 mg of LH on days 17, 18 and 19. A

range of 4 to 55 corpora lutea was produced by this sequence of injections.

Scanlon, Sreenan and Gordon (1968) treated 85 heifers and four cows with 3000 IU of PMS on day 16 of the cycle to induce high superovulation. An average of nine ovulations was produced and 68 percent of the recovered ova were fertilized.

Superovulation and Superfetation

PMS has been the most commonly used hormone preparation for inducing superovulation in cattle; however, a variety of other FSH preparations have been used. From the standpoint of inducing superovulation and superfetation for practical application, PMS has the advantage that a single injection of PMS remains active much longer than other sources of FSH.

Willett and Buckner (1953) found that one injection of PMS on the 15th or 16th day of the cycle gave comparable ovulation rates to five daily injections of sheep or swine FSH. In this study, 2000 IU of PMS on the 16th day of the cycle gave predominantly two egg ovulations.

Comparisons of dose-response relationships among the different sources of gonadotrophins should be made with great caution because of the apparent amount of variation in biologic activity and different procedures for standardization of the hormone preparations. Within limits, there appears to be a linear relationship between dosage level of PMS or FSH and the superovulatory response (Gordon, Williams and Edwards, 1962; Bellows, Anderson and Short, 1969). However, Hammond and Bhattachanya (1944) reported no relationship between number of corpora lutea and dosages of 1000 to 5000 IU of PMS.

A single injection of 1000 to 3000 IU of PMS on the 15th to the 17th day of the estrous cycle has been used most often for inducing superovulation for superfetation. More two egg ovulations and a higher fertilization rate have been obtained with 1000 IU than with 2000 or 3000 IU of PMS (Peli and Castelli, 1962; Denny, 1964).

Hafez et al. (1964) treated 79 heifers with 1500 IU of PMS on day 17 of the cycle. Natural mating and insemination at the subsequent estrus resulted in a conception rate of 65 percent. Of the total treated, 11 percent had twin embryos and 13 percent had triplet, quadruplet or quintuplet embryos at 30 to 60 days gestation. A similar treatment of 19 cows produced two sets of twins, one set of quadruplets and one set of quintuplets with a 76.5 percent conception rate at the post-PMS estrus (Arbeiter, 1962).

Hafez, Jainudeen and Lindsay (1965) reported mean ovulation rates of 5.4 and 6.9 following administration of 1500 or 2000 IU of PMS, respectively, on day 16 of the estrual cycle. Schwartz and Shelby (1969) reported a similar, but somewhat lower, ovulation rate of 4.72 following a single injection of 1500 IU of PMS on day 16 of the cycle and Jainudeen and Hafez (1966) reported an average of 3.7 corpora lutea with this same level of PMS. A considerable lower ovulation rate of 1.71 was obtained by Gordon et al. (1962) when 1600 IU were given on day 16 of the cycle.

An extensive field trial, using a single injection of PMS to induce superovulation and superfetation, was conducted in Wales in 1959 to 1960 (Gordon et al., 1962). Hormone treatment consisted of a single subcutaneous injection of 800, 1000, 1200, 1600 or 2000 IU of PMS on day 16 or 17 of the cycle. Information on ovulation, conception

and pregnancy rates were obtained on 416 of the 525 cows treated. Estrus was observed in approximately 75 percent of the treated cows 4 to 6 days following PMS injection. Rectal palpation performed 6 weeks after breeding revealed that 76.2 percent conceived and 30.3 percent had multiple pregnancies. The percentage of multiple pregnancies at 6 weeks gestation increased with each increase in PMS level. There were no differences in conception in cows producing single or multiple ovulations. On the basis of total cows pregnant at 6 weeks gestation, 19.2 percent had twins, 8.8 percent had triplets and 1.9 percent had quadruplets. There were a total of 44 multiple births (35 sets of twins, eight sets of triplets and one set of quadruplets). Ovulation rate was determined by rectal palpation. Mean ovulation rates and ranges for 800, 1000, 1200, 1600 and 2000 IU of PMS, respectively, were: 1.43, 1 to 5; 1.77, 1 to 15; 2.5, 1 to 17; 2.17, 1 to 15; 3.97, 1 to 25. It was noted that cows with two egg ovulations usually had only one fetus at 6 weeks gestation and ovulations of more than 10 eggs rarely produced multiple pregnancies. In addition, cows with more than three fetuses at 6 weeks usually lost all of them prior to parturition. The best time for diagnosing multiple pregnancies was at 42 days gestation; however, 32.5 percent of the cows palpated resorbed or aborted the fetuses shortly after rectal examination. Attempts to control the number of embryos to two by rupturing the excess amniotic vesicles caused termination of the pregnancy. This procedure was performed rectally at 6 weeks gestation in 19 cows carrying three or four fetuses. Seventeen of the 19 cows expelled or resorbed all of their fetuses following the procedure.

Based on the results of this study by Gordon et al. (1962), 2000

IU of PMS was suggested as being most desirable for inducing multiple pregnancies. A single injection of PMS at levels above 2000 IU during the follicular phase of the cycle has usually resulted in overstimulation of the ovary (Dowling, 1949; Hafez and Ishibashi, 1964; Jainudeen et al., 1966; Scanlon et al., 1968).

Although Gordon et al. (1962) attained only limited success in inducing multiple births, the study indicated that the induction of multiple pregnancies can be accomplished under field conditions. They concluded that induction of multiple pregnancies would be more suitable for practical application, if a procedure for inducing only two fetuses could be determined.

Most attempts to induce multiple pregnancies have employed a single injection of PMS or a sequence of FSH injections during the follicular phase of the cycle. Such a procedure usually results in a higher number of ovulations and a greater range in ovulation rates than feasible for practical application.

A more promising regime to induce a high percent of two and three egg ovulations was reported by Schilling and Holm (1963). Cows were given 1000 to 1500 IU of PMS on day 5 followed by 2000 IU of PMS and enucleation of the corpus luteum on day 16 to 18 of the cycle. All cows exhibited estrus 3 to 5 days following the second PMS injection and were given 4000 IU of LH intravenously. Of the 11 cows receiving this treatment, two failed to ovulate, one ovulated one ovum, four ovulated two ova and four ovulated three ova. Fertility was not determined in this study. The reason these workers chose this sequence of injections was based on the following rationale. It was assumed that several follicles begin development during or immediately after estrus,

with the one destined to ovulate continuing to grow, while the others become atretic. The purpose of the first subovulatory injection was to prevent the atresia that normally occurs with all but one of several follicles stimulated during or just after the previous estrus. The second injection would presumably provide sufficient additional gonadotrophic stimulation to induce maturation of these follicles, thus producing more than one ovulation. The findings of Choudary, Gier and Marion (1968) and Marion, Gier and Choudary (1968) that the growth rate of follicles from a diameter of 1 to 12 mm is constant and independent of cyclic hormonal stimulation during the luteal phase of the estrual cycle would not support this hypothesis.

A sequence of injections similar to that of Schilling and Holm (1963) has been used by others in an attempt to induce limited superovulation (Hafez et al., 1965; Turman, Renbarger and Stephens, 1968; Schwartz and Shelby, 1969).

Hafez et al. (1965) reported a mean ovulation rate of 4.8 following injection of 750 IU of PMS on day 5 and 1500 IU of PMS on day 16 of the cycle and a mean of 6.0 ovulations when the second injection was increased to 2000 IU. A sequence of two PMS injections resulted in lower ovulation rates than a single injection of the same level of PMS given on day 16 of the cycle. The PMS treatments, mean ovulation rates and ranges of ovulation rates were: (1) 1500 IU on day 16: 5.3, 2 to 9; (2) 750 IU on day 5 plus 1500 IU on day 16: 4.8, 1 to 12; (3) 2000 IU on day 16: 10.0, 2 to 14; (4) 750 IU on day 5 plus 2000 IU on day 16: 6.0, 2 to 8. These workers did not find that an injection of LH on day of estrus was necessary or that it was necessary to enucleate the corpus luteum in order to obtain a superovulatory response.

A similar ovulation rate was obtained by Schwartz and Shelby (1969) when 1500 IU of PMS were injected on day 5 and 2000 IU of PMS on day 16 followed by 1000 IU of HCG on day of estrus. An average of 6.56 corpora lutea were produced from this sequence of injections with a range from 0 to 16.

The highest percentage of induced multiple births reported in the literature was obtained by a sequence of two subcutaneous injections of PMS followed by a single intravenous injection of HCG on day of estrus (Turman et al., 1968). Twenty-three multiple births (12 sets of twins, eight sets of triplets, two sets of quadruplets and one set of quintuplets) were produced from the treatment of 81 beef cows. The cows were treated with 1500 IU of PMS on either day 3, 4, 5 or 6 and 2000 IU of PMS on either day 16, 17 or 18 of the cycle. On day of estrus, the cows were bred naturally and given 2500 IU of HCG intravenously. Conception rate at first post-PMS estrus was 64.2 percent. Ovulation rate was not determined in this study and all cows were allowed to calve. There were 24 twins, 12 triplet, 3 quadruplet and 2 quintuplet live calves from the 23 multiple births. Multiple births occurred in 46.7%, 46.7% and 53.8% of cows injected first on day 4, 5 or 6, respectively, and in 55.5%, 52.9% and 30.8% of those injected on day 16, 17 or 18, respectively. One set of twins and one set of triplets were produced from the treatment of seven cows which received the first PMS injection on day 3 of the cycle.

Prior to this experiment these workers had conducted a preliminary study using a similar sequence of injections (Turman et al., unpublished data). Six of eight cows conceived at first post-PMS estrus and three produced twin calves. Four of eight heifers similarly treated

and slaughtered at 45 days postbreeding had two or more embryos.

Gonadotrophin Injections Following Estrus Synchronization

Estrus detection for proper timing of the gonadotrophin injections and individual treatment of the cows requires considerable labor and expense. Since a rather precise timing of the injections during the estrual cycle is required to obtain desirable results, the successful combination of gonadotrophin injections with estrus synchronization would make the induction of multiple births more suitable for practical application. The use of oral progestogens for synchronizing estrus without gonadotrophin has produced promising results in recent years (Parker et al., 1965; Hansel et al., 1966; Turman et al., 1967; Wiltbank and Kasson, 1968).

Lamond (1964) reported low fertility in heifers treated with six daily injections of progesterone prior to a single injection of PMS. Decreased fertility has also been reported in heifers fed MAP prior to receiving PMS (Jainudeen and Hafez, 1966). Conception at post-PMS estrus following MAP feeding was 14 percent compared to 57 percent in the unsynchronized group. Although fertility was decreased, a satisfactory ovulatory response was obtained by feeding 180 mg of MAP for 18 days prior to a single intramuscular injection of PMS. Mean ovulation rates for 1000, 1500 and 3000 IU of PMS were 2.8, 4.4 and 6.2, respectively. PMS was administered 24 hours following the last MAP feeding.

Lamond and Hill (1969) synchronized estrus by feeding 0.5 mg of MGA for 18 days. A single injection of 3500 IU of PMS on the 14th day of MGA feeding and mating at the first posttreatment estrus gave an

average of 6.7 corpora lutea and an average fertilization rate of 95 percent for the recovered ova.

A series of FSH injections in combination with MGA (Bellows, 1969; Reynolds et al., 1969) or in combination with norethandrolone (Vincent and Mills, 1970) have given some promising results for inducing limited superovulation and superfetation.

The most desirable results obtained by Bellows et al. (1969) was with a total of 6.25 mg of porcine FSH administered in equal doses two times daily for five successive days, beginning on the eighth day of MAP feeding. MAP was fed for 11 days with the first day of MAP feeding designated as day 1. A single intramuscular injection of 5 mg of estradiol valerate was given on the second day of MAP feeding to induce regression of the corpus luteum (Wiltbank and Kasson, 1968). Average ovulation rate, range of ovulation rate and fertility in this group of heifers was 2.1, 1 to 4 and 94 percent, respectively. FSH treatment levels were total dosages of 3.12, 6.25, 12.50, 25.00, 50.00 or 75.00 mg. A single injection of 75 mg of FSH given on day 10 resulted in a small increase in follicular development and no multiple ovulations. This same level administered two times daily on days 9, 10 and 11 gave an average ovulation rate of 1.3 with a range of one to two. Ovarian response was linear from 6.25 to 25 mg of FSH given two times daily on days 8, 9, 10, 11 and 12; however, the response seemed to plateau at 25 mg.

Pregnancy rate at 30 days postbreeding has been determined in cows treated with MAP, estradiol valerate and 6.25 mg of FSH, administered two times daily on days 8, 9, 10, 11 and 12 with an 11 day MAP feeding period (Reynolds et al., 1969). Fifty-seven percent of the cows were

pregnant and three of 15 had twin embryos.

Vincent and Mills (1970) used various levels and different injection sequences of porcine FSH in combination with norethandrolone, a progestogen, for inducing multiple ovulations in beef cattle. In two of the trials, norethandrolone was injected intramuscularly at the rate of 5 mg daily for 4 days beginning on the 14th to the 16th day of the cycle. FSH dosages from 6.25 to 15 mg were divided into five or 10 equal dosages (once or twice daily) and given intramuscularly for 5 days beginning at the first norethandrolone injection. In two other trials, estrus was synchronized with 10 daily injections of 5 mg of norethandrolone and a single injection of 5 mg of estradiol valerate on the second day of the norethandrolone injections. FSH or Vetrophin (FSH-LH activity) in saline or CMC (1% sodium carboxymethyl cellulose) was given as a single injection on the last day of norethandrolone treatment. Ninty-three FSH treated cows produced 10 sets of twins and two sets of triplets. Fifty-one percent of the FSH treated cows had multiple ovulations and average conception was 56 percent compared to 46 and 73 percent in norethandrolone and control cows, respectively. Mean ovulation rate, determined by rectal palpation, ranged from 1.4 to 3.0 for the various levels and injection sequences of FSH. FSH and Vetrophin produced a similar ovarian response.

Effect of Body Weight, Season and Interval from the Exogenous Gonadotrophin to Subsequent Estrus on Ovarian Response

Numerous reports have indicated that there is no relationship between body weight and ovarian response to exogenous gonadotrophins

(Gordon et al., 1962; Turman et al., 1968; Bellows et al., 1969; Vincent and Mills, 1970).

Gordon et al. (1962) reported no differences in ovulation rates and multiple births among seasons of the year, but lower fertility and increased embryonic mortality have been reported in cows bred during the hot summer months (Stott and Williams, 1962).

Time from injection of the gonadotrophin to the subsequent estrus will affect the superovulatory response (Hammond, 1949; Brock and Rowson, 1952; Gordon et al., 1962; Hafez et al., 1963; Scanlon et al., 1968; Turman et al., 1968). An interval of 2 or 3 days between injection of PMS or FSH and subsequent estrus has resulted in a more restricted level of superovulation than longer intervals (Hammond, 1949; Brock and Rowson, 1952; Hafez et al., 1963.

Brock and Rowson (1952) reported that the percentage of ovulations increased directly with the delay from the PMS injection to subsequent estrus. Cows which exhibited estrus 2 days after receiving 3000 IU of PMS had an average ovulation rate of 2.5 compared to 15 for cows exhibiting estrus 7 days after PMS. Injection of LH intravenously 4 days post-PMS gave a mean ovulation rate of 8.7 compared to 14.3 when HCG was given 7 days post-PMS.

Scanlon et al. (1968) found that the mean ovulation rate increased from 2.0 to 12.7 as the interval between 3000 IU of PMS and subsequent estrus increased from 2 to 5 days.

Turman et al. (1968) reported a higher frequency of multiple births in cows when estrus occurred 3 to 5 days following the second PMS injection, which was given on day 16, 17 or 18 of the cycle.

Graves and Dzuik (1968) found that ovulation had occurred in

dairy cows within 40 hours after receiving HCG, although the cows had not exhibited estrus.

Although it has been suggested that the level of LH production in the cow is sufficient to induce ovulation of more than one follicle (Hammond, 1955; Gordon et al., 1962; Hafez et al., 1965), these findings would suggest that administering a source of LH intravenously 2 to 3 days after PMS or FSH could be used to restrict the number of multiple ovulations.

Refractoriness to Exogenous Gonadotrophins

Induction of multiple ovulations more than once during a breeding season and more than one year in the life of a cow is an important consideration in the practical application of induced multiple births. Cows exhibit refractoriness to repeated injections of PMS and other sources of FSH, but a limited superovulatory response can usually be obtained.

The degree of refractoriness may depend on the interval between the gonadotrophin injections. Hafez et al. (1964) reported no superovulatory response to 2000 IU of PMS given during the second estrual cycle following initial treatment with 1500 IU of PMS. Jainudeen et al. (1966) also reported complete ovarian refractoriness following treatment with 1500 to 2000 IU of PMS when the injections were repeated at 18 to 40 day intervals. However, Jainudeen et al. (1966) found no ovarian refractoriness to PMS when cows were retreated with 3000 IU of PMS 170 to 206 days following initial treatment with 1500 or 2000 IU of PMS. Gordon et al. (1962) reported that PMS treatment during one year did not affect the superovulatory response during the follow-

ing year. Contrary to these findings, Willett and Buckner (1953) found that long periods without administering gonadotrophins failed to eliminate the refractoriness. However, an increased dosage during successive treatments partially overcame the refractoriness.

The type and purity of the gonadotrophin may affect ovarian refractoriness to exogenous gonadotrophins. Successive injections of sheep and swine pituitary extracts failed to produce a superovulatory response in dairy cows (Willett, et al., 1952a), but repeated injections of PMS gave decreasing, but some superovulatory response in successive estrual cycles (Willett and Buckner, 1952). Onuma et al. (1969) found that the superovulatory response in calves, exhibiting refractoriness to repeated injections of FSH, could be increased five fold with the use of PMS following FSH. Repeated injections of purified swine FSH gave a decreased superovulatory response in cows retreated during the same breeding season (Vincent and Mills, 1970).

The literature indicates that the determination of a sequence of gonadotrophin injections that will result in a high percent of two egg ovulations is one of the requirements for developing a practical program suitable for the induction of multiple births.

CHAPTER III

GENERAL PROCEDURE

This study was conducted in four consecutive trials from November, 1968 to April, 1970 at the Fort Reno Livestock Research Station, El Reno, Oklahoma. The experimental animals included a total of 80 beef heifers and 55 beef cows. Since they were of different ages and breeds and were maintained under different conditions in each trial, the animals and their management will be described in the respective trials. Likewise, since the treatments of each successive trial were determined, to a large extent, by the results of the previous trial, the methods and procedures that were unique to each trial will be discussed with the trial. However, a number of factors were common to all trials and will be discussed in this section.

The PMS, HCG and 6-Chloro-Δ^6-17-acetoxyprogesterone (CAP) were supplied by the Eli Lilly Company, Greenfield, Indiana. The gonadotrophins were lyophilized, standardized preparations. Standardization was carried out by the Eli Lilly Company prior to shipment. The biologic activity of the PMS was 100 IU per milligram, and the HCG was 80 IU per milligram.

Precautions were taken to protect the PMS and HCG from moisture and heat prior to time of injection. The stock bottles, containing the lyophilized materials, were sealed with tape and stored in the refrigerator in a cotton lined, sealed container. Prior to usage,

the proper dosage of PMS and HCG were weighed on Mettler scales (Type B balance), placed in 15 ml test tubes, stoppered and stored in the refrigerator of the Physiology laboratory. The test tubes containing the PMS and HCG were transported in a styrofoam cooler from the laboratory in Stillwater to the Fort Reno Research Station.

The PMS was reconstituted with 10 ml of double distilled water, and the HCG with 10 ml of physiological, isotonic (0.9%) sodium chloride solution immediately prior to injection. All PMS injections were made subcutaneously in the girth region of the animal approximately 15 to 20 inches from the midline of the back while HCG injections were intravenously via the jugular vein.

Estrus was synchronized by feeding CAP for 18 days, 10 mg per day the first 16 days and 5 mg per day the last 2 days. CAP feeding was initiated without regard to stage of estrous and all animals in a given treatment group were fed during the same time period.

The CAP was in a soybean meal premix and 4.54 grams of the premix contained 10 mg of CAP. The daily dosage of CAP for each animal was placed into individual plastic containers. The animals in the estrus synchronized groups were fed in individual stalls once daily at approximately the same time each day. Each animal's daily allottment of CAP was mixed with 1/4 to 1/2 pound of milo.

Artificial insemination of the heifers in Trial III and the cows in Trial IV was made with semen from two bulls of proven fertility supplied by the Codding-Noba Laboratory, Foraker, Oklahoma. The semen was processed by standard quality control procedures used by artificial insemination bull studs and was stored in liquid nitrogen prior to being used. Inseminations with semen from the two bulls were distri-

buted about equally among the different treatment groups in each trial. The semen was thawed in ice water for approximately 20 minutes before the inseminations. It was deposited into the body of the uterus, except in a few heifers in which the breeding tube could not be passed more than one-half the way through the cervix. A second insemination was made if the semen was not deposited in the uterus or at least two-thirds through the cervical canal.

Estrual behavior in all trials was determined by the use of vasectomized bulls, which ran with the animals at all times, and by visual observation. Visual observations were made twice daily, early morning and late afternoon, throughout each trial and three times daily during the more critical periods, such as, the dates when the animals were due to return to estrus. Almost constant observation for estrus was made for three to four days following withdrawal of the progestogen.

In the non-synchronized groups, the PMS injections were given on day 5 and day 17 of the estrual cycle with day of estrus counted as day 0. In the synchronized groups, average date of estrus following withdrawal of the CAP was counted as day 0 for all animals in the group and the PMS injections were given on day 5 and day 17 during the estrual cycle following CAP feeding.

Ovulation rate was determined by counting the number of corpora lutea 5 to 13 days after administering HCG. This was done in Trial I by means of a vaginal laparotomy, in Trial II by slaughtering the heifers and in Trials III and IV by means of a high lumbar laparotomy. The presence of luteinized follicles was noted, but only luteal tissue with a definite corpus luteum structure was counted as a corpus luteum.

The Chi Square procedure was used for statistical analysis of the ovulation rate data (Steel and Torrie, 1960). Simple correlations and analysis of variance were determined as described by Steel and Torrie (1960).

CHAPTER IV

TRIAL I

Materials and Methods

This trial was conducted from November, 1968 to March, 1969. Sixteen multiparous Hereford and two Angus cows, which had failed to rebreed during a previous 90 day breeding season, were used in this study. None of the cows had received PMS or other hormones previously. All cows were observed for estrus at least two estrual cycles prior to treatment. Following the third or fourth estrus, the ovaries were rectally palpated immediately prior to or on the day of expected estrus to determine if a large follicle was present. The ovaries were then palpated 1 to 3 days following estrus to confirm that ovulation had occurred. Only cows exhibiting 16 to 24 day estrual cycles and ovulating at the time of estrus were included in the study.

The cows were maintained on native pasture and were fed 2 pounds of 43% cottonseed cake and 5 pounds of ground milo per day. All cows had been receiving the cake and milo a minimum of 50 days prior to the PMS injections.

The gonadotrophin injections were timed from a non-synchronized estrus. All cows were given 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the cycle followed by 2500 IU of HCG either on day of estrus or on the third day following second PMS (20th day of the cycle), disregarding the occurrence of estrus. Individual cows were

designated to receive HCG on day of estrus or on the third day following second PMS at the time of the first PMS injection by an alternating procedure. All cows scheduled to receive HCG on day of estrus, but which were not observed in estrus by the seventh day following second PMS, were given HCG on the seventh day (24th day of the cycle).

The ovaries were rectally palpated in cows which showed signs of estrus following the first PMS injection to determine if ovulation or extreme follicular development had occurred.

Number of corpora lutea were determined by performing a vaginal laparotomy 5 to 10 days following the HCG injection. A laparotomy technique described by Hansel (personal communication) was used in this trial. A similar procedure has been briefly described by Frank (1947), MacDonald (1952), Casida (1960) and others. However, a detailed description of the procedure and postoperative care used in this trial has not been reported.

An incision was made through the left and right lateral portions of the vaginal musculature and the peritoneum 2 to 3 inches posterior to the cervix. The vagina muscle was first scored with a scapel blade, which was mounted in a cork. The scapel blade was withdrawn from the lumen of the vagina and an opening was made through the vagina muscle to the peritoneum by blunt dissection of the muscles with the finger. The scapel blade was then used to make an incision through the peritoneum and the opening into the body cavity was enlarged to about 2 inches in diameter by blunt dissection with the finger and thumb. The lumen of the vagina was flooded with physiological saline after the incisions were made to remove all blood from the area, although very little hemorrhage was encountered.

Each ovary was pushed into the lumen of the vagina by manipulation with one hand in the rectum and one hand in the vagina. After the ovary was forced into the vagina, it was held with one hand and observed through a speculum inserted into the vagina. The ovaries were washed thoroughly with physiological saline before and after observation and were pushed back into the body cavity immediately following observation.

No type of anesthesia was administered prior to performing the laparotomy, but 10 ml of a two percent procaine hydrochloride solution were injected into the vagina musculature following the laparotomy to aid in preventing intestinal prolapse. Twenty milliliters of penicillin-streptomycin antibiotic (Penstrep) were administered intramuscularly at the time of surgery and on the second and third days postsurgery.

The incisions were permitted to heal without sutures or further attention. The reproductive organs were manipulated by rectal palpation on the second and fourth days after laparotomy to prevent adhesions of the organs. The vaginal incisions were examined through a speculum for signs of infection and hemorrhage 3, 7 and 14 days following the laparotomy and the incisions were evident at 14 days only by a small amount of fibrous tissue. The laparotomy had no apparent effect on the reproductive activity, as evidenced by the fact that all cows returned to estrus during the subsequent cycle.

Results and Discussion

This trial was initiated to determine ovulation rate following treatment with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17

of the estrual cycle with 2500 IU of HCG given on day of estrus or on the third day following the second PMS injection.

Ovulation rates for all cows in the trial are presented in Table I. Since the objective of this study was to determine a sequence of gonadotrophin injections that would induce a maximum number of two or three egg ovulations, the tabular results of the ovulation rates are presented as the number of cows with each number of corpora lutea, rather than average ovulation rates. Nine (50%) of the cows had either two or three corpora lutea, six had more than three and only one cow had a single corpus luteum. Mean ovulation rate was 3.38 for all cows that ovulated.

TABLE I

OVULATION RATES OF COWS TREATED WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 OF THE ESTRUAL CYCLE AND WITH 2500 IU OF HCG ON DAY OF ESTRUS OR DAY 3 OR 7 POST-PMS (TRIAL I)

Post-PMS HCG Treatment	Treated	Number of Cows with No. of Corpora Lutea/Cow 1	2	3	4	5	6	Cystic Ovaries
Day of Estrus	8	0	0	4	2	1	0	1
7th Day	2	0	0	1	0	0	1	0
3rd Day	8	1	3	1	0	2	0	1
Total	18	1	3	6	2	3	1	2

The cows that received HCG on the seventh day post-PMS were included with the group receiving HCG on day of estrus for treatment comparisons, because they were due to receive HCG on day of estrus.

There were no statistically significant differences ($P < .01$) in ovulation rates between cows receiving HCG on day of estrus and those receiving HCG on the third day post-PMS. However, all cows that received HCG on day of estrus and ovulated had three or more corpora lutea, while three of the eight cows that received HCG on the third day had two corpora lutea. This suggests that the injection of HCG on day 3 post-PMS may result in more two egg and fewer three or more egg ovulations. Average ovulation rates were 2.86 and 3.78, respectively, for cows receiving HCG on the third day or on day of estrus post-PMS. This would agree with earlier findings that a shorter interval between the injection of PMS or FSH and subsequent estrus will result in a more restricted level of superovulation than longer intervals (Hammond, 1949; Brock and Rowson, 1952; Hafez et al., 1963; Scanlon et al., 1968).

In the group of cows given HCG on day of estrus, the number of days from second PMS to estrus and the number of cows for each were: 1 day, 1; 3 days, 4; 4 days, 1; 5 days, 1; 6 days, 1. Two cows developed cystic follicles on both ovaries following hormone treatment and two cows exhibited estrus following the first PMS injection. One of the cows that developed cystic follicles received HCG on day of estrus, which occurred 3 days after second PMS. This cow had not exhibited estrus during the PMS treated cycle. The other cow with cystic follicles exhibited estrus 4 days following the first PMS and received HCG on the third day after second PMS. Neither cow was laparotomized

because of the extreme size (approximately 3 inch diameter) of the ovaries. Twenty-one days following HCG, the ovaries of both cows had returned to near normal size. The other cow, which had exhibited estrus during the PMS treated cycle, received HCG on day 7 following second PMS and ovulated six ova. Neither cow, which exhibited estrus following first PMS, ovulated at that time.

The results of this trial indicated that a satisfactory ovulation rate can be obtained when HCG is given on the third day following second PMS, disregarding the occurrence of estrus.

CHAPTER V

TRIAL II

Materials and Methods

This trial was conducted in June and July, 1969. Thirty-four nulliparous, yearling beef heifers ranging in weight from 625 to 873 pounds were employed in this study. The breeds represented were Hereford, Angus and Hereford-Angus-Holstein crossbreds. The heifers had been wintered on wheat pasture and were observed in estrus at least once prior to the experiment. They were confined to a feedlot on full-feed prior to and during the experiment and were randomly allotted to the treatments on the basis of weight and breed.

The treatments for all heifers in the trial are shown in Table II. All heifers received 1500 and 2000 IU of PMS and 2500 IU of HCG. PMS injections for the 17 non-synchronized heifers were timed from day of estrus for each heifer. The PMS injections were initiated in the non-synchronized group on the last day of CAP feeding in the synchronized group. Eight heifers received HCG on day of estrus following second PMS and nine received HCG on the third day following second PMS, disregarding the occurrence of estrus.

Seventeen of the heifers were synchronized by feeding CAP. In addition to determining average date of estrus following withdrawal of the progestogen, average date of ovulation was determined by rectal palpation of the ovaries. Average day of estrus was the third day

following the last day of CAP feeding. Thus, the PMS injections were given to all animals in this group on day 8 and day 20 post-CAP (approximately day 5 and day 17 of the cycle). Nine heifers received HCG on day of estrus and eight on the third day following second PMS.

TABLE II

TREATMENT AND NUMBER OF HEIFERS IN TRIAL II

Treatment		No. Heifers
Non-synchronized:		17
1500 IU PMS[a] day 5 and 2000 IU PMS day 17 post-estrus	2500 IU HCG[b] day of estrus post-PMS	8
	2500 IU HCG[b] day 3 post-PMS	9
Synchronized:[c]		17
1500 IU PMS[a] day 8 and 2000 IU PMS day 20 post-CAP	2500 IU HCG[b] day of estrus post-PMS	9
	2500 IU HCG[b] day 3 post-PMS	8

[a] PMS injected subcutaneously.

[b] HCG injected intravenously.

[c] Fed CAP for 18 days.

Heifers in both the non-synchronized and synchronized groups were randomly assigned to receive HCG on day of estrus or on the third day post-PMS at the beginning of the trial. Heifers in both groups due to receive HCG on day of estrus, but which were not observed in estrus by the seventh day following second PMS, were given HCG on the seventh day.

During the CAP feeding period, the heifers in the synchronized group were fed individually each morning. After the CAP was eaten, a full feeding of the concentrate ration was put into the self-feeders. The feed was removed each night and the total amount weighed and the heifers in the non-synchronized group were group fed the average amount comsumed by the heifers in the synchronized group. Following the CAP feeding period, all heifers were returned to the large feedlot pens.

Ovaries of all heifers were rectally palpated 3 days before and 3 days following the first and second PMS injections to determine the immediate effects of the PMS injections on follicular activity.

All heifers were slaughtered and the reproductive tracts recovered 5 to 13 days following HCG administration. The number of corpora lutea, number and size of follicles and weight of the ovaries and corpora lutea were determined.

The analysis of variance method was used for analysis of the ovarian and corpora lutea weight data. Sum of squares for synchronization adjusted for time of HCG and time of HCG adjusted for synchronization were determined by the Abbreviated Doolittle procedure (Steel and Torrie, 1960).

Results and Discussion

This trial was initiated to determine ovulation rates following treatment with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle followed by 2500 IU of HCG on day of estrus or 3 days post-PMS with the PMS injections timed from either a natural or a synchronized estrus. The PMS injections in the synchronized groups were given during the cycle following CAP feeding because Jainudeen and Hafez (1966), Turman et al. (1967), and others have reported higher conception rates at the second estrus following withdrawal of oral progestogens than at the estrus immediately following withdrawal of the progestogen.

Ovulation rates for all heifers in Trial II are shown in Table III. Average ovulation rate was 1.77 for all treatments.

No apparent differences in ovulation rates were noted between heifers given PMS timed from a non-synchronized or a synchronized estrus. Six of the 17 (35%) heifers in the synchronized group had two corpora lutea compared to five of 17 (29%) in the non-synchronized group. The numbers of heifers in the synchronized and non-synchronized groups with either two or three corpora lutea were eight (47%) and five (29%), respectively. The numbers of corpora lutea of heifers in the two groups are compared graphically in Figure 1.

Although the differences between ovulation rates of heifers given HCG on the third day or on day of estrus post-PMS were not statistically significant ($P > .10$), there was some evidence ($\chi^2 = 4.0$) that HCG, given on the third day, induced more two egg ovulations. Eight of the 17 (47%) heifers in the group given HCG 3 days post-PMS had two corpora lutea compared to three of 17 (18%) in the group that received HCG on

day of estrus (Figure 2). In addition, only one heifer (6%) given HCG on the third day had more than two corpora lutea, while four (24%) heifers given HCG on day of estrus ovulated more than two ova. This supports the hypothesis based on observations made in Trial I that HCG injected on day 3 post-PMS increases the proportion of two egg ovulations.

TABLE III

OVULATION RATES OF HEIFERS TREATED WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 OF THE ESTRUAL CYCLE AND 2500 IU OF HCG ON DAY OF ESTRUS OR DAY 3 POST-PMS (TRIAL II)

Treatment	Number of Heifers					
	Total Treated	No. of Corpora Lutea/Heifer				
		1	2	3	4	5
Non-Synchronized:[a]						
HCG-Day of Estrus Post-PMS	8	6	1	0	1	0
HCG-3rd Day Post-PMS	9	4	4	0	1	0
Synchronized:[b]						
HCG-Day of Estrus Post-PMS	9	4	2	2	0	1
HCG-3rd Day Post-PMS	8	4	4	0	0	0

[a] PMS injections were timed from a non-synchronized estrus.

[b] PMS injections were timed from average day of post-CAP estrus.

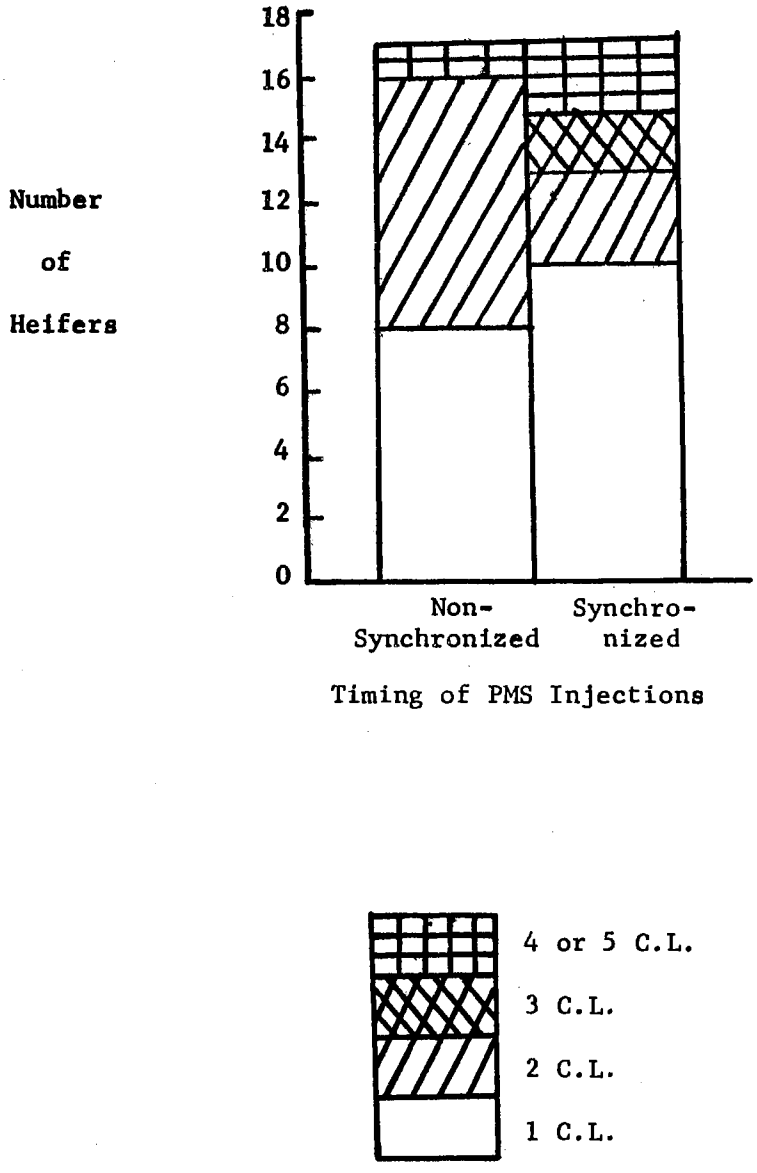

Figure 1. Number of Heifers with Each Number of Corpora Lutea Following PMS Injections, 1500 and 2000 IU on Day 5 and 17 of the Estrual Cycle, Timed from a Non-Synchronized or an Estrus Synchronized by Feeding CAP, with 2500 IU of HCG Injected on Day of Estrus or Day 3 Post-PMS

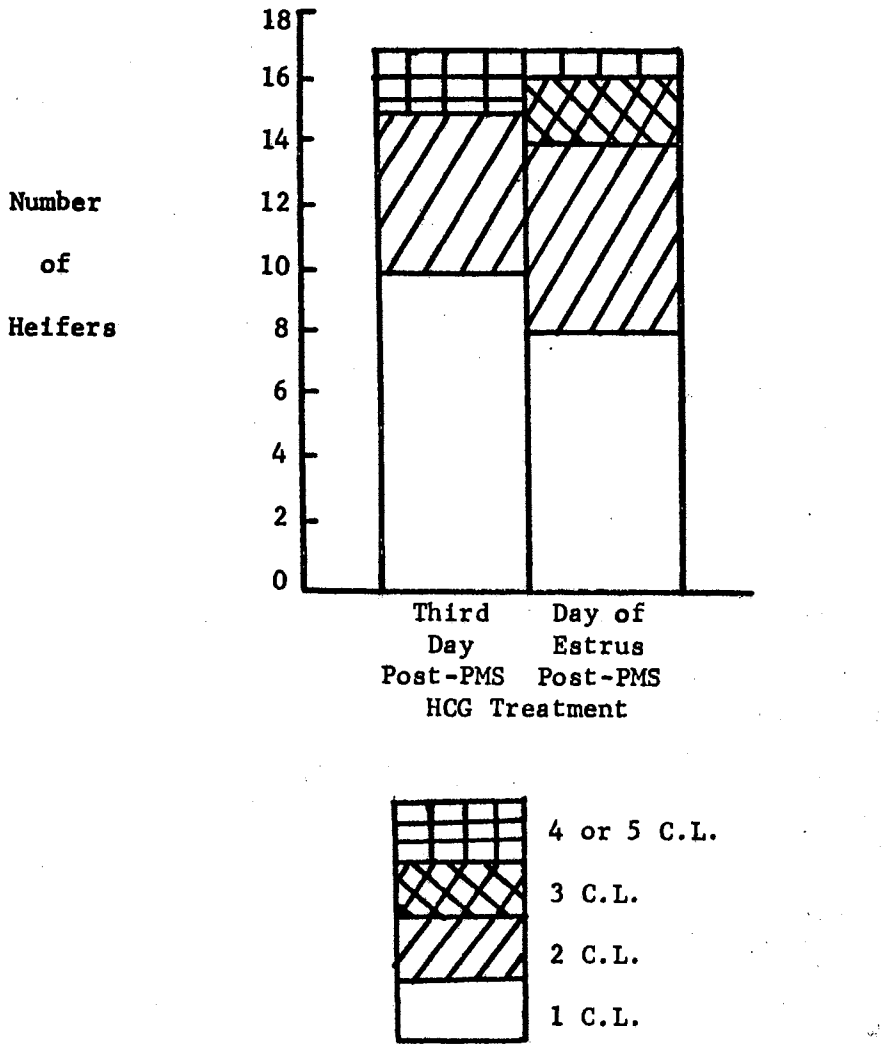

Figure 2. Number of Heifers with Each Number of Corpora Lutea Following 2500 IU of HCG Injected on the Third Day or on Day of Estrus Following a Sequence of Two PMS Injections, 1500 IU on Day 5 and 2000 IU on Day 17 of the Estrual Cycle

Average ovulation rate was 1.65 in the group given HCG 3 days post-PMS and 1.82 in the group given HCG on day of estrus post-PMS. By comparing these values with the proportion of two egg ovulations, it can be seen that average ovulation rate does not accurately reflect the number of two egg ovulations. The group given HCG on the third day had 29 percent more two egg ovulations and 18 percent fewer three or more egg ovulations than the group given HCG on day of estrus post-PMS.

Average interval from second PMS to the occurrence of estrus was 4 days in the group given HCG on day of estrus. Six of the 17 heifers given HCG on day of estrus, received HCG 4 or more days following second PMS. The number of days from second PMS to HCG and the number of heifers for each were: 2 days, 1; 3 days, 10; 4 days, 1; 5 days, 1; 6 days, 2; 7 days, 2. The two heifers given HCG on the seventh day did not exhibit estrus; one was in the synchronized and one was in the non-synchronized group. Ovulation rates of these heifers were included in Table III with the HCG on day of estrus group.

All of the heifers in the synchronized group had at least one corpus luteum and 9 of 17 (53%) had multiple ovulations following PMS and HCG treatment; indicating that the PMS injections can be timed from a synchronized estrus.

Twelve of the 17 (70%) heifers fed CAP were observed in estrus on the third or fourth day following withdrawal of the progestogen. Two heifers were not observed in estrus following withdrawal of CAP; however, they were rectally palpated on the third day post-CAP and both heifers had uterine tone indicative of estrus and both had one large follicle. Following PMS and HCG, one of these heifers ovulated two ova and one ovulated one ovum. Two heifers exhibited estrus 5

days post-CAP and one on the seventh day. Each of these heifers had one corpus luteum following PMS and HCG treatment.

Rectal palpation of the ovaries 3 days prior to the first PMS injection revealed that all heifers in the non-synchronized group had a corpus luteum and not more than one large follicle. All heifers in the synchronized group, except the three that exhibited estrus 5 or 7 days post-CAP, also had a corpus luteum and not more than one large follicle 3 days before the first PMS injection, indicating that ovulation had occurred following withdrawal of the CAP.

The first PMS injection stimulated follicular development in 30 of the 34 heifers. The ovaries were palpated and either the presence of follicles or an increase in ovarian size relative to the size prior to the PMS injection was considered to be an indication of follicular development. There was no detected relationship between subsequent ovulation rate and amount of follicular stimulation following the first PMS injection.

There were fewer large follicles 3 days prior to the second PMS injection (day 14) than 3 days following the first PMS injection (day 8). Very few large follicles were detectable prior to the second PMS injection. Since follicles greater than 10 to 12 mm diameter constantly undergo atresia during a normal estrual cycle (Choudary et al., 1968; Marion et al., 1968) these large follicles apparently had undergone at least partial atresia from the first to the second PMS injection. None of the heifers exhibited estrus or ovulated following first PMS.

Large follicles were present on the ovaries of 24 of the 34 heifers 3 days following second PMS. There was a good relationship

between the number of large follicles at this time and the number of large follicles and corpora lutea on the ovaries at slaughter, but not necessarily the number of corpora lutea. Eighty percent of the heifers with large follicles 3 days post-PMS had either large follicles or more than one corpus luteum at the time of slaughter.

The mean luteal-free ovarian weights per heifer, the mean weight of the luteal tissue per ovary, the number of heifers with one or more follicles $\geq$ 10 mm diameter and the number of heifers with one corpus luteum having one or more follicles $\geq$ 10 mm diameter are shown in Table IV.

The weight of the ovary is influenced to a great extent by the amount of the luteal tissue and the number and size of the follicles it contains; therefore, the ovary weights were expressed in Table IV on a luteal-free basis. The follicles 10 mm or greater were measured, but the total follicular fluid of each ovary was not determined. Assuming a random variance in ovary weights among the treatment groups; the luteal-free ovarian weight per heifer should give some indication of total follicular development following PMS and HCG treatment.

Sixteen of the 18 heifers with one corpus luteum had one or more follicles $\geq$ 10 mm diameter, but there were no differences among treatments in the number of heifers with follicles 10 mm or greater. There were no significant differences ($P > .10$) in the ovarian weights among treatments, however, there was some evidence to indicate that HCG given on the third day post-PMS resulted in lower ovarian weights. The difference in the mean ovarian weights between the synchronized and non-synchronized groups was apparently due to the decidedly lower weights in the non-synchronized group given HCG on the third day, but

TABLE IV

DEVELOPMENT OF THE CORPORA LUTEA AND FOLLICLES FOLLOWING TREATMENT WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 OF THE ESTRUAL CYCLE AND WITH 2500 IU OF HCG ON DAY OF ESTRUS OR DAY 3 POST-PMS (TRIAL II)

Treatment	No. Heifers	Mean Luteal-free Ovarian Wt/Heifer ± SE(g)	Mean CL Wt/Ovary ± SE(g)	No. Heifers with 1 or more Follicles >10 mm dia.	No. Heifers with 1 CL	No. Heifers with 1 CL and 1 or more Follicles >10 mm dia.
Non-Synchronized[a]						
HCG-Day Estrus Post-PMS	8	10.48±1.38	3.57±1.10	6	6	5
HCG-3rd Day Post-PMS	9	9.16±1.25	2.17±1.04	7	4	4
Synchronized[b]						
HCG-Day Estrus Post-PMS	9	11.64±1.25	3.33±1.04	7	4	4
HCG-3rd Day Post-PMS	8	10.47±1.38	3.63±1.10	5	4	3
Non-Syn.[a] & Syn.[b]						
HCG-Day Estrus Post-PMS	17	11.06±0.96	3.42±0.76	12	10	9
HCG-3rd Day Post-PMS	17	9.82±0.96	2.80±0.76	13	8	7

[a] PMS injections timed from a non-synchronized estrus.

[b] PMS injections timed from a synchronized estrus.

the HCG x synchronization interaction did not approach significance. The luteal-free ovarian weight per heifer for the group given HCG on the third day was 11.06 ± 0.96 grams compared to 9.82 ± 0.96 grams for those given HCG on day of estrus. Since the mean interval from second PMS to day of HCG administration was one day longer in the group given HCG on day of estrus, it seems logical that heifers in this group would have more follicular development than those receiving HCG at a shorter interval from the second PMS injection. In addition, heifers given HCG on the third day post-PMS had less (non-significant $P > .10$) luteal tissue per ovary, 2.80 ± 0.76 grams, than those given HCG on day of estrus, 3.42 ± 0.76 grams. This might suggest that administration of HCG prior to estrus will induce ovulation of partially mature follicles; however, Hafez and Ishibashi (1964) reported no effect of PMS and HCG on the maturation patterns of oocytes. No information is available on the relationship between the size of the corpus luteum and morphology of the ovum. It should be noted that the mean weight of corpora lutea from single ovulations was 4.36 ± 0.84 grams compared to 2.59 ± 0.92 grams for corpora lutea from multiple ovulations. However, most reports indicate that conception rates of induced multiple ovulated cows are approximately the same as non-gonadotrophin treated cows (Arbeiter, 1962; Gordon et al., 1962; Hafez et al., 1964; Turman et al., 1968). These findings are further supported by the results of Bellows et al. (1968) and Lamond and Hill (1969) who reported fertilization rates of 95 and 94 percent, respectively, of ova from gonadotrophin treated cows.

Mean luteal tissue weight from each heifer with multiple ovulations was 6.63 grams compared to 4.36 grams for single ovulations.

There was no relationship between number of corpora lutea for heifers with multiple ovulations and weight of each corpus luteum.

PMS injections timed from a synchronized estrus followed by HCG on the third day post-PMS gave the most promising results (50% single and 50% double ovulations) in this trial. The number of heifers with a single corpus luteum and one or more large follicles prior to and following HCG administration suggests that a higher level of HCG might increase the number of multiple ovulations.

CHAPTER VI

TRIAL III

Materials and Methods

This trial was conducted from August, 1969 to February, 1970. Forty-six yearling Hereford and Angus heifers ranging in weight from 579 to 814 pounds were randomly allotted to treatment groups on the basis of weight and breed. The heifers had been wintered on wheat pasture and were on native pasture when the experiment was initiated. Milo was fed at the rate of 2 pounds per head per day during the initial phase of the study and increased to 10 pounds following the first sequence of PMS injections. All heifers were put into the feedlot 14 days following the last laparotomy.

The study was conducted in two replicates, initiated 21 days apart, with 24 heifers in replicate 1 and 22 heifers in replicate 2. Table V shows the initial treatments and number of heifers for the combined replicates.

Estrus was synchronized in all heifers by feeding CAP. The PMS injections were timed from the average day of estrus following withdrawal of the CAP. Thus, all gonadotrophin treated heifers in each replicate received PMS and HCG and were inseminated on the same calendar dates. Thirty heifers received 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle following CAP feeding.

TABLE V

INITIAL TREATMENT AND NUMBERS OF HEIFERS IN TRIAL III

Group	No. Heifers	1st[a] PMS	Lap. Day 10[b]	2nd[c] PMS	HCG Day 3 Post-PMS	Insem-inated[d]	Lap. 11 Days Post-HCG
		(IU)		(IU)	(IU)		
1	15	1500	Yes	2000	4000	Yes	Yes
2	15	1500	No	2000	4000	Yes	Yes
3	16	None	Yes	None	None	No	Yes

[a]Given on day 7 post-CAP in replicate 1 and day 8 post-CAP in replicate 2.

[b]Five days following the first PMS injection.

[c]Given on day 19 post-CAP in replicate 1 and day 8 post-CAP in replicate 2.

[d]Inseminated at the time of HCG administration and 24 hours later.

Five days following the first PMS injection, day 10 of the estrual cycle, a high lumbar laparotomy was made on the control group and one-half of the PMS treated group to determine ovarian response to the first PMS injection. The number of follicles $\geq$ 5 mm diameter were counted and the diameter of each follicle was estimated. Numbers and sizes of follicles $\geq$ 5 mm in the control and PMS treated groups were analyzed by the Student's t test. The locations of the follicles on the ovaries were diagrammed to determine which follicles continued to develop and ovulate.

All PMS treated heifers received 4000 IU of HCG and were inseminated on the third day following second PMS. A second insemination was made 24 hours later. The heifers were observed for estrus, but

the inseminations were made without regard to the occurrence of estrus. The control heifers did not receive PMS and were not inseminated at this time.

A high lumbar laparotomy was performed on all heifers 11 days following HCG administration to determine number of corpora lutea, location of the corpora lutea on the ovaries and number, location and size of follicles.

Upon return to estrus following the second laparotomy, all heifers in the control group and all PMS treated heifers failing to conceive following initial PMS treatment were given PMS and HCG and were inseminated as shown in Table VI. The PMS injections were timed from the individual heifer's estrus. All heifers were given 1500 and 2000 IU of PMS on days 5 and 17, respectively, during the second estrual cycle following the initial PMS treatment cycle. HCG was administered on the third day following second PMS and the heifers were inseminated at the time of HCG administration and 24 hours later. A high lumbar laparotomy was performed 5 to 12 days following HCG administration to determine ovulation rate and number and size of follicles.

Conception was determined by absence of estrus behavior and rectal palpation of the ovaries. The ovaries of all heifers, which were not observed in estrus within 30 days following insemination, were rectally palpated 30 and 60 days postinsemination. Palpations were made to determine if the same number of corpora lutea were at the same location on the ovaries as they were at the time of the laparotomy following insemination. In addition, the uterine horns were rectally palpated 60 and 90 days post-insemination.

TABLE VI

TREATMENTS ADMINISTERED TO THE CONTROL HEIFERS AND THE PMS TREATED HEIFERS THAT FAILED TO CONCEIVE FOLLOWING THE FIRST SEQUENCE OF PMS INJECTIONS (TRIAL III)

Group	No. Heifers	PMS Day 5 Post-Estrus (IU)	PMS Day 17 Post-Estrus (IU)	HCG Day 3 Post-PMS (IU)	Insem-inated[a]	Lap. 5-12 Days Post-HCG
Control[b]	15	1500	2000	4000	Yes	Yes
Repeat[c] PMS	19	1500	2000	4000	Yes	Yes

[a]Inseminated at the time of HCG administration and 24 hours later.

[b]Heifers had not previously received PMS or HCG.

[c]Heifers were previously treated with PMS and HCG.

All heifers diagnosed pregnant at 30 days postinsemination were slaughtered and the reproductive tracts were recovered 90 days post-insemination. Number and viability of the fetuses, number of corpora lutea and condition of the uterus were determined at this time.

The high lumbar laparotomy procedure used in this trial and in Trial IV was that of Rhynes (personal communication). It was a modification of the procedures described by Guard (1953) and Frank (1959).

The site of incision was between the last rib and the external angle of the ilium. The hair was clipped from the operative area and the area was scrubbed and saturated with an antiseptic soap. The skin and underlying tissues in the immediate incision area were anesthetized by infiltration with a local anesthetic, Xylocaine or Procaine. The incision in the skin was started approximately 4 inches ventral to the

transverse process of the lumbar vertebrae and 4 inches caudal to the last rib and extended downward 6 inches. To facilitate suturing, the incision through the muscle layers and peritoneum was made in line with the skin and was longest through the skin with a gradual shortening through the muscle layers and the peritoneum. Hemorrhage was controlled by clamping hemostats on the severed blood vessels.

The ovaries were grasped with the hand and held in approximately their normal position while being observed through a speculum inserted into the body cavity. To minimize dangers of infection and adhesions, the speculum and hand were washed in antiseptic soap and rinsed with physiological saline prior to insertion into the body cavity, and the ovaries were rinsed with physiological saline following observation.

The incision was closed with number 60 Vetafil by a through and through, cruciate type of stitch. The operative area was washed with antiseptic soap and covered with iodine. Penstrep (10 ml) was injected into the incision area and intramuscularly (10 ml) following the laparotomy.

Results and Discussion

This trial was initiated to determine: (1) Ovulation rate following two PMS injections timed from a synchronized estrus with HCG given on day 3 post-PMS; (2) The effect of first PMS on follicular development; (3) Conception rate and embryo survival; (4) Ovarian refractoriness to PMS when PMS treatment was repeated during the second estrual cycle following the initial treatment.

Forty-five of the 46 heifers were observed in estrus within four days from last day of CAP feeding. One heifer, which failed to eat

CAP for four days and exhibited estrus during the CAP feeding period, was not observed in estrus following withdrawal of CAP. Average day of estrus following withdrawal of CAP was the third day in replicate 1 and the second day in replicate 2. Thus, the PMS injections were made on days 8 and 20 post-CAP in replicate 1 and days 7 and 19 post-CAP in replicate 2. In replicate 1, eight heifers exhibited estrus on day 2, 14 on day 3 and two on day 4 post-CAP. In replicate 2, five heifers exhibited estrus on day 1, 15 on day 2 and one on day 3 post-CAP.

Since there were no significant differences in ovulation rates of heifers in replicates 1 and 2, the ovulation rates of the 30 heifers from both replicates following the first sequence of PMS injections are shown in Table VII.

TABLE VII

OVULATION RATES OF HEIFERS FOLLOWING INITIAL TREATMENT WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 OF THE ESTRUAL CYCLE TIMED FROM A SYNCHRONIZED ESTRUS AND FOLLOWED BY 4000 IU OF HCG ON DAY 3 POST-PMS (TRIAL III)

Lap.[a] following 1st PMS	Total Treated	Number of Heifers — Number of Corpora Lutea/Heifer						
		0	1	2	3	4	5	6
Yes	15	1	5	5	2	0	0	2
No	15	0	4	7	1	1	0	2
Total	30	1	9	12	3	1	0	4
				50%			16.6%	

[a] High lumbar laparotomy performed on day 10 of the PMS treated cycle.

Twelve (40%) of the heifers had two corpora lutea, three heifers (10%) had three and five heifers (16.6%) had more than three corpora lutea. Average ovulation rate was 2.33. The one heifer that failed to ovulate had not exhibited estrus following withdrawal of CAP.

The level of HCG in this trial was increased to 4000 IU because the results of Trial II suggested that a higher level of HCG might increase the number of multiple ovulations in heifers responding with only a single ovulation and because 4000 IU of PMS were used by Schilling and Holm (1963). Although the proportion of heifers with two and three egg ovulations in this trial (50%) was very similar to the heifers that received HCG on the third day in Trial II (47%), 16.6 percent of the heifers in this trial had more than three ovulations compared to 5.8 percent in Trial II.

Only one-half of the PMS treated heifers were laparotomized following first PMS in an effort to evaluate the effects of the laparotomy on subsequent ovulation rate and conception. The laparotomy at this time did not affect subsequent ovulation rate. Nine heifers, laparotomized following first PMS, had multiple ovulations following HCG administration, compared to 11 heifers, which were not laparotomized following first PMS.

There was good evidence that the first PMS injection stimulated follicular development (Figure 3). The PMS treated heifers had a significantly greater ($P < .01$) number of follicles $\geq$ 10 mm diameter than the control heifers 5 days following first PMS. Only nine of 16 heifers in the control group had one or two follicles $\geq$ 10 mm and only one heifer had a 12 mm follicle; while in the PMS treated group, 14 of the 15 heifers had at least one follicle $\geq$ 12 mm and eight had

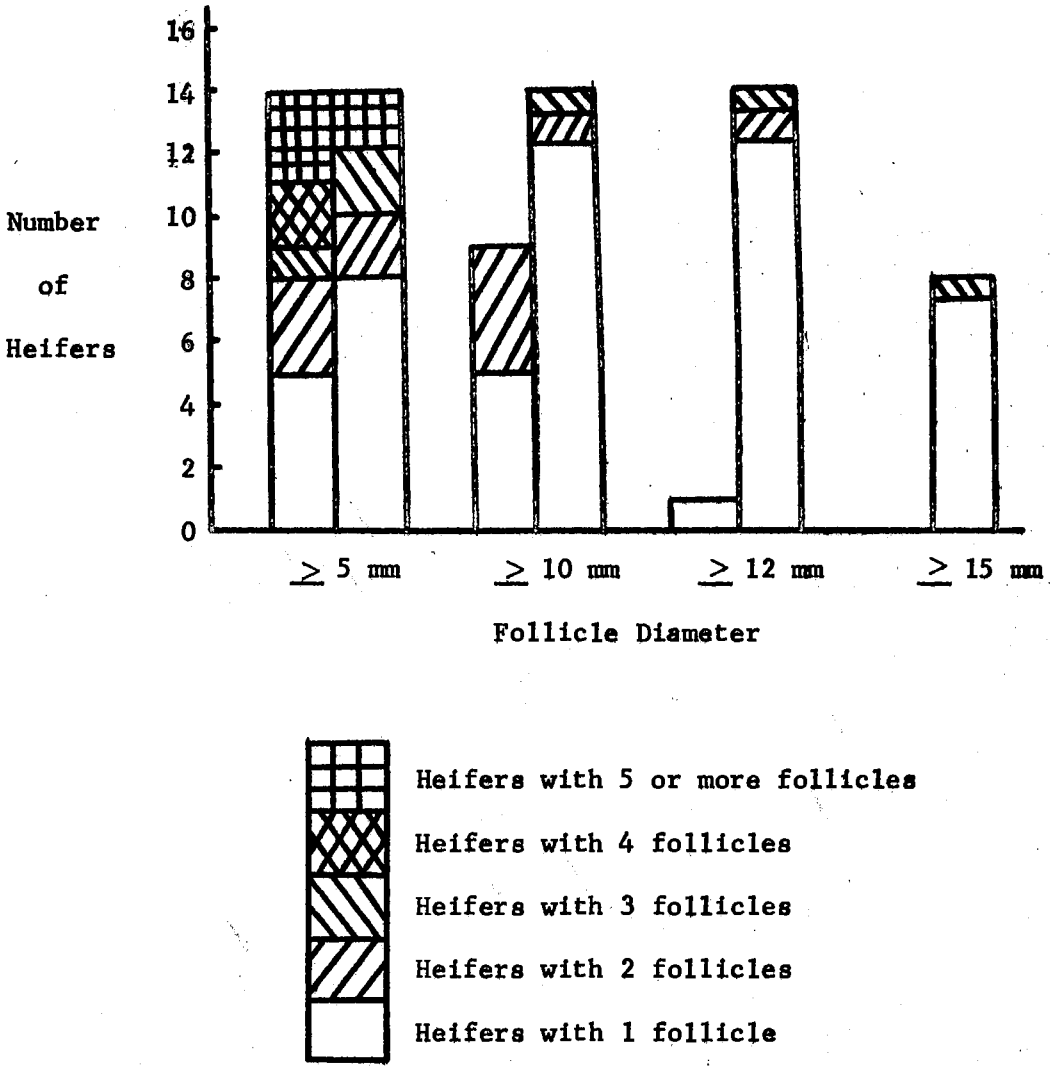

Figure 3. Number of Heifers with Different Numbers and Sizes of Follicles on Day 10 of the Estrual Cycle in Control Heifers and in Heifers Injected with 1500 IU of PMS on Day 5 of the Estrual Cycle

at least one follicle $\geq$ 15 mm.

There were fewer heifers in the PMS treated group with two or more follicles greater than 5 mm but less than 10 mm diameter than in the control group. In the PMS treated group, 6 of 14 heifers had more than one 5 mm follicle and 2 of 14 had more than one follicle $\geq$ 10 mm compared to 9 of 14 and 4 of 9, respectively, in the control group. This suggests that the first PMS injection stimulated follicles which were $\geq$ 5 mm at the time of the injection.

The significance of the large follicles following first PMS in terms of subsequent ovulation is not clear since the follicles greater than 10 mm on the 10th day of the cycle were not at the same place on the ovaries where the corpora lutea were located following ovulation. In most instances, the follicles greater than 10 mm on the 10th day of the PMS treated cycle had regressed by the time of the laparotomy following HCG, which was 20 days later. This would agree with the findings of Choudary *et al.* (1968) and Marion *et al.* (1968) that follicles $\geq$ 10 mm during a normal cycle become atretic prior to subsequent ovulation.

There were significantly greater ($P< .01$) numbers of follicles $\geq$ 10 mm in the PMS treated heifers 10 days following HCG than 5 days following the first PMS injection (Figure 4). The comparisons between numbers and sizes of follicles in Figure 4 were in the same 15 heifers.

There was only a small difference between numbers of follicles $\geq$ 5 mm after first PMS and after HCG, but only two heifers had more than one follicle $\geq$ 10 mm following first PMS compared to 12 heifers with more than one follicle $\geq$ 10 mm following HCG. Following first PMS only 7 heifers had one follicle $\geq$ 15 mm and one had 3 follicles $\geq$

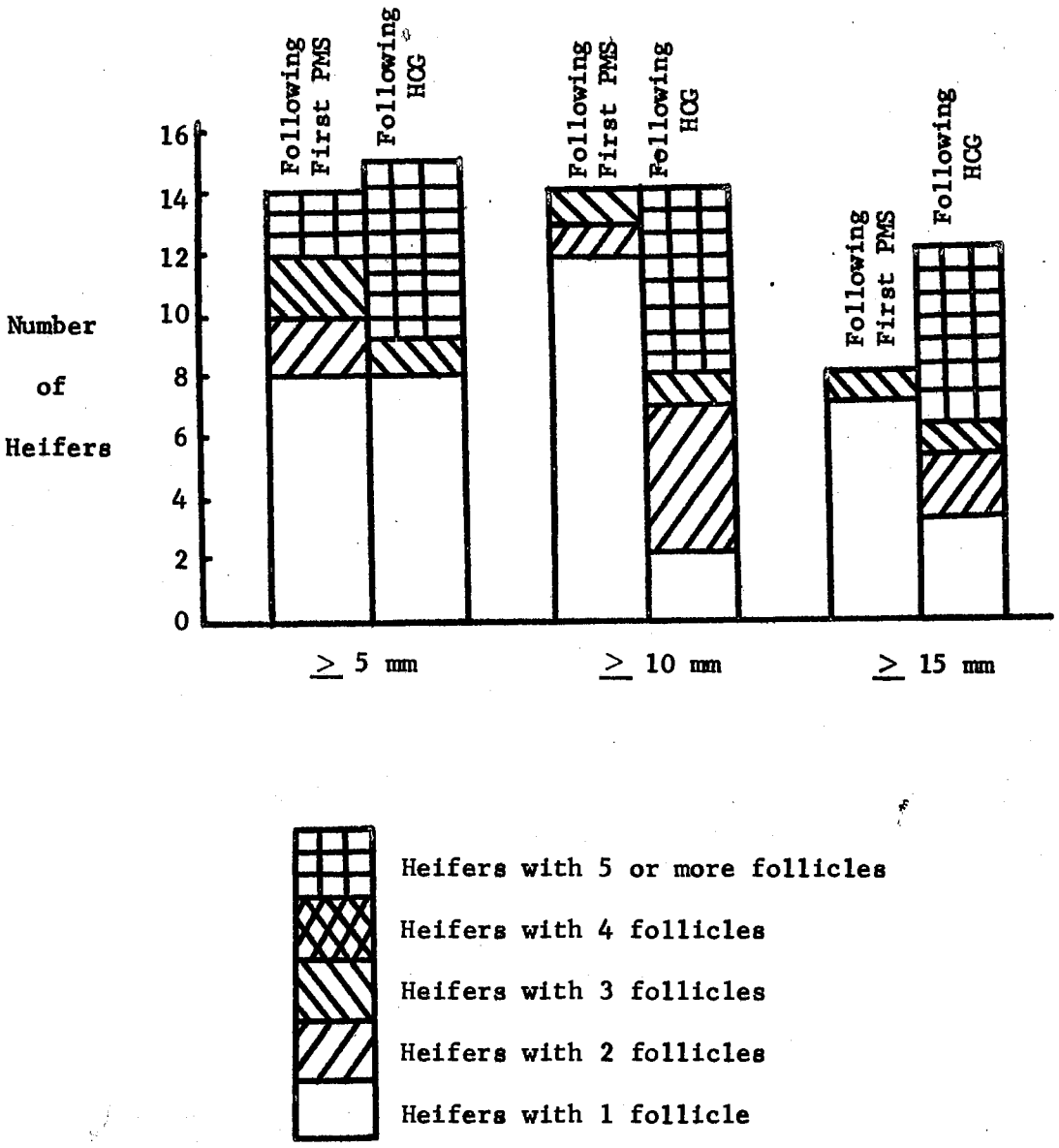

Figure 4. Number of Heifers with Different Numbers and Sizes of Follicles 5 Days Following Injection of 1500 IU of PMS and 11 Days Following Administration of 2000 IU of PMS on Day 17 and 4000 IU of HCG on Day 3 Post-PMS

15 mm, but following HCG three, two, one and six heifers had one, two, three or five or more, respectively, follicles $\geq$ 15 mm.

Following HCG administration, only 14 percent of the corpora lutea and 11 percent of the follicles greater than 10 mm were located on the ovary where 5 to 8 mm follicles were present on the 10th day of the PMS treated cycle. In addition, the corpora lutea were not at the same place on the ovaries where follicles greater than 10 mm were located following first PMS. This suggests that the second PMS injection stimulated different follicles than were stimulated by the first injection, or that the first PMS injection only slightly stimulated some follicles which were less than 5 mm in diameter 5 days following the first PMS injection.

Table VIII shows the ovulation rates of the control heifers following PMS treatment and the PMS retreated heifers that failed to conceive following the first sequence of PMS injections. The control heifers had been subjected to two laparotomies prior to PMS treatment, but had not previously received gonadotrophins; consequently, they served as controls for evaluating refractoriness to the second sequence of PMS injections. One of the control heifers did not return to estrus within 60 days following the second laparotomy and one of the PMS treated heifers, diagnosed by rectal palpation as open at 30 and 60 days post-insemination did not return to estrus following initial PMS treatment. These heifers were not included in Table VIII.

The 19 heifers showed some refractoriness to the second sequence of PMS injections. Only 51.6 percent of the retreated heifers had two or more corpora lutea compared to 80 percent of the control group. Mean ovulation rates were 1.95 and 3.0 in the retreated and control

groups, respectively. These findings are contrary to those of Hafez et al. (1964) and Jainudeen et al. (1966), who reported complete refractoriness to a single injection of 1500 or 2000 IU of PMS given on day 16 of the cycle during the first or second cycle following initial PMS treatment.

TABLE VIII

OVULATION RATES OF THE CONTROL HEIFERS AND THE PMS RETREATED HEIFERS FOLLOWING TREATMENT WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 OF THE ESTRUAL CYCLE WITH 4000 IU OF HCG ON DAY 3 POST-PMS (TRIAL III)

Previous Treatment	Total Treated	Number of Heifers: Number of Corpora Lutea/Heifer								
		0	1	2	3	4	5	6	7	8
Control[a]	15	1	2	4	1	5	1	0	1	0
				33.3%				46.6%		
PMS[b]	19	1	8	8	0	0	1	0	0	1
				41.1%				10.5%		

[a]Heifers had not previously received PMS or HCG.

[b]Heifers were previously treated with PMS and HCG.

In the control group, five of the 15 (33.3%) heifers had either two or three corpora lutea, but seven (46.6%) had more than three corpora lutea; while in the retreated group, 8 of 19 (42.1%) heifers had two egg ovulations and only 2 of 19 (10.5%) had more than two. The two heifers with no corpora lutea had a corpus albicans at the time of the laparotomy, indicating that the PMS injections were not properly

timed from the previous estrus.

The ovulatory response of the retreated heifers was more similar than the control heifers to the response of the heifers following initial PMS treatment in this trial and those in Trial II that received HCG on the third day. The response of the control heifers; however, was very similar to the cows in Trial I that received HCG on the third day.

Conception rate was 52.2 percent for all heifers in the trial. Ten of the 30 (33.3%) conceived following the initial sequence of injections and seven of the 10 (36.8%) heifers from this group conceived following the second sequence of injections. Seven of the 15 (46.7%) heifers in the control group conceived to the inseminations following PMS treatment. The numbers of heifers in the different groups pregnant at 30, 60 and 90 days postinsemination are shown in Table IX.

Two inseminations were made following second PMS because the results of Trial II indicated that 59 percent of the heifers would exhibit estrus and presumably ovulate on the second or third day following second PMS. In addition, Graves and Dzuik (1968) found that ovulation occurred within 40 hours after HCG administration. No relationship was noted between conception rate and the occurrence of estrus following second PMS.

Of the 24 heifers pregnant 30 days postinsemination, only 9 were pregnant at 60 days and only 5 were pregnant at 90 days postinsemination. Fetal resorption occurred in the 15 heifers, which were pregnant at 30 days but not pregnant at 60 days, as evidenced by soft, enlarged and distended uterine horns at 60 days postinsemination.

TABLE IX

NUMBERS OF HEIFERS PREGNANT 30 AND 60 DAYS POSTINSEMINATION AND NUMBER OF FETUSES 90 DAYS POSTINSEMINATION IN HEIFERS TREATED WITH PMS AND HCG[1] AND INSEMINATED ON DAYS 3 AND 4 POST-PMS (TRIAL III)

Group	Total	Pregnant 30 Days Postinsem.	Pregnant 60 Days Postinsem.	90 Days Postinsem.		
				Resorbed[b] Fetuses	Fetuses	
					Single	Multiple
Initial PMS[c] Treated	30	10	5	7	1	2
Second PMS[d] Treated	19	7	2	7	0	0
Control[c]	15	7	2	4	0	2

[1]All heifers had been treated with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 with 4000 IU of HCG given on day 3 post-PMS.

[b]Uterine condition at slaughter indicated that resorption or abortion had occurred.

[c]Heifers had not previously received PMS or HCG.

[d]Second sequence of PMS and HCG injections.

Examination of the reproductive tracts from the 19 heifers, diagnosed pregnant 30 days postinsemination but which were not pregnant 90 days postinsemination, revealed that 18 of the 19 had been pregnant. The condition of the reproductive tracts ranged from those having corpora lutea of pregnancy, dried blood in the uterine horns and unrepaired endometrial mucosa to enlarged uterine caruncles.

Although there was a higher conception rate in heifers with single ovulations (47.3%) than in those with multiple ovulations (37.5%), there was no relationship between number of ovulations and embryonic survival (Table X). The five heifers pregnant at 90 days had each

ovulated more than one ova. The numbers of corpora lutea and number of fetuses in the five heifers pregnant at 90 days postinsemination were: 2 CL, 1 fetus; 3 CL, 1 fetus; 5 CL, 2 fetuses; 4 CL, 3 fetuses; 4 CL, 3 fetuses.

TABLE X

NUMBERS OF HEIFERS IN EACH OVULATION RATE GROUP OF THE TOTAL TREATED WITH PMS AND HCG[1] AND INSEMINATED ON DAYS 3 AND 4 POST-CAP AND OF THOSE PREGNANT AT 30, 60 AND 90 DAYS POSTINSEMINATION (TRIAL III)

Group	Total Heifers	Number of Corpora Lutea/Heifer							
		1	2	3	4	5	6	7	8
Total Heifers[a]	61	19	24	4	6	2	4	1	1
Pregnant at 30 days	24	9	6	1	5	2	1	0	0
Pregnant at 60 days	9	1	4	1	2	1	0	0	0
Pregnant at 90 days	5	0	1	1	2	1	0	0	0

[1]All heifers treated with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 with 4000 IU of HCG given on day 3 post-PMS.

[a]Total includes ovulation rates of 19 heifers treated two times and 27 heifers treated once.

The reason for the high embryo mortality could not be determined. The laparotomy following the first PMS injection, during the initial sequence of injections, decreased conception and may have decreased embryo survival. Seven of the 10 heifers that conceived at this time were not laparotomized following first PMS and the three heifers from

this group with fetuses at 90 days postinsemination were not laparotomized following first PMS. Thus, the laparotomy following insemination as well as that following first PMS may have contributed to embryo mortality. The fact that embryonic mortality in 15 of the 19 heifers occurred between 30 and 60 days pregnancy would suggest that the laparotomy following insemination was not the causative factor. However, the corpora lutea of pregnancy were present at 90 days in four of the heifers in which resorption had previously occurred; one prior to 60 days and three after 60 days. This could mean that the causative factor exerted a sustained luteolytic effect and would explain how the laparotomy 10 days postinsemination could result in fetal resorption 20 or more days later.

Gordon et al. (1962) reported that rectal palpation at 42 days pregnancy caused abortion of 32.5 percent of the pregnancies; therefore, rectal palpation at 30 and 60 days may have caused or contributed to the high rate of embryonic mortality. It seems likely that both the laparotomy and rectal palpation were responsible for the embryo and fetal mortality.

CHAPTER VII

TRIAL IV

Materials and Methods

This trial was conducted from October, 1969 to April, 1970. Thirty-seven multiparous, non-lactating beef cows ranging in weight from 870 to 1310 pounds were randomly allotted to the treatments shown in Table XI on the basis of weight, breed and previous years calving performance. Thirty of the cows, 17 Herefords and 13 Hereford-Angus crossbreds, had weaned calves immediately prior to initiation of the experiment and had not been exposed to a bull following calving. Seven of the cows, four Herefords and three Angus, had been eliminated from another project because they had failed to conceive during a 90 day breeding season. These cows had calved the previous year, were cycling normally and there were no apparent reasons for their failure to conceive. The cows were maintained on native pasture and were fed 5 pounds of milo plus 2 pounds of 43 percent cottonseed cake per head per day, except during the CAP feeding period when they received 2 pounds of milo per head per day.

All cows were fed CAP for 18 days and the PMS injections were timed from average day of estrus following withdrawal of the CAP. The ovaries were palpated 8 days post-CAP feeding to determine if ovulation had occurred. Eighteen cows received 1000 IU of PMS and 19 received 1500 IU of PMS on day 8 post-CAP. All cows were given

2000 IU of PMS on day 20 post-CAP and 4000 IU of HCG on day 23 post-CAP. Inseminations were made at the time HCG was injected and 24 hours later. A high lumbar laparotomy was performed 10 days following HCG administration to determine number of corpora lutea and number and size of follicles greater than 10 mm diameter.

TABLE XI

TREATMENTS AND NUMBER OF COWS IN TRIAL IV

No. Cows	PMS: Post-CAP[a] Day 8	Day 20	HCG Day 3 Post-PMS	Insem-inated[b]	Lap. 10 Days Post-HCG
	(IU)	(IU)	(IU)		
18	1000	2000	4000	Yes	Yes
19	1500	2000	4000	Yes	Yes

[a]All cows fed CAP for 18 days with PMS injections given during the estrual cycle following CAP feeding.

[b]Inseminated at the time of HCG administration and 24 hours later.

All cows that returned to estrus within 45 days postinsemination were retreated as shown in Table XII. The PMS injections were timed from a non-synchronized estrus and all cows received 1500 and 2000 IU of PMS on days 5 and 17, respectively, followed by 4000 IU of HCG on the third day post-PMS. Inseminations were made at the time of HCG administration and 24 hours later. A high lumbar laparotomy was made 5 to 12 days following HCG to determine number of corpora lutea.

TABLE XII

TREATMENT OF COWS THAT FAILED TO CONCEIVE FOLLOWING THE FIRST SEQUENCE OF PMS INJECTIONS (TRIAL IV)

No. Cows[a]	PMS: Post-estrus[b] Day 5	Day 17	HCG Day 3 Post-PMS	Insem-inated[c]	Lap. 5-12 Days Post-HCG
	(IU)	(IU)	(IU)		
27	1500	2000	4000	Yes	Yes

[a]All cows had previously received PMS and HCG.

[b]The PMS injections timed from estrus of cows failing to conceive to inseminations following initial PMS.

[c]Inseminated at the time of HCG administration and 24 hours later.

Following the initial sequence of gonadotrophin injections, conception was determined by absence of estrus behavior and was confirmed by rectal palpation of the ovaries at 30 days and of the uterine horns at 60 and 90 days postinsemination. Following the second sequence of injections, conception was determined by absence of estrus behavior and rectal palpation at 60 and 90 days postinsemination.

Results and Discussion

This trial was initiated to determine: (1) The effects of two levels of the first PMS on ovulation rate following the second PMS injection; (2) Conception and embryo survival in cows treated with two PMS injections timed from a synchronized estrus with HCG given on day 3 and inseminations made on days 3 and 4 post-PMS; (3) Ovarian refractoriness to a second sequence of PMS injections.

Twenty-eight of the 37 cows exhibited estrus following CAP with-

drawal, five on day 1, 11 on day 2, five on day 3, four on day 4 and three on day 6; therefore, the PMS injections were given on days 8 and 20 post-CAP. Of the nine cows not detected in estrus following CAP withdrawal, corpora lutea were not detectable when palpated on day 8 in three of the cows, but six had a corpus luteum indicating that ovulation had occurred.

Ovulation rates following initial gonadotrophin treatment are shown in Table XIII. Mean ovulation rate was 2.30 for all cows with little difference between the levels of first PMS. Mean ovulation rate was 2.22 for cows given 1000 IU of PMS on day 5 and 2.37 for those given 1500 IU at this time. The cow with no corpus luteum was detected in estrus during the CAP feeding period, on the sixth day following CAP withdrawal and on the third day following first PMS.

TABLE XIII

OVULATION RATES OF COWS FOLLOWING INITIAL TREATMENT WITH 1000 OR 1500 IU OF PMS ON DAY 8 POST-CAP, 2000 IU OF PMS ON DAY 20 POST-CAP AND 4000 IU OF HCG ON DAY 3 POST-PMS (TRIAL IV)

Level of First PMS (IU)	Total Treated	Number of Cows: Number of Corpora Lutea/Cow									
		0	1	2	3	4	5	6	7	8	9
1000	18	1	7	4	2	3	0	0	1	0	0
				33.3%				22.2%			
1500	19	0	6	7	4	1	0	0	0	0	1
				57.9%				10.5%			

Although there was no significant difference ($P > .10$) between the two levels of first PMS in numbers of cows with each number of corpora lutea, there was some evidence that 1500 IU gave more two and three and fewer four or more egg ovulations than 1000 IU. Six of the 18 (33.3%) cows given 1000 IU of PMS on day 5 had either two or three corpora lutea and four (22.2%) had four or more corpora lutea compared to 11 of 19 (57.9%) and two of 19 (10.5%), respectively, in the group given 1500 IU on day 5. The ovulation rates of the cows given 1500 and 2000 IU of PMS were very similar to those of the heifers in Trial III following initial PMS treatment.

There were no differences in number of cows with large follicles or luteinized follicles between the levels of first PMS. In the group given 1000 IU of PMS, 15 of 18 cows had one or more follicles greater than 10 mm and four of 18 had one or more luteinized follicles compared to 15 of 19 cows with one or more large follicles and five of 19 with luteinized follicles in the group given 1500 IU of PMS on day 5. All cows with luteinized follicles in both first PMS level groups had at least one normal corpus luteum.

Ovulation rates of the cows following the second sequence of PMS injections are shown in Table XIV. Although a control group was not used, it appeared that the cows were somewhat refractory to the second sequence of injections. Only 37 percent of the cows gave a super-ovulatory response following the second sequence of PMS injections compared to 68.4 percent following initial treatment with 1500 and 2000 IU of PMS. Eight of the 27 (29.6%) cows had either two or three corpora lutea and two of the 27 (7.4%) had more than three corpora lutea. Although the ovulation rates in this trial and Trial III were

very similar following initial PMS treatment, the superovulatory response following the second sequence of injections in this trial (37.0%) was considerably lower than in Trial III (52.6%). There was no difference in ovulatory response following the second sequence of PMS injections between cows treated with 1000 or 1500 IU of PMS on day 5 during the initial sequence of injections.

TABLE XIV

OVULATION RATES OF COWS FOLLOWING A SECOND SEQUENCE OF PMS AND HCG INJECTIONS[1]

Total Treated	Number of Cows: No. of Corpora Lutea/Cow					
	0	1	2	3	4	5
27	2	15	6	2	1	1
			29.6%		7.4%	

[1]Treated with 1500 and 2000 IU of PMS on days 5 and 17 post-estrus, respectively, and 4000 IU of PMS on day 3 post-PMS.

There was no relationship between number of cows with multiple ovulations following the second sequence of PMS injections given during the second or third estrual cycle following initial PMS treatment. Six of 15 (40.0%) cows treated during the second cycle had two or more corpora lutea compared to four of 12 (33.3%) treated during the third estrual cycle.

Conception rate, based on non-return to estrus by 45 days post-

insemination, was 48.6 percent for all cows in the trial. Nine of the 37 (24.3%) cows conceived following the first sequence of gonadotrophin injections and nine additional cows conceived following the second sequence of injections.

There appeared to be some relationship between the occurrence of estrus following the second PMS injection and conception following the initial sequence of injections. Six of the 13 cows exhibiting estrus following second PMS conceived, while only three of the 24 cows, failing to exhibit estrus following second PMS, conceived at first post-PMS insemination. This might mean that the cows which are near the time of estrus and have follicles ready to ovulate at the time of HCG administration are more likely to conceive than those which are not near time of normal estrus when HCG is administered.

Rectal palpation of the nine cows that did not return to estrus following the initial sequence of gonadotrophin injections revealed that the corpora lutea were located at the same place on the ovaries 30 days postinsemination as they were at the laparotomy 10 days following insemination. This was interpreted as confirmation that they had conceived. However, at 60 days all nine cows had apparently resorbed their conceptus, as evidenced by enlarged, soft and distended uterine horns. Similar condition of the uterine horns at 60 days postinsemination in five of the nine cows, which had not exhibited estrus within 60 days following the second sequence of injections but which were not pregnant 60 days postinsemination, indicated that these cows had been pregnant. The four cows pregnant at 60 days postinsemination were also pregnant at 90 days postinsemination.

Although this trial was not designed to determine the cause of

embryonic mortality, it appears that the laparotomy 10 days following insemination was at least partially responsible. The nine cows which were retreated with PMS and which did not return to estrus 45 days following breeding were not rectally palpated 30 days postinsemination. However, five had resorbed fetus(s) when palpated at 60 days postinsemination. In addition, 12 of the 27 cows that received a second sequence of gonadotrophin injections returned to estrus between 31 and 43 days following the initial inseminations, but the corpora lutea were not at the same place on the ovaries at 30 days as they were 10 days postinsemination. This suggests that early embryonic mortality may have occurred in these 12 cows and would indicate the laparotomy following the insemination may have been the causative factor.

CHAPTER VIII

GENERAL RESULTS AND DISCUSSION

The ovulation rates for the 79 heifers and 37 cows in all trials treated with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle are shown in Table XV. The ovulation rate was 2.36 with an ovulatory response of 44.4 to 57.9 percent two or three egg ovulations in seven of the nine groups. The most promising and consistent ovulation rates were obtained when the PMS injections were timed from a synchronized estrus and HCG was administered on day 3 post-PMS. Fifty-two percent of these 38 heifers and 19 cows had two or three egg ovulations and only 12.3 percent had four or more egg ovulations.

Although there was no significant difference ($P > .10$) in ovulation rates between heifers and cows, there was some evidence ($\chi^2 = 9.0$) that the cows gave a higher superovulatory response than the heifers (Figure 5). Average ovulation rate was 2.68 for the cows and 2.22 for the heifers. There were 75.6 percent of the cows with two or more ovulations compared to 60.8 percent of the heifers, but the heifers had more two egg and fewer three or more egg ovulations than the cows. In the heifers, there were 34.2 percent two egg ovulations and 26.6 percent three or more egg ovulations compared to 27.0 and 48.6 percent two and three or more egg ovulations, respectively, in the cows.

There was no significant difference ($P > .10$) in ovulation rates

TABLE XV

OVULATION RATES OF COWS AND HEIFERS IN ALL TRIALS TREATED WITH 1500 IU OF PMS ON DAY 5 AND 2000 IU OF PMS ON DAY 17 WITH HCG GIVEN ON DAY OF ESTRUS OR DAY 3 POST-PMS

Treatment	Trial	Total Treated	Number of Animals: Number of Corpora Lutea/Animal									
			0	1	2	3	4	5	6	7	8	9
HCG-3rd Day												
PMS Timed from a Syn. Estrus												
Cows	IV	19	0	6	7	4	1	0	0	0	0	1
					57.9%				10.5%			
Heifers	II	8	0	4	4	0	0	0	0	0	0	0
					50.0%					0%		
Heifers	III	30	1	9	12	3	1	0	4	0	0	0
					50.0%				13.1%			
PMS Timed from a Non-Syn. Estrus												
Cows	I	8	1	1	3	1	0	2	0	0	0	0
					50.0%				25.0%			
Heifers	II	9	0	4	4	0	1	0	0	0	0	0
					44.4%				11.1%			
Heifers	III	15	1	2	4	1	5	1	0	0	1	0
					33.3%				46.6%			
HCG-Day Estrus												
PMS Timed from a Syn. Estrus												
Heifers	II	9	0	4	2	2	0	1	0	0	0	0
					44.4%				11.1%			
PMS Timed from a Non-Syn. Estrus												
Cows	I	10	1	0	0	5	2	1	1	0	0	0
					50.0%				40.0%			
Heifers	II	8	0	6	1	0	1	0	0	0	0	0
					12.5%				12.5%			

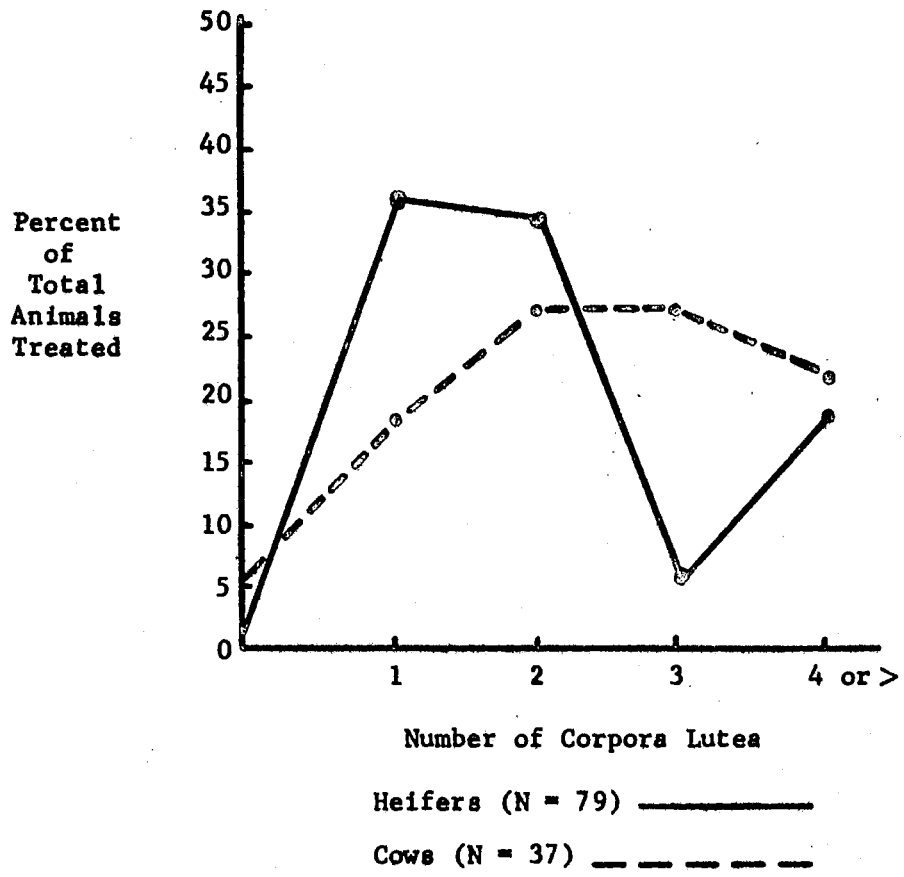

Figure 5. Ovulation Rates of Heifers or Cows in All Trials Treated with 1500 IU of PMS on Day 5 and 2000 IU of PMS on Day 17 of the Estrual Cycle and 2500 or 4000 IU of HCG Administered on Day of Estrus or Day 3 Post-PMS

of heifers and cows when the PMS injections were timed from a synchronized or a non-synchronized estrus (Figure 6). Turman et al. (1968) found no difference in number of multiple births when the first PMS injection was given on either day 4, 5 or 6 and the second injection given on either day 16 or 17 of the estrual cycle; suggesting that the PMS injections could be given on the same days if the previous estrus had occurred within a two or three day period in a high percent of the group. Thus, no difference in ovulatory response between the synchronized and non-synchronized groups would be expected since most of the cows and heifers exhibited estrus within a three day period following withdrawal of the CAP. The percentage of animals with two or three egg ovulations was similar for the two groups, but there was a higher percentage with four or more egg ovulations when the PMS injections were timed from a non-synchronized estrus. Average ovulation rates were 2.21 and 3.35 when the PMS injections were timed from a synchronized or a non-synchronized estrus, respectively.

The heifers and cows given HCG on the third day post-PMS had more two and fewer three or more egg ovulations than those given HCG on day of estrus, but the differences between the numbers of animals with each number of corpora lutea were not significantly different ($P > .10$) (Figure 7). Average ovulation rate was 2.35 for those given HCG on the third day and 2.41 for those given HCG on day of estrus. In the groups given HCG on the third day post-PMS, 38.2 percent had two egg ovulations and 29.2 percent had three or more egg ovulations compared to 11.1 and 48.1 percent, respectively, in the groups given HCG on day of estrus.

There was no relationship between body weight and ovulation rate of the cows and heifers following initial treatment with 1500 and 2000

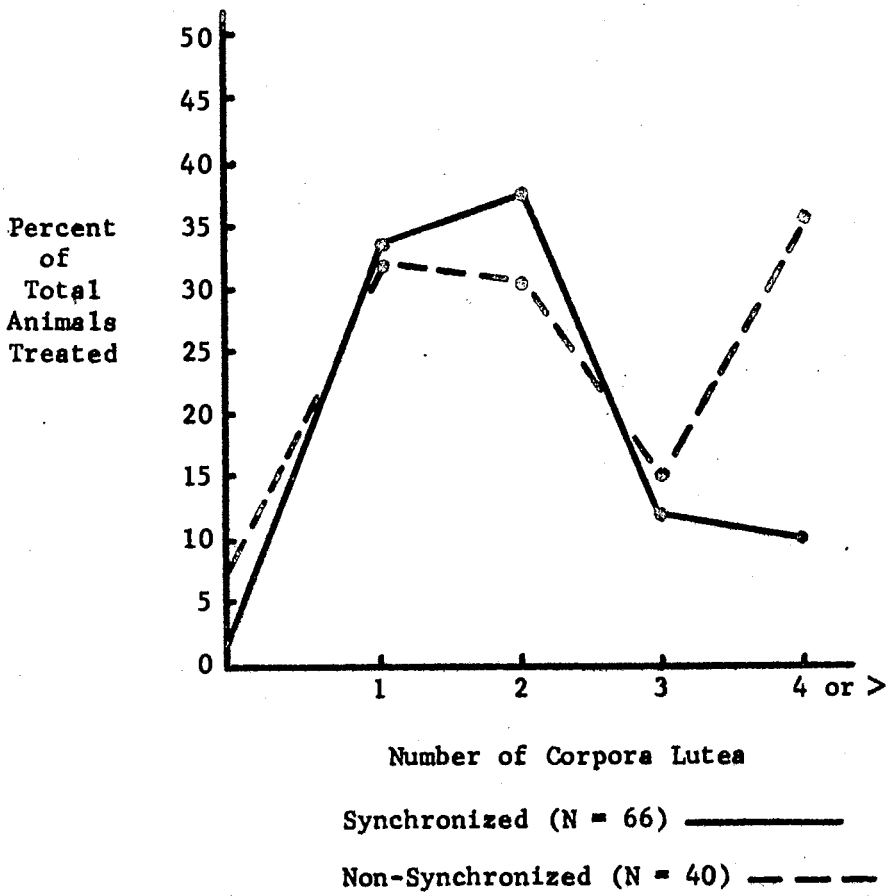

Figure 6. Ovulation Rates of Heifers and Cows in All Trials Treated with 1500 IU of PMS on Day 5 and 2000 IU of PMS on Day 17 of the Estrual Cycle with 2500 or 4000 IU of HCG Administered on Day of Estrus or Day 3 Post-PMS. The PMS Injections were Timed from Either a Synchronized or a Non-synchronized Estrus

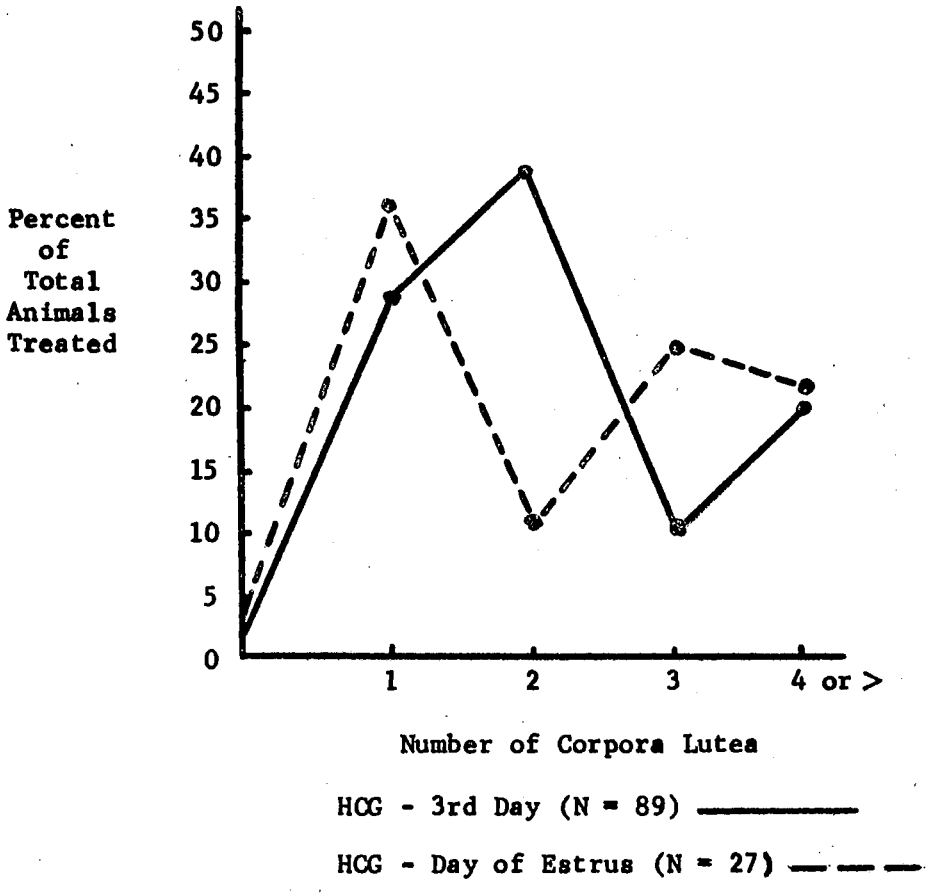

Figure 7. Ovulation Rates of Heifers and Cows in All Trials Treated with 1500 IU of PMS on Day 5 and 2000 IU of PMS on Day 17 of the Estrual Cycle and Given 2500 or 4000 IU of HCG on the Third Day or Day of Estrus Post-PMS

IU of PMS with HCG given on the third day post-PMS. The body weights of the heifers and cows were grouped according to ovulation rates (1, 2, 3 or 4-plus corpora lutea) in Trials II, III and IV and analyzed by analysis of variance. There were no significant differences ($P > .10$) in body weights of cows and heifers with different numbers of corpora lutea within trials, but there was a difference ($P < .01$) among trials. The difference among trials was probably due to the difference in body weights between the cows and heifers.

There was some evidence ($r = 0.69$) that the ovulation rate of 19 heifers following the second sequence of PMS injections was associated with the ovulation rate following the first sequence of injections, but this association ($r = 0.15$) was not found in the 25 cows which were retreated with PMS.

There was little difference in ovulation rates of the straightbred and crossbred heifers and cows receiving comparable PMS and HCG treatment. Average ovulation rate in 39 straightbreds was 2.31 compared to 2.0 in 31 comparably treated crossbreds.

The results of this study does not support the theory proposed by Schilling and Holm (1963) that the purpose of the first subovulatory injection is to prevent atresia that normally occurs with all but one of several follicles stimulated during or just after the previous estrus. In Trial III, it was observed that the second PMS injection stimulated different follicles than were stimulated by the first injection. The mechanism, if any, by which the first injection has an effect on the subsequent ovulation rate was not clearly determined in this study. However, there is the suggestion that it induces a partial refractoriness to the second injection. This is supported by the

results in Trial IV in which it was observed that cows given 1000 IU of PMS on day 5 and 2000 IU of PMS on day 17 had a higher percentage of four or more egg ovulations than those given 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17. Hafez et al. (1965) reported lower ovulation rates with 750 and 1500 or 2000 IU of PMS given on days 5 and 16, respectively, than with a single injection of 1500 or 2000 IU of PMS on day 16. Furthermore, lower mean ovulation rates and a lower range in ovulation rates were obtained in this study than in other studies in which a single injection of 2000 IU of PMS were administered on day 16 or 17 of the cycle (Gordon et al., 1962; Hafez et al., 1964; Hafez et al., 1965; Schwartz and Shelby, 1969).

The mean ovulation rate in this study was similar to that reported by Schilling and Holm (1963), but considerably lower than reported in other studies using a similar sequence of injections (Schwartz and Shelby, 1969; Hafez et al., 1965). It should be noted that differences in biologic activity of different PMS preparations at the time of usage can exist and could be responsible for these differences in ovulatory response.

CHAPTER IX

SUMMARY AND CONCLUSIONS

The objectives of this study were: (1) To determine the ovulation rate of cows and heifers given a sequence of two subcutaneous injections of PMS followed by a single intravenous injection of HCG with the PMS injections timed from a synchronized or a non-synchronized estrus and with HCG administered on day of estrus or on the third day following the second PMS injection; (2) To determine the conception rate to artificial insemination of all cows on two consecutive days following injections of PMS and HCG timed from a synchronized estrus; (3) To determine the degree of ovarian refractoriness to a second sequence of PMS injections; (4) To study the effects of the first PMS injection on follicular development.

Data were obtained from 80 heifers and 55 beef cows employed in four consecutive trials. The study was conducted so that the treatments of each successive trial were determined, to a large extent, by the results of the previous trial. Ovulation rates were determined 5 to 13 days following HCG administration by examining the ovaries by means of a laparotomy or by recovering the reproductive tracts at the time of slaughter.

In Trial I, 18 cows were treated with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle followed by 2500 IU of HCG given on day of estrus, in 10 cows, or on the third day following

second PMS, disregarding the occurrence of estrus, in 8 cows. More two egg and fewer three or more egg ovulations were produced when HCG was given on the third day than when HCG was given on day of estrus. Percentages of cows with two, three and four or more egg ovulations, respectively, were: 37.5, 12.5 and 25.0 for cows given HCG on the third day post-PMS; 0, 50 and 40 for cows receiving or scheduled to receive, HCG on day of estrus.

In Trial II, 34 heifers were treated with 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle followed by 2500 IU of HCG on day of estrus or 3 days post-PMS. The PMS injections were timed from either a synchronized or a non-synchronized estrus. Estrus was synchronized by feeding CAP for 18 days, 10 mg per head per day for 16 days and 5 mg the last two days.

Eight of the 17 (47.0%) heifers given HCG on the third day post-PMS had two egg ovulations and only two (11.8%) heifers had three or more egg ovulations compared to 3 of 17 (17.6%) with two egg ovulations and four of 17 (23.5%) with three or more egg ovulations in the group given HCG on day of estrus. Thus, further substantiating the observations made in Trial I that HCG given on the third day is followed by fewer three-plus egg ovulations. All heifers given the PMS injections timed from a synchronized estrus had at least one corpus luteum and eight of the 17 (47%) had either two or three corpora lutea, indicating that the PMS injections can be timed from a synchronized estrus. Four of the eight synchronized heifers that received HCG on the third day had two corpora lutea and four heifers had one corpus luteum. This treatment appeared to be the most promising and was further evaluated in Trial III. Sixteen of the 18 heifers with one corpus luteum had

one or more follicles greater than 10 mm diameter at the time of slaughter, suggesting that a higher level of HCG might stimulate these follicles to ovulate and, thus, increase the number of two and three egg ovulations.

In Trial III, 46 heifers were fed CAP for 18 days. Thirty of the heifers were given 1500 and 2000 IU of PMS on days 5 and 17, respectively, of the estrual cycle following CAP feeding and 16 heifers were used as controls to study ovarian response to the first PMS injection. A high lumbar laparotomy was made on all control and one-half of the PMS treated heifers 5 days following first PMS to determine number, size and location of the follicles on the ovaries. All PMS treated heifers received 4000 IU of HCG and were inseminated on day 3 post-PMS. A second insemination was made 24 hours later. All PMS treated heifers that failed to conceive as evidenced by return to estrus, plus the heifers in the control group were given 1500 IU of PMS on day 5 and 2000 IU of PMS on day 17 of the estrual cycle and 4000 IU of HCG on the third day post-PMS. All heifers were inseminated at the time of HCG injection and 24 hours later.

The PMS treated heifers had a significantly ($P < .01$) greater number of follicles $\geq$ 10 mm diameter than the control heifers 5 days following first PMS. Following the second PMS injection, 40 percent of the heifers had two corpora lutea and only 16.6 percent had three or more. Average ovulation rate was 2.33 for the 30 heifers. The corpora lutea were not at the same place on the ovaries where follicles greater than 10 mm had been observed following first PMS. Only 14 percent of the corpora lutea and 11 percent of the follicles greater than 10 mm were located on the ovary where 5 to 8 mm follicles were present

on the 10th day of the PMS treated cycle. These observations suggest that the second PMS injection stimulated different follicles than were stimulated by the first injection.

The 19 heifers which failed to conceive following the first sequence of injections, showed some refractoriness to the second sequence of PMS injections. Only 51.6 percent of the retreated heifers had two or more corpora lutea compared to 80 percent of the control group and ovulation rates were 1.95 and 3.0 in the retreated and control groups, respectively.

Twenty-four (52.2%) of the heifers in the study conceived. Ten of the 30 (33.3%) heifers conceived following the initial sequence of injections and seven of the 19 (36.8%) heifers from this group conceived following the second sequence of injections. Seven of the 15 (53.3%) heifers in the control group conceived to the inseminations following PMS treatment.

Embryo mortality occurred between 30 and 60 days postinsemination in 15 of the 24 heifers and between 60 and 90 days postinsemination in four of the heifers. Although there was a higher conception rate in heifers with single ovulations (47.3%) than in those with multiple ovulations (37.5%), there was no relationship between ovulation rate and embryonic mortality. The reason for the high embryonic mortality could not be determined, but there was some evidence to indicate the laparotomy following the insemination and the rectal palpations at 30 and 60 days postinsemination as the contributing factors.

In Trial IV, 37 beef cows were treated with 1000 or 1500 IU of PMS on day 5 followed by 2000 IU of PMS on day 17 of the estrual cycle with the PMS injections timed from a synchronized estrus. All cows

received 4000 IU of HCG and were inseminated on day 3 post-PMS. A second insemination was made 24 hours later. All cows that failed to conceive were retreated with 1500 and 2000 IU of PMS on days 5 and 17, respectively, with the PMS injections timed from a non-synchronized estrus. The HCG injections and inseminations were the same as those following the initial sequence of PMS injections.

Cows given 1500 IU of PMS on day 5 had more two and three (57.9% vs. 33.3%) and fewer four-plus (10.5% vs. 22.2%) egg ovulations than those given 1000 IU of PMS on day 5.

The cows were somewhat refractory to the second sequence of injections. Only 37 percent of the cows gave a superovulatory response following the second sequence of injections and the mean ovulation rate was 1.56; however, 29.6 percent had two or three egg ovulations.

Conception rate was 48.6 percent for all cows in the trial. Embryonic mortality occurred in 14 of the 18 cows between 30 and 60 days postinsemination and only four of the cows were pregnant at 90 days postinsemination. It appeared that the laparotomy following the insemination contributed to the high rate of embryonic mortality in Trial IV.

Analysis of variance of the body weight of the animals grouped according to ovulation rate in Trials II, III and IV revealed that there was no relationship ($P > .10$) between body weight and ovulation rate of the cows and heifers within trials.

There was little difference in ovulation rates of the straightbred and crossbred heifers and cows receiving comparable PMS and HCG treatment. Average ovulation rate in 39 straightbreds was 2.31 compared to 2.0 in 31 comparably treated crossbreds.

The results of this study suggest that the first PMS injection makes the animals refractory to the second PMS injection. Thus, resulting in lower ovulation rates and a lower range in ovulation rates than when a single injection of PMS is given on day 16 or 17.

Desirable and repeatable ovulation rates were obtained when 1500 and 2000 IU of PMS were given on days 5 and 17, respectively, with the injections timed from a synchronized estrus and HCG administered on the third day following second PMS. This treatment in 38 heifers and 19 cows resulted in 1.7, 33.3, 40.4, 12.3 and 12.3 percent of the heifers and cows with zero, one, two, three or four-plus egg ovulations, respectively. It appears that these ovulation rates would be acceptable for practical application if the ova produced are capable of undergoing normal fertilization and development. However, conception rate was only 36.0 percent to inseminations following this treatment. It appeared that the laparotomies reduced the conception rate, but there was also some evidence in Trial III to indicate that either all the ova in the multiple ovulated heifers were not fertilized or implanted or that some of the embryos in the multiple pregnancies were resorbed. More work is needed to determine fertilization rates, implantation rates and embryo survival, and ways to improve the same, before such a sequence of injections can be considered suitable for practical application.

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Candidate for the Degree of

Doctor of Philosophy

Thesis: OVULATION RATE OF COWS AND HEIFERS TREATED WITH EQUINE GONADOTROPHIN (PMS) AND CHORIONIC GONADOTROPHIN (HCG)

Major Field: Animal Breeding Minor Field: Physiology

Biographical:

Personal Data: Born at Scotts Hill, Tennessee, November 29, 1942, the son of William C. and Ruby W. Laster. Married Brenda Moore June 10, 1960 and have two children, Jeffery and Dana.

Education: Received the Bachelor of Science degree from the University of Tennessee, with a major in Agricultural Education in August, 1963. Received the Master of Science degree, with a major in Animal Science, from the University of Kentucky in December, 1964.

Experience: Graduate Assistant at the University of Kentucky, 1963-1964. Assistant County Extension Agent in Larue County Kentucky, 1964-1965. Assistant Bull Stud Manager Armour Beef Cattle Improvement Program at Morris, Illinois, 1965. Research Specialist at the University of Kentucky, Agricultural Experiment Station, Lexington, 1966-1968. Graduate Assistant at Oklahoma State University, 1968-1970.

Member: American Society of Animal Science, Alpha Zeta, Gamma Sigma Delta and Associate Member of Sigma Xi.

Date of Degree: May, 1971.