# INDUCTION OF MULTIPLE BIRTHS IN BEEF COWS BY INJECTION OF PREGNANT MARE SERUM (PMS) AND HUMAN CHORIONIC GONADOTROPIN (HCG)

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# CHAPTER I

## INTRODUCTION

Present day production costs represent a dilemma to the cattleman, since under conventional management practices cow productivity cannot keep pace with rising production costs. He is consequently forced to either operate on an increasingly narrow margin of profit or seek feasible means of increasing his net returns. One very promising way of accomplishing this would be to maternally increase the number of calves weaned over the 85% to 90% annual calf crop now obtained.

The development of practical methods for inducing multiple births in beef cows may offer a revolutionary means for increasing reproductive efficiency within an economic unit without drastically increasing the costs of operation. Ovarian stimulation by exogenous gonadotropic hormones has been repeatedly demonstrated in cattle. The use of similar hormones to obtain multiple births has met with only limited success.

One such gonadotropic hormone preparation, pregnant mare serum (PMS), has produced encouraging results under practical conditions in inducing multiple births. However, attempts to limit these multiple births to twins and triplets have been generally unsuccessful. These studies have, however, succeeded in identifying many of the problems associated with multiple births, and have suggested possible areas of research that may offer solutions to some of these problems.

The objectives of this study were to test a sequence of gonadotropin injections combined with estrus synchronization that would limit multiple births to twins and triplets, and yet prove to be practical for the beef producer. Also, problems associated with artificially induced multiple pregnancies and subsequent births were studied.

## CHAPTER II

## LITERATURE REVIEW

### Incidence of Natural Twinning

Inductive methods for obtaining multiple births are necessitated by the low rate of natural occurrence of twinning in all breeds of cattle. The incidence of natural twin births is approximately 0.4% in beef breeds and 1.8% in dairy breeds (Erb et al., 1960). Johansson (1932) and Belić (1941) concluded from numerous European herd book registry records that heritability estimates of twinning tend to vary among all breeds and even between herds within a breed. Erb et al. (1960) reported that, after 30 years of intensive selection for the trait from 1,905 Holstein-Friesian cows, very little increase in the incidence of multiple births had occurred. Donald and Anderson (1953) as well as Mechling and Carter (1964) reported similar results. Mechling and Carter (1964) reported on the results of over 25 years selection pressure applied to a Virginia non-purebred Angus herd by selecting cows born as twins, out of twins, or twin related. The resulting twinning rates were similar to that found in any beef herd. In general, it appears that the heritability of the incidence of natural twinning is probably too low for improvement through selection (Lush, 1925; Lush, 1945; Erb et al., 1960).

### Role of the Gonadotropins

Smith and Engle (1927) and Zondek and Aschheim (1927), working independently of each other, outlined the hormonal relationships between the adenohypophysis and the gonads. The classic work of Casida (1934), using ovine and equine pituitary extracts to induce ovulation in immature female rats and rabbits, has provided the basis for present day use of exogenous gonadotropins to stimulate ovarian activity.

Casida et al. (1943) were the first to demonstrate superovulation in the bovine by use of exogenous gonadotropins. Ovulation was evoked in prepuberal heifers and mature 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.

## Equine Gonadotropin

The exogenous gonadotropin most commonly used to stimulate ovarian activity in domestic mammalian farm animals has been pregnant mare serum (PMS). The classic description of PMS given by Cole and Hart (1930) reported the presence of gonadotropic activity in blood serum from mares at progressive intervals of gestation as measured by effect on sex maturation changes in immature female rats. Equine gonadotropin first appears at the thirty-seventh to the forty-second day of pregnancy (Cole and Hart, 1930) reaching a peak concentration between the fiftieth and seventieth days of gestation (Cole and Hart, 1930; Day and Rowlands, 1940; Schmidt-Elmendorff et al., 1962) and generally decreasing to minimum levels of concentration during the sixth and seventh months (Day and Rowlands, 1940; Cole, 1963). It is secreted by the structures known as the endometrial cups of the uterus. These circular structures on the surface of the endometrium around the point of attachment of the fetus secrete a highly viscous gel which may contain many thousand international units of gonadotropin per gram.

Equine gonadotropin is extracted from the blood of pregnant mares, or may be obtained directly from the endometrial cups of the gravid uterus (Cole, 1936; Rowlands, 1965; Butt, 1967). The products of different extraction procedures vary markedly in biological activity. For example, one such procedure for endometrial cup extraction via freeze-drying yields about 100 I.U. per milligram (Morris, 1964), whereas a cellulose chromatography and gel filtration technique results in about 15,000 I.U. per milligram preparation (Butt, 1967). A more practical technique consists of alcohol precipitation of PMS from blood serum, adsorption chromatography on Permutit columns (ion exchange resin) and then adsorption of protein impurities on barium carbonate precipitated _in situ_, and counter-current distribution in a system of ammonium sulfate ethanol, n-propanol and water. The resulting preparation with a potency of 16,000 I.U. per milligram appeared to be quite homogenous following ultracentrifugation and electrophoresis (Ligault-Démare _et al._, 1958; Jutisz, 1965).

A complete chemical analysis of PMS by Bourrillon and Got (1960) indicates the material to be 48.6% carbohydrates and 10.4% sialic acid, with a molecular weight of about 70,000. The hormone has properties of both FSH and LH with a predominance of the former. Unlike the pituitary gonadotropins, PMS cannot be separated into two entities (Turner, 1966; Van Tienhoven, 1968). The inseparable nature of the hormone and its constituents limits standardization procedures to bioassay for determination of its biological activity (Bell, 1966; Butt, 1967). The rat ovarian weight test is presently the most commonly employed assay due to its simplicity and accuracy (Cartland and Nelson, 1938; Butt, 1967). Initially, Cole and Erway (1941) proposed a 48 hour method of assay based upon the ovarian weight response of 25 day old immature female rats. A slight modification involves a two point assay using younger (22 day old) rats. This procedure is more rapid and simpler than the older 96-hour minimal response assays. It is more specific and probably more accurate than assays based on uterine weight (Cole and Bigelow, 1967). Unlike HCG, PMS remains in the blood and lymph and is practically absent from the urine. The larger molecular size offers some explanation for PMS not crossing the fetal placental barrier. Thus, PMS *in vivo* exerts a more prolonged gonadotropic effect than HCG or purified extracts of FSH and LH (Cole, 1936; Folley and Malpress, 1944; Turner, 1966).

## Human Chorionic Gonadotropin

The presence of a gonadotropin in the urine of pregnant women was first described by Aschheim and Zondek (1927). The biological activity of this hormone was found to resemble that of luteinizing hormone (LH) and was named human chorionic gonadotropin (HCG) (Leonard and Smith, 1934; Engle, 1939).

HCG is a glycoprotein consisting of about 21% carbohydrate and 8.5% sialic acid with a molecular weight of about 30,000 (Morris, 1955; Got and Bourrillon, 1960).

The major site of production of HCG is the Langhans cells in the cytotrophoblast villi of the placental chorion (Diczfalusy and Troen, 1961; Midgely *et al.*, 1963; Pierce and Midgely, 1963). HCG can be detected as early as the twenty-fourth day (day one is the first day of last menstruation) and rises to a peak (100 to 120 I.U. per ml of serum) at about the fiftieth day, remains at this concentration for about 14 days and drops to a low level before the ninetieth day (Rowlands, 1964). Until recent replacement by immunological assays (Butt, 1967) HCG served as criteria for such human pregnancy tests as Aschheim-Zondek, Friedman and Galli-Mainini (Turner, 1966).

HCG can be extracted from the urine of pregnant women by adsorption on benzoic acid and separated out by ion exchange chromatography (Butt, 1967). Got and Bourrillon (1960a, 1960b) used a purified benzoic acid extract procedure and obtained a homogenous product of biological potency ranging from 12,000 to 13,000 I.U. per milligram. Having a small molecular size, HCG besides being filtered through the kidney may also be transported through the fetal chorion.

## Superovulation for Ova Recovery

Superovulation and ova recovery have been accomplished by various levels of gonadotropins in both prepuberal (Marden, 1952; Black et al., 1953; Avery et al., 1962; Jainudeen et al., 1966; Onuma et al., 1969) and postpuberal females (Dowling, 1949; Dziuk et al., 1958; Avery et al., 1962; Rowson, Moor and Lawson, 1969; Sreenan, 1969; Vincent et al., 1969), for the purpose of ova transplantation. Recovery of fertilizable ova from a genetically superior calf might shorten the generation interval by allowing earlier progeny tests (Onuma et al., 1969). Fertilizable ova which could be transplanted from a superior cow to a host cow could increase prolificacy of desired animals which would ultimately bring about genetic improvement (Avery et al., 1962). Unlike present requisites for inducing multiple births, ova recovery technique would require production of maximum fertilizable ova. However, as suggested by Rowson, Lawson and Moor (1969) egg transplants might someday be the key to multiple birth production since both the number and quality of calves could be controlled.

## Superovulation for Multiple Births

Since the initial reports of Casida (1934) and Casida et al. (1943) different gonadotropins and combinations of gonadotropins have been used to induce multiple ovulations in cattle. The ovarian response to exogenous gonadotropins is dependent on the stage of the cycle at which treatment is initiated and whether or not the corpus luteum is removed prior to or during treatment (Hafez et al., 1965). However, expression of the corpus luteum is generally a hazardous procedure (Dawson, 1961).

PMS is probably the most commonly used exogenous gonadotropin for inducing bovine superovulation and plural births largely because of its relative inexpensiveness and prolonged retention within the body (Folley and Malpress, 1944; Rowson, 1951; Gordon et al., 1962). PMS simplifies the multiple injection schedule required by most other FSH-like gonadotropins to possibly a single or split injection sequence.

From studies considering various dosage levels of different gonadotropins, some indication of linear relationship between dosage level and ovarian response has been noted. Bellows et al. (1969) observed a linear dose-response relationship between dosage of FSH and the ovarian characteristics studied. Preliminary work with PMS by Hammond (1949) demonstrated the importance of dose level. However, results from dosages of 1,000 I.U. to 5,000 I.U. of PMS failed to show a relationship in number of corpora lutea with dosage level (Hammond and Bhattacharya, 1944). In contrast, Gordon et al. (1962) recorded increasing ovulatory responses from dose levels of 800 I.U. up to 2,000 I.U. PMS given as one subcutaneous injection. The level of 800 I.U. gave a response that was too low, while those levels nearer 2,000 I.U. gave more variation in response. Lamond and Clark (1960) using dose levels of 900 to 1,000 I.U., noted that small increases in doses gave progressive increases in ovulations.

The type of gonadotropin used for obtaining plural births from superovulation may have an effect on ovulatory response. Dziuk et al. (1958) concluded that purified extracts of FSH gave more satisfactory results and were more predictable than PMS. Hammond and Bhattacharya (1944) found equine pituitary extracts to compare favorably with PMS for inducing superovulation. However, Rowson (1951) and Hammond (1959) recommended the use of PMS over that of other gonadotropins. Rowson considered the advantages of PMS, especially its stability in the body, effectiveness and lower cost, to outweigh the advantages of pituitary extracts. Willett and Buckner (1953) demonstrated that not only is one injection of PMS on day 15 or 16 of the cycle cheaper than five daily injections of sheep FSH but that it produces a similar ovulatory response. A predominance of 2- egg ovulations was obtained with 2,000 I.U. PMS given on day 16 of the cycle.

The phase of the estrous cycle during which gonadotropic hormones are given can affect response of the ovary. Dowling (1949) concluded that higher fertilization rates could be expected from gonadotropin treatments administered during the follicular phase of the cycle than from treatment given in the luteal phase. Similar findings were reported by Willett et al. (1925b), Avery et al. (1962) and Scanlon et al.

(1968).

Hammond (1949) also recognized the importance of the timing of the gonadotropic injection. He suggested that injection be timed at about 3 to 4 days before estrus. Equally as important to correct timing of injection is a possibility of decreased fertilization rates and increased abortions if more than two or three ovulations occur (Hammond, 1949; Gordon et al., 1962). For example, Lotti and Galli (1961) reported that 12 cows produced 5 singles, 3 sets of normal twins and 4 cows aborted either triplets or quadruplets. This followed the injection of 500 I.U. of FSH 3 to 4 days later. When 1,000 I.U. of FSH was given as a single injection 2 to 3 days before estrus, 8 cows produced normal twins, 3 produced normal triplets and 2 aborted (2 fetuses at 6 months and 4 fetuses at 7 months).

A single injection of PMS on the fifteenth to the seventeenth day of the cycle has been used most often for inducing superfetation (Petrov, 1960; Arbeiter, 1962; Gordon et al., 1962; Hafez et al., 1964). PMS administered at the rate of 1,000 I.U. late in the follicular phase proved more optimal for two egg ovulations and fewer abortions when compared to 2,000 I.U. (Pelli and Castelli, 1962) or 3,000 I.U. of PMS (Denny, 1964). Gordon et al. (1962) attained only limited success in inducing multiple births in beef and dairy cattle with a single PMS injection. This extensive field trial included treatment of 525 cows of which pregnancy data were collected from 416 cows through various cooperating farmers in Wales. Hormone treatment consisted of a single subcutaneous injection of 800, 1,000, 1,200, 1,600 or 2,000 I.U. of PMS on day 16 or 17 of the cycle. Following hormone treatment ovulation rates were determined by rectal palpations. These rates ranged from 1 to 25 averaging 1.47 to

3.97 ovulations. Both range and average number of ovulations increased with the dosage level. Manifestations of estrus were observed in about 75% of the treated cows 4 to 6 days after PMS injection. Artificial insemination resulted in a conception rate of 76.2% with 30.3% multiple pregnancies as determined by rectal palpation at 6 weeks gestation. At this time 19.2% had twins, 8.8% had triplets and 1.9% had quadruplets. However, these workers believed that palpation resulted in 32.5% of those cows pregnant either aborting or resorbing, and a final result of 44 multiple births at parturition (35 sets of twins, 8 sets of triplets and 1 set of quadruplets). Based on the results of this field trial 2,000 I.U. of PMS is probably the most optimal level tested for inducing superfetation. This level of PMS, though, has been indicated by other researchers as being too high for optimal results (Dowling, 1949; Hafez and Ishibashi, 1964; Jainudeen et al., 1966; Scanlon et al., 1968).

Superovulation and multiple births have also been obtained from lower dosage levels of PMS. Hammond (1949) reported that 1,500 I.U. PMS provided sufficient follicular growth for twin ovulations.

Ovulation rates for this or a similar dose level of PMS given late in the follicular phase of the cycle used in different studies has not been consistent. For example, 1,600 I.U. produced an average ovulation rate of 1.71 for Gordon et al. (1962) whereas, Hafez, Jainudeen and Lindsay (1965) reported a mean ovulation rate of 5.4 following administration of 1,500 I.U. of PMS on day 16 of the estrual cycle. Jainudeen and Hafez (1966) reported a mean of 3.7 ovulations using the same level of PMS. Schwartz and Shelby (1969) observed an average rate of 4.72 following a single injection of 1,500 I.U. of PMS on day 16 of the cycle.

Arbeiter (1962), using a single PMS injection of 1,500 I.U. reported a conception rate of 76.5% at the first post-PMS estrus and produced 2 sets of twins, 1 set of quadruplets and 1 set of quintuplets from the 19 cows originally treated. Hafez et al. (1964) treated 79 cows with a single injection of 1,500 I.U. PMS on day 17 of the cycle. Use of natural and artificial insemination at the post-PMS estrus resulted in a 65% conception rate. At 30 to 60 days gestation, palpation indicated of the total cows treated 11% were pregnant with twins, and 13% with triplets, quadruplets or quintuplets.

Wide ranges in ovulation rates and unpredictable variation in litter size resulting from either a sequence of FSH injections or a single injection of PMS has prompted work to develop a more promising regime. Schilling and Holm (1963) reported that ovulations could be limited to two or three eggs by giving a sequence of two PMS injections, one of 1,500 I.U. on day 5 after heat followed by 2,000 I.U. on day 16 to 18 of the cycle. All cows exhibited estrus 3 to 5 days following the second PMS injection and were given 4,000 I.U. of LH intravenously. The results indicate 8 of 11 cows producing multiple ovulations none of which had in excess of three ovulations. These researchers also using various combinations of progesterone with PMS, but the results of those treatments including progesterone were not very successful. Further studies attempting to either limit or control superovulation using a sequence of injections similar to that of Schilling and Holm (1963) have been reported (Hafez et al., 1965; Schwartz and Shelby, 1969; Turman et al., 1971; Laster et al., 1971b).

Following PMS subcutaneous injections of 750 I.U. on day 5 and 1,500 I.U. on day 16 of the cycle, Hafez et al. (1965) reported a mean ovulation rate of 4.8. The mean was increased to 6.0 ovulations when the second injection was increased to 2,000 I.U. In some cases 750 I.U. HCG were given intravenously on either day 20 or day of estrus. However, HCG was found unnecessary in order for ovulation to occur.

Schwartz and Shelby (1969), using 18 nulliparous beef heifers, reported a similar ovulation rate of 6.56, with a wider range, following 1,500 I.U. of PMS given on day 5 and 2,000 I.U. of PMS on day 16 followed by 1,000 I.U. of HCG on day of estrus or day 21 of the cycle. The lower ovulation rate of 4.72 was obtained using 1,500 I.U. of PMS in one injection on day 16 of the cycle. Fertilization rates in the two treatments to inseminations were 11% and 22%, respectively. Low fertilization rates and small numbers of multiple births may have been affected by high lumbar laparotomy performed from 8 to 12 days following insemination.

The highest percent of multiple births reported to date 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., 1970; Turman et al., 1971). PMS was administered at the rate of 1,500 I.U. on either day 4, 5 or 6 and 2,000 I.U. on either day 16, 17 or 18 of the cycle. On day of estrus the cows were bred by natural service and given an intravenous injection of 2,500 I.U. of HCG. Of 81 cows treated, 52 (64.2%) conceived to the first estrus after the second injection of PMS. These 52 cows produced 29 singles and 23 multiple births (12 sets of twins, 8 sets of triplets, 2 sets of quadruplets and 1 set of quintuplets). This study ultimately yielded approximately a 173% calf crop on the basis of those cows conceived to the post-PMS estrus or 109% calf crop weaned for all cows treated. It was found that there were no significant differences in response of cows in terms of numbers of multiple births associated with whether the first PMS injection was given on day 4, 5 or 6 and whether the second injection was given on day 16 or 17. However, a second injection on day 18 appeared to be too late for optimal results. Due to the nature of this study, to evaluate the response of PMS on the basis of multiple births, no attempt was made to determine ovulation rate following PMS treatment.

In a more recent study, Laster et al. (1971b) treated 16 cows with 1,500 I.U. PMS on day 5 and 2,000 I.U. of PMS on day 17 of the cycle. HCG was given at the rate of 2,500 I.U. on day of estrus in 8 cows or on the third day following second PMS, disregarding the occurrence of estrus, in 8 cows. The mean ovulation rate was 3.38 for all cows that ovulated or 3.57 for those receiving HCG on day of estrus and 2.86 for those receiving HCG on day 3 post-PMS. 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.

Gonadotropin Treatment Combined With Estrus Synchronization

The success of synchronizing estrus prior to treatment could lead to more precise timing of the injections, plus reduce much of the labor involved with estrus detection and individual handling of cows within a herd. Also, additional attention required by multiple births at time of parturition might be concentrated within a smaller period of time with the aid of estrus synchronization. Synchronization by progestogens without superovulation has shown encouraging results (Parker et al., 1965; Hansel et al., 1966; Turman et al., 1967; Wiltbank and Kasson, 1968). Hansel et al. (1966) utilizing 408 beef cows recorded higher conception rates to one service in non-synchronized cows (59.8%) than in MAP (6-α-methyl-17-acetoxyprogesterone) treated cows (48.7%) or CAP (6-chloro-$\Delta^6$-17-acetoxyprogesterone) treated cows (34.8%) when bred on the synchronized estrus. Similar unsatisfactory conception rates were obtained from a sequence of 6 daily injections of progesterone followed by a single injection of PMS (Lamond, 1964). However, Zimbleman (1963), Hansel et al. (1966) and Turman et al. (1967) using either CAP or MAP had more satisfactory fertility on the subsequent estrus to the post-synchronization estrus. Van Blake et al. (1963) and Hansel et al. (1966) suggest that MAP-fed cows return to estrus earlier following withdrawal of the hormone than do cows fed CAP.

Synchronization of estrus by use of oral progestogens prior to administration of PMS has resulted in decreased fertility. Jainudeen and Hafez (1966) reported that MAP feeding was followed by a 14% conception rate compared to a 57% conception rate for unsynchronized cattle at the post-PMS estrus. From 2 to 6 days after withdrawal of the hormone, estrus was synchronized in over 90% of those cows fed 180 mg of MAP for 18 days without interference to ovarian response from exogenous gonadotropins. PMS was administered 24 hours following the last MAP feeding at the rate of either 1,000, 1,500 or 3,000 I.U. prior to 1,000 I.U. of HCG given on day of estrus.

Recent studies have offered promising results from estrus synchronization combined with FSH treatment. Bellows et al. (1969) indicated the most desirable results for various levels of FSH tested when MAP was fed 11 days with estradiol valerate given on day 2 followed by FSH injected twice daily on days 8, 9, 10, 11 and 12.

Reynolds et al. (1969) compared MGA (6-α-methyl-6-dehydrc-16-methylene-17-acetoxyprogesterone) to MAP feeding in conjunction with estradiol and FSH treatment. The first estrus after MAP and MGA resulted in a 55.3% conception rate, or 33% twin births and 17% quintuplet births compared to a 45.6% conception rate or 20% twin births based on 30 day slaughter data for the progesterone schedule, respectively.

Using the schedule demonstrated by Bellows et al. (1969) and Reynolds et al. (1969), where estradiol and FSH injections were given in conjunction with MAP feeding, Bellows et al. (1970) reported the results obtained from 43 beef heifers. Twenty-four of 43 heifers (56%) calved from the first breeding. These heifers produced 19 singles and 5 sets of twins for a 121% calving rate on the basis of those conceived to the first estrus after the last FSH injection or a 90% calving rate for all cows treated.

Vincent and Mills (1970) utilized 93 lactating beef cows in testing various levels and different injection sequences of porcine FSH in combination with norethandrolone, a progestogen, for inducing multiple births. Norethandrolone was injected intramuscularly at the rate of 5 mg daily for 4 days beginning on day 14 to 16 of the cycle in two trials. Fifty-one percent of the FSH treated cows had multiple ovulations with an average of 1.9 corpora lutea per cow. Average conception in a 32 day period was 56% compared to 46% and 65% in norethandrolone and control cows, respectively. Similar ovarian response was noted among all treatments. Ten sets of twins and two sets of triplets resulted from the 52% conception rate to the first breeding (natural service).

Vincent (unpublished data) using FSH dosages from 6.25 to 15 mg divided into equal doses administered these once a day or twice a day for 5 days beginning at the first norethandrolone injection. A schedule of 10 daily injections of 5 mg norethandrolone and a single injection of

5 mg estradiol on the second day of the norethandrolone injections was followed in two trials. Porcine FSH or Vetrophin (ovine FSH-LH activity) in saline or CMC (1% sodium carboxymethyl cellulose, used to prolong absorption of gonadotropins) was given as a single injection on the last day of norethandrolone treatment. A third trial, all animals received 250 mg norethandrolone implants and injections of estradiol on day 0. The implants were removed on day 10 and each animal was given subcutaneous injections of 5 mg norethandrolone in 1 cc of sesame oil and 10 mg FSH in 5 cc CMC. A portion of these received 500 mcg DES (diethylstilbesterol) on day 12.

These three trials, attempting to combine a single FSH injection schedule with artificial insemination, yielded results less promising than those reported by Vincent and Mills (1970). Although overall conception rate was low (43%), the administration of pituitary gonadotropins as a single injection did produce desirable ovulation response. In this case, approximately 29 of 109 cows conceived to the first estrus after gonadotropin treatment to produce 5 multiple births (4 sets of twins and 1 set of triplets). Many of those cows used in these trials were known to be sub-fertile or had been treated with gonadotropins prior to the start of this study. Such factors may have influenced the low conception rates and poor gonadotropin response, whereas, the shortened 32 day breeding season could have affected the results of both Vincent and Mills (1970), and Vincent (unpublished data).

Utilizing artificial insemination and estrus synchronization, Laster et al. (1971b) used an injection schedule similar to the procedure demonstrated by Turman et al. (1971). However, PMS subcutaneous injections were made on days 5 and 17 of the estrus cycle and were timed from either a natural or synchronized estrus. Intravenous injections of HCG were made on day of estrus or on day 3 following the second PMS injection disregarding the occurrence of estrus. Treatment with 1,500 I.U. PMS on day 5 and 2,000 I.U. PMS on day 17 of the cycle, counting day 3 post-CAP as day 0, with 2,500 or 4,000 I.U. HCG given on day 3 following the second PMS injection gave the most promising and repeatable ovulation rates. Fifty-seven cows and heifers had 52.7% two and three-egg ovulations. However, conception rate to insemination which occurred at time of estrus or on days 3 and 4 after second PMS injection was low, being only 36%. High fetal mortality rate and low conception rate may have been due to the high lumbar laparotomy performed 5 days after the first PMS injection and again 5 to 12 days after HCG to determine ovarian response.

## Factors Affecting Multiple Births

Age and parity of the cow may influence artificial induction of twinning. Gordon _et al._ (1962) suggested that with increasing age there was an increasing incidence of multiple ovulations, and also that multiple pregnancies are more easily sustained later in life. Johansson (1932) working with numerous European breed records, found a rapid rise in the frequency of multiple births up to 5 years of age followed by a slower increase in the frequency up to 10 to 11 years of age.

Multiple births are probably unaffected by seasons of the year (Gordon et al., 1962) with the exceptions of lower fertility and increased prenatal mortality (Scott and Williams, 1962; Vincent and Mills, 1970).

Time interval between the final PMS or FSH injection and the subsequent estrus can affect ovarian response (Hammond, 1949, 1959; Brock and Rowson, 1952; Gordon et al., 1962; Hafez et al., 1963). Up to 7 days after PMS treatment ovulations increase directly with the interval of delay (Brock and Rowson, 1952). Brock and Rowson (1952) failed to stimulate superovulation after a 7 day interval while Turman et al. (1971) obtained ovarian response as late as the eighth day. Turman et al. (1971) noted that 65% of cows conceiving at the first post-PMS estrus did so on day 3, 4 or 5 post-PMS and produced 87% of the multiple births.

Generally, HCG or some other forms of exogenous LH has been used in conjunction with various gonadotropin induction regimes for multiple births. Supporting evidence suggests that sufficient LH is available in the cow to induce multiple ovulations (Hammon, 1955; Gordon et al., 1962; Hafez et al., 1965), thus exogenous LH may serve to limit or control multiple ovulations.

Wiltbank et al. (1961) found that in non-gravid cows regression of the corpus luteum would occur approximately 2 to 7 days subsequent to an injection of 5 mg estradiol valerate. This knowledge offers the possibility for more efficient synchronization of the estrual cycle and may allow increased accuracy in the timing of the gonadotropin injections for induction of multiple births. For example, estradiol combined with estrus synchronization and FSH treatment has been used successfully for inducing superfetation and multiple births (Reynolds et al., 1969; Bellows et al., 1970; Vincent, unpublished data). Rowson (1951) and Greenwald (1968) concluded that estrogen may affect fertility in that administration of the hormone slows or even stops the passage of ova through the oviducts following ovulation. In contrast, Hafez et al. (1963) noted that estrogen included in a gonadotropin schedule for inducing superfetation had no effect on fertility, although when given before PMS as compared to administration after PMS, subsequent decreases and increases in ovulation rates were recorded.

The need for a safe and effective diagnostic method for identifying prenatal litter size has been prompted by high rates of prenatal mortality and abortions in cows supporting multiple fetuses. Ultrasonic detection, though useful in sheep and pigs, has not produced favorable results in cattle (Fraser and Robertson, 1968; Hulet, 1968). Rectal palpation, the technique most commonly used to detect pregnancy and fetal number, has been associated with increased fetal resorption and abortions (Gordon et al., 1962). These researchers did report that day 42 of pregnancy was the most effective time for palpating to determine the occurrence of multiple fetuses. Turman et al. (1971) reported good prenatal survival following a single rectal palpation for determination of pregnancy performed 3 to 5 months post-breeding.

Failure of conception to occur following one sequence of PMS or other source of FSH injections would encourage additional treatments in order that superovulation lead to superfetation. However, repeated treatment with exogenous gonadotropins has been demonstrated to cause either partial or total ovarian refractoriness (Hafez et al., 1964; Laster et al., 1971b; Vincent and Mills, 1970). The degree of ovarian refractoriness appears to vary according to type and form of gonadotropin. Gordon et al. (1962) reported that PMS treatment during one year did not affect the superovulatory response of the following year. Willett and Buckner (1953), contrary to Gordon et al. (1962), noted a failure in long intervals of time to eliminate the refractoriness of the ovaries to gonadotropins, although progressive increases in dose level according to successive gonadotropin treatments resulted in superovulatory response.

CHAPTER III

# MATERIALS AND METHODS

## General Procedure

This study was conducted from May, 1970, to May, 1971, at the Fort Reno Livestock Research Station, El Reno, Oklahoma. The experimental animals were 65 lactating, multiparous beef cows consisting of 17- 3 year old Hereford cows, 12- 7 to 9 year old Hereford cows, 25- 4 year old Angus-Holstein crossbred cows and 11- 4 year old Angus-Hereford crossbred cows. The cows had calved in February, March and April and were maintained under range conditions on native grass and bermuda grass pastures. The cows were divided into two groups so that each cow was allowed a post-partum interval of at least 50 days before starting treatment. Within each of the two post-partum groups cows were assigned to one of four different treatment groups on the basis of breed of cow, age of cow, sex of calf, post-partum interval and post-partum ovarian activity. Post-partum ovarian activity or evidence of recycling was determined in all cows prior to treatment group assignment and after a 50 day elapsed post-partum interval by rectal palpation.

### Hormones Used

Hormone preparations used in this study were pregnant mare serum (PMS), human chorionic gonadotropin (HCG), and 6-chloro-$\Delta^6$-17-acetoxy-progesterone (CAP) provided by the Eli Lilly Company, Greenfield, Indiana. The gonadotropins were lyophilized and had been standardized prior to shipment to a stated potency of 100 I.U. PMS and 80 I.U. HCG per mg. However, after treatments had been completed Eli Lilly Company discovered an error in calculation so the actual biological activity of the PMS was 72 I.U. per mg. A soybean meal premix served as a carrier for the CAP so that 4.54 gm of premix contained 10 mg of CAP or each 2.27 gm contained 5 mg CAP.

Storage and Administration of Gonadotropins

Precautionary measures were taken to protect the gonadotropic hormones from heat, moisture and excessive light prior to injection. The lyophilized gonadotropins were stored in tape sealed bottles within cotton-filled, sealed canisters and refrigerated at $3^{o}C$. Prior to injection the gonadotropic hormones were weighed on a Mettler scales (Type B balance) into predetermined individual dosages which were placed in 15 ml dry test tubes, stoppered, tape-sealed and stored in the refrigerator of the physiology laboratory. A cotton padded styrofoam cooler was used for transporting the PMS and HCG from the laboratory in Stillwater to the Fort Reno Research Station.

The PMS and HCG were reconstituted with 10 ml of double distilled water and 10 ml of physiological isotonic solution, respectively, immediately prior to injection. Care was taken to assure complete homogeneity of solution.

PMS injections were administered subcutaneously in the girth region or in the neck region of the animal, whereas, the HCG was injected intravenously into the jugular vein.

## Estrus Synchronization

Synchronization of estrus was accomplished by feeding CAP for 18 days, 10 mg per day the first 16 days and 5 mg per day the remaining 2 days. All cows that were fed CAP were started together without regard for individual stage of estrous cycle. Cows in estrus synchronization groups were fed individually in restraining stalls once daily at about the same time each day. Each daily dosage of CAP was weighed into individual packets and at time of feeding was mixed into approximately 1.5 lbs ground milo.

## Breeding Procedure

Detection of estrus in all treatments was determined visually by the combined appearance of clear mucus (Müürsepp, 1967), vulvular swelling and standing to be ridden by vasectomized bulls. Visual observations were made at least twice daily, in early morning and late afternoon, and more frequently during the more critical periods, such as the dates when the animals were due to return to estrus and following the withdrawal of CAP.

Frozen semen from an Angus bull of proven fertility obtained from American Breeders Service was used for the treatment groups bred by artificial insemination. Following an approximately 20 minute ice-water thawing of the liquid nitrogen frozen semen, the semen was deposited in the body of the uterus by means of a breeding tube.

Angus bulls of proven fertility were used in treatments bred by natural service. Periodic rotation of bulls among treatments was exercised to increase the activity of each bull and to decrease possible fertility variation effects among bulls.

In all four treatment groups following the post-PMS estrus each cow was placed in a pasture with a fertile bull until September 1, which resulted in a breeding season of 60 to 90 days duration.

Feeding

The cows were wintered on native grass pastures at Fort Reno. During approximately the last two months of gestation or from January to calving the cattle were supplemented at the rate of about 2.5 lbs of 21% alfalfa-cottonseed meal pellets and 20 lbs of alfalfa hay and bermuda grass hay per head per day.

## Specific Procedure

This study consisted of one trial composed of four treatment groups as indicated in Table I.

In the non-synchronized treatment group, the PMS injections were given on day 5 (first PMS) and day 17 (second PMS) of the estrual cycle with day of estrus counted as day 0. Previous work had indicated that most cows synchronized by feeding CAP would be in estrus on days 2, 3 and 4 following the withdrawal of CAP. Therefore, in the synchronized treatment groups day 3 was designated as average day of post-CAP estrus and was counted as day 0 for all animals in the group. The PMS injections were given on day 8 and day 20 post-CAP which was equivalent to days 5 and 17 of the cycle. In all treatment groups, the PMS levels were 1,080 I.U. and 1,440 I.U. for first and second PMS injections, respectively.

Treatment group I included 15 multiparous beef cows. The gonadotropin injections were timed from a non-synchronized estrus. Each cow

TABLE I

TREATMENT SCHEDULE[a]

| Treatment Group | No. Cows | Synchronized[b] | Time of Injection: PMS Injections[c] 1080 I.U. | PMS Injections[c] 1440 I.U. | HCG[d] 2500 I.U. | Breeding: Time | Breeding: Type |
|---|---|---|---|---|---|---|---|
| I | 15 | No | Day 5 post-estrus | Day 17 post-estrus | Day of estrus | 1st. post-PMS estrus | Natural |
| II | 16 | Yes | Day 8 post-CAP[e] | Day 20 post-CAP | Day of estrus | 1st. post-PMS estrus | Natural |
| III | 16 | Yes | Day 8 post-CAP | Day 20 post-CAP | Day of estrus | 1st. post-PMS estrus | A.I. |
| IV | 18 | Yes | Day 8 post-CAP | Day 20 post-CAP | Day 3 post-PMS | Days 3 and 4[f] post-PMS | A.I. |

[a]Treatments imposed June, 1970.

[b]10 mg CAP/head/day for 16 days, followed by 5 mg/head/2 days fed individually.

[c]Subcutaneously

[d]Intravenously

[e]Average day of estrus was 3 days post-CAP.

[f]All cows inseminated both days regardless of occurrence of estrus.

was placed in a lot with a fertile bull immediately following administration of the second PMS injection. On the day of the first post-PMS estrus, when a cow was observed to have mated, she was given an intravenous injection of 2,500 I.U. of HCG and mated to a second bull.

In Treatment group II, 16 cows received gonadotropin injections timed from a synchronized estrus. Breeding procedure and HCG administration were the same as that used in the first treatment.

Sixteen cows, representing Treatment group III were also treated with PMS timed from a synchronized estrus. However, the cows were artificially inseminated on day of estrus following the second PMS injection at which time 2,500 I.U. of HCG were injected intravenously.

The 18 cows in the Treatment group IV also received PMS timed from a synchronized estrus, but in this case all PMS injected cows were administered 2,500 I.U. HCG on day 3 post-PMS and were inseminated twice: the time of the HCG injections and 24 hours later.

Pregnancy was determined by rectal palpation as described by Wisnicky (1948) 4 to 5 months post-PMS breeding or approximately 30 days after removal of the fertile bulls.

Starting in November all cows were weighed every 28 days following an overnight shrink away from feed and water in an effort to maintain progressive weight gains by detecting and preventing critical weight losses and to attempt to establish maintenance requirements for those cows with multiple fetuses. In March, or at the onset of the calving period, the interval between weighing was reduced to 14 days. Any cow having calved 3 days prior to the scheduled day for weighing or any cow expected to calve within the next 3 days after weighing was not weighed until the next scheduled weigh period. Weighing was discontinued on an individual cow basis following parturtion, and for the entire herd on May 1.

Survival of multiple births was aided by confining such cows and calves to bedded, covered stalls provided with heat lamps. Weak calves received approximately one pint of colostrum, obtained from the Oklahoma State University dairy herd and stored in frozen form until needed, via stomach tube and 1 cc of epinephrine (1 cc = 1 mg adrenalin) given intramuscularly.

Any cow failing to immediately accept all or part of her litter was stanchioned periodically to enable calves to nurse until she would accept the calves. All multiple births and their dams were confined for observation for at least one week before being turned out to pasture. Reproductive tracts from all dead multiple birth heifers were examined for possible freemartin characteristics. With the exception of one cow, who was allowed to raise her own triplets, efforts were made to insure that as many as possible of the cows that calved multiples reared twins. Calves were reared without access to creep or supplemental feed. All bull calves were castrated at approximately 2 months of age.

## Statistical Analyses

The data were analyzed by analysis of variance (Steel and Torrie, 1960). Duncan's New Multiple Range Test was used to determine differences among group means of gestation lengths and birth weights within the various types of births (Steel and Torrie, 1960). Survival rates among the treatment groups were analyzed by a chi-square analysis to determine if there were differences. The "F" test was used to test for differences between birth weights from natural service and those from artificial insemination. Subsequent to a two sample comparison from a binomial population a "t" test was used to determine if there were differences in numbers of multiple births among the treatment groups on the basis of total cows treated and also on the basis of cows conceiving to the first estrus after second PMS. Conception rates among the different treatments were analyzed by the same procedure.

A two sample comparison from a binomial population was utilized in order that differences between one proportion of one sample $n_1$ and another proportion of a second sample $n_2$ might be detected. Differences are described in terms of successes and failures, where,

$P_1-P_2$ = is the difference between the successes (multiple births or conceptions) in two different independent proportions.

$f_1-f_2$ = is an unbiased estimate of $P_1-P_2$.

$f_i$ = an unbiased estimate of $P_i$.

$f_i = \frac{Y_i}{n_i}$

$Y_i$ = the observed number of successes in each successive sample (multiple births or conceptions).

$n_i$ = the size of each successive sample (total cows in each treatment group).

The variance of $f_i$ is estimated by

$$s^2_{f_i} = \frac{f_i\ (1-f_i)}{n_i}$$

The variance of the difference of $f_1-f_2$ is the sum of their variances.

$$s^2_{f_1-f_2} = \frac{f_1\ (1-f_1)}{n_1} + \frac{f_2\ (1-f_2)}{n_2}$$

The estimated standard deviation of their differences is

$$s_{f_1-f_2} = \sqrt{s^2_{f_1-f_2}}$$

The calculated t value was computed as

$$t = \frac{f_1-f_2}{s_{f_1-f_2}}$$

# CHAPTER IV

# RESULTS AND DISCUSSION

## General Comments

Initially, 69 cows were included in this study. Four were found to have been pregnant at time of gonadotropin treatment and consequently were rejected from the analyses. No untreated control animals were used.

## Conception Rates and Response to PMS

Of the 65 cows treated, 21 (32.3%) conceived at the first post-PMS estrus (Table II). Conception rates at the first post-PMS estrus for Treatments I, II, III and IV were: 10 (66.7%) of 15 cows treated, 5 (31.3%) of 16 cows treated, 3 (18.8%) of 16 treated and 3 (16.7%) of 18 treated, respectively. All cows that conceived to the first post-PMS estrus subsequently calved, with the exception of one animal in Treatment I. Eleven cows were found to be not pregnant at palpations carried out in October and November. Fifty-two of the 54 pregnant cows that were wintered calved.

In the non-synchronized treatment group the conception rate as determined by palpation at approximately 120 days was 100%. These results compare favorably to previous conception rates from non-synchronized PMS treated cows (Hammond, 1949; Gordon et al., 1962; Hafez, Sugie and Hunt, 1963; Hafez et al., 1964). However, reports from Hafez et al. (1965) and Schwartz and Shelby (1969) have implicated PMS with lowered fertility

TABLE II

SUMMARY OF THE BREEDING PERFORMANCE OF ALL COWS TREATED WITH PMS AND HCG AND THE CALVING PERFORMANCE OF COWS CONCEIVING TO NATURAL SERVICE OR A.I. AT THE FIRST POST-PMS ESTRUS

| Treatment Group | Body wt. (lb) | Total treated | No. conceived at first post-PMS estrus | No. open (60-90 days) | No. of cows conceiving at first post-PMS estrus producing | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Singles | Twins | Trips | Quads |
| I | 1020 | 15 | 10 | 0 | 5 | 2 | 1 | 1 |
| | | | | | | | 4 | |
| II | 1003 | 16 | 5 | 2 | 1 | 3 | 1 | 0 |
| | | | | | | | 4 | |
| III | 1000 | 16 | 3 | 5 | 1 | 1 | 1 | 0 |
| | | | | | | | 2 | |
| IV | 972 | 18 | 3 | 4 | 1 | 1 | 1 | 0 |
| | | | | | | | 2 | |
| Total for all cows | | 65 | 21 | 11 | 8 | 7 | 4 | 1 |
| | | | | | | | 12 | |
| Live calves[a] | | | | | 8 | 11 | 9 | 2 |

[a]Calves alive at 2 weeks of age

in both heifers and cows.

As determined by rectal palpation at approximately 120 days Treatment I appeared to be superior in conception rate at the first post-PMS estrus over those treatments (II, III and IV) which utilized estrus synchronization (Table II). For example, conception rate at the first post-PMS estrus among the various treatment groups was significantly higher in Treatment I than in Treatments II ($P < .05$), III ($P < .01$) or IV ($P < .01$).

Conception rate for the 60 to 90 day breeding season (Table II) was again higher in Treatment I than in either Treatment III or IV ($P < .05$). These differences were noted at time of palpation when 2 cows in Treatment II, 5 in Treatment III and 4 in Treatment IV were found to be open. Thus, the progestogen used for estrus synchronization may have affected fertility and conception rates in Treatments II, III and IV. A further factor that could have affected fertility in Treatments III and IV was artificial insemination technique. Laster et al. (1971b), using a schedule similar to Treatments III and IV, also observed low conception rates.

As indicated in Table III estrus synchronization was accomplished in 48 (96%) of the 50 cows in Treatment groups II, III and IV. Estrus occurred in all 48 cows within 2 to 5 days after withdrawal of CAP. The majority of these cows (35 of 48) showed estrus on day 3 post-CAP. Consequently, it does not appear that lowered conception rates among those animals fed CAP (Treatments II, III and IV) were generally associated with a poorly timed gonadotropin treatment.

A factor that may have contributed to the low overall conception rate in Treatments II, III and IV was the fact that the breeding season

TABLE III

SYNCHRONIZATION OF ESTRUS IN LACTATING BEEF COWS BY CAP FEEDING[a]

| Treatment group | No. treated | No. of cows showing estrus[b] within five days after CAP feeding — Post-CAP (days) Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|---|
| II | 16 | 0 | 1 | 8 | 4 | 1 |
| | | | | (14)[c] | | |
| III | 16 | 0 | 0 | 16 | 0 | 0 |
| | | | | (16) | | |
| IV | 18 | 0 | 4 | 11 | 3 | 0 |
| | | | | (18) | | |
| All groups | 50 | 0 | 5 | 35 | 7 | 1 |
| | | | | (48) | | |

[a]CAP (6-chloro-$\Delta^{6}$-17-acetoxyprogesterone) was fed, individually, for 18 days, 10 mg per head per day the first 16 days and 5 mg per head per day the last 2 days. CAP feeding was initiated without regard to stage of the estrus cycle.

[b]Estrus was determined visually and by vasectomized bulls.

[c]Number in parentheses indicates total cows synchronized in each treatment group.

for this particular group of cows occurred during June and July, when the ambient temperatures reached daily highs of 33 to 42$^{o}$C. Long et al. (1969) and Dunlap and Vincent (1971) have offered good evidence that hot weather has an adverse effect on fertility.

The response in terms of multiple births observed in the treatment groups were not significantly different. However, Treatment group II did result in 3 sets of twins and 1 set of triplets of 5 cows pregnant after the first estrus following PMS. Multiple births as presented in Table II were also obtained in Treatment groups III (2 sets) and IV (2 sets). These data indicate that PMS injections can be effectively timed from a synchronized estrus. This conclusion is in agreement with findings of Laster et al. (1971b).

Of the total conceptions at first post-PMS estrus shown in Table IV, 61.8% (13) occurred in Angus-Holstein crossbred cows, 23.8% (5) in Hereford cows of various ages and 14.4% (3) in Angus-Hereford crossbred cows, respectively. These conceptions resulted in 6, 4 and 2 sets of multiple births from the Angus-Holstein crossbreds, Hereford straightbreds and Angus-Hereford cows, respectively. Conception rate to service at the first estrus after PMS treatment was singificantly higher ($P < .01$) in Angus-Holstein crossbreds than in straightbred Hereford cows. The difference between the two crossbred groups was not statistically different. There were no significant differences between any of the breeding groups in numbers of multiple births.

The data obtained in this study did not reveal a relationship between body weight and response to PMS as measured by numbers of multiple births. As shown in Table II very little difference existed in mean body weight between the various breeding groups. No trends between PMS

TABLE IV

1971 CALVING PERFORMANCE BY BREED AND AGE GROUP OF ALL COWS TREATED WITH PMS IN 1970

| Breed of cows | Age[a] (years) | Body[b] wt. (lbs) | Total treated | No. Conceiv. at 1st. Post-PMS estrus | No.[c] Open | No. of Cows Producing | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Single | Twin | Trip. | Quad. |
| Hereford | 3 | 948.5 | 17 | 3 | 3 | 0 | 2 | 0 | 1 |
| | | | | | | | | 3 | |
| Hereford | 7-9 | 1156 | 12 | 2 | 2 | 1 | 1 | 0 | 0 |
| | | | | | | | | 1 | |
| Ang. x Hol. | 4 | 944 | 25 | 13 | 3 | 6 | 3 | 3 | 0 |
| | | | | | | | | 6 | |
| Ang. x Heref. | 4 | 950 | 11 | 3 | 3 | 1 | 1 | 1 | 0 |
| | | | | | | | | 2 | |
| Total | | | 65 | 21 | 11 | 8 | 7 | 4 | 1 |
| | | | | | | | | 12 | |
| Live Calves | | | | | | 8 | 11 | 9 | 2 |

[a]Age of cows when treated.

[b]Body weight at time of second PMS injection.

[c]After a 60 to 90 day breeding season.

response and age of cow as well as post-partum interval and ovarian cyclicity were found.

## Time Interval From Second PMS to Estrus

Based on those cows showing estrus after the second PMS injection, the majority of conceptions (79.0%) and 81.8% of the multiple births occurred in cows conceiving on days 2, 3 or 4 after the second PMS injection (Figure 1).

The data reported in Figure 1 is similar to observations from various other studies. However, most other studies have suggested that an interval of at least 3 days is most desirable. Turman _et al_. (1971) suggested that the most effective treatment would be to induce estrus 3 to 5 days post-PMS. Hammond (1949), Gordon _et al_. (1962) and Hafez, Jainudeen and Lindsay (1965) reported that an interval from PMS injection to estrus of at least 3 days was necessary for satisfactory ovulatory response to PMS. Hafez _et al_. (1965) noted ovulatory response highest when the interval was 4 days. Hammond (1949) suggests that more 2-egg ovulations occur after an interval of 3 to 4 days between PMS injection and estrus. Laster _et al_. (1971b) concluded that shorter intervals between the injection of PMS and subsequent estrus resulted in a more restricted level of superovulation than longer intervals.

## Survival Rates

Numbers of live calves in Table II are for those living at two weeks of age. Most death losses occurred at time of parturition and none later than 4 days post-parturition. Calf losses for all cows wintered consisted of 1 single birth, 3 twin birth calves, 3 triplet birth calves

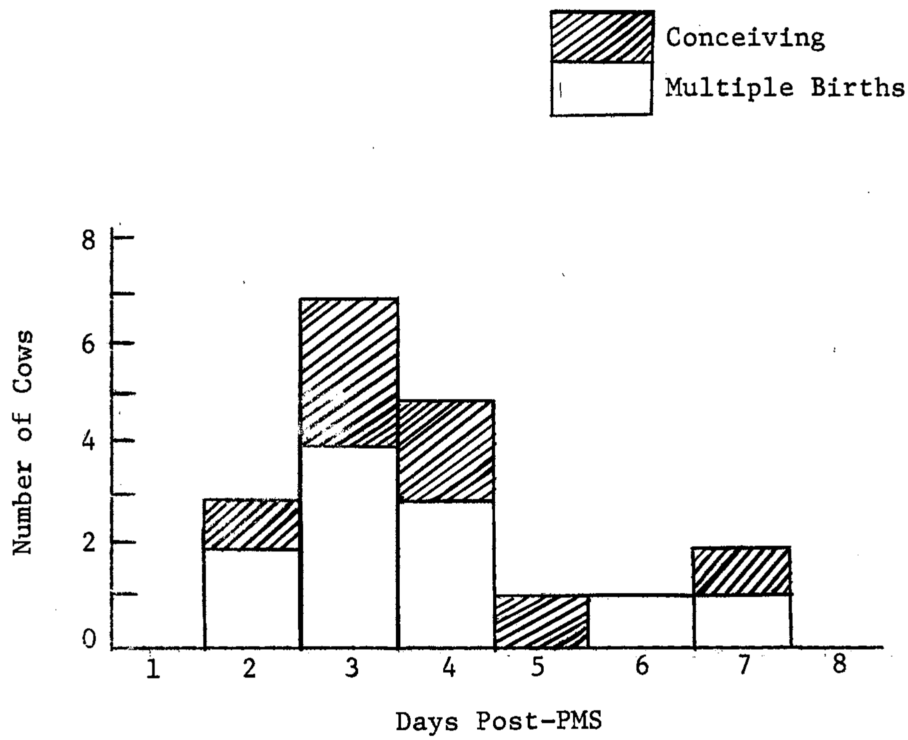


Figure 1. The Numbers of Cows Conceiving on Each Day Following the Second PMS Injection and the Conceptions Resulting in Multiple Births

and 2 calves from the quadruplet birth. Contrary to William's (1948) findings, parturient mortality in multiple births did not appear to be related to dystocia. However, dystocia was associated with the parturition of two multiple birth calves.

Livability of single calves was higher than the various multiple births. They were significantly higher than the quadruplet calves ($P < .05$) and approached significance for twins and triplets ($P < .1$). The survival rates reported in Table II for triplets (75%) and quadruplets (50%) were higher than that obtained by Gordon et al. (1962) or Turman et al. (1971) but lower than those rates reported by Vincent and Mills (1970).

After being affected by primary bloat, one cow aborted twins at 7 months of gestation and died two days later. This in part explains the low survival rate (78.6%) for twin births. Turman et al. (1971) reported that survival rates for twin births (100%) were the same as for single births (100%).

Incidence of prenatal mortality, as represented by abortions or cows failing to calve after positive rectal palpation, was markedly lower than that observed by Gordon et al. (1962) and Vincent and Mills (1970). In the present study, of the 54 cows found pregnant after the regular breeding season one cow aborted at seven months and two failed to calve the following year. In contrast, Vincent and Mills (1970) reported that of 52 pregnant cows, two aborted and four failed to calve. Gordon et al. (1962) reported that 41 of 126 pregnant cows aborted or resorbed either multiple or single births after the cows were diagnosed pregnant at six weeks of gestation.

Despite calf losses (Table V), calving rate was markedly affected by multiple births in this study (Table II). A total of 61 live calves

## TABLE V

## INFLUENCE OF TYPE OF BIRTH ON CALVING STATISTICS

| Type of birth | No. of sets | Avg. gestation length (days) | Avg. Birth Weight[a] Male (lbs) | Avg. Birth Weight[a] Female (lbs) | Percent retained placentas | Percent calf loss |
|---|---|---|---|---|---|---|
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | | |
| Single | 8 | 283.6 ± 1.7 | 84.8 ± 2.8 | 73.7 ± 4.4 | 0.0% | 0.0%[b] |
| Twin | 6 | 281.7 ± 1.9 | 60.2 ± 2.6 | 62.7 ± 4.5 | 50.0% | 8.3%[c] |
| Triplet | 4 | 271.0 ± 2.4 | 56.2 ± 3.5 | 48.6 ± 2.9 | 75.0% | 25.0%[d] |
| Quadruplet | 1 | 272.0 ± 4.7 | 33.0 ± 5.5 | 32.0 ± 5.5 | 0.0% | 50.0%[e] |
| Level of significance | | $(1>3^{**}>4^{*}, 2>3^{**})$ | $(1>2^{**}, 1>3>4^{**})$[f] | $(1>3, 1>4^{**}, 2>3^{*}>4^{**})$[g] | | |
| | | | $(1>2^{**}, 1>3>4^{**}, 2>3^{**})$[h] | | | |

[a]Calf weights apply to all calves born.

[b]Those cows failing to calve are not included.

[c]One twin birth aborted at 7 mo. is excluded, one twin calf died at birth.

[d]Three triplet calves from different litters died at birth.

[e]Two calves died at birth, all four calves were very weak at birth.

[f]Differences between male calves of singles, twins, triplets and quadruplets were tested for significance.

[g]Differences between female calves of the various birth categories were tested for significance.

[h]Differences between groups of singles, twins, triplets and quadruplets were tested for significance.

*Significant at $P < .05$.
**Significant at $P < .01$.

were obtained from 54 cows wintered or from 65 cows originally treated. These births resulted in a calving rate of 142.9% for live calves at two weeks of age for those 21 cows conceiving to the first estrus after PMS, 113.0% for all cows wintered or 93.8% for all cows treated.

Four cows having conceived prior to PMS treatment were found to have uninterrupted pregnancies. These results agree with previous findings of Casida et al. (1943) who concluded that the effects of PMS will not alter the functional corpus luteum of pregnancy. Based on this study, cows carrying in excess of twin fetuses were not predisposed to increased abortion rates. This is contrary to such reports as Hammond (1949), Lotti and Galli (1961) and Gordon et al. (1962).

## Gestation Lengths and Birth Weights

Significant differences were observed in gestation lengths and birth weights (Table V). Gestation length of 283.6 days for singles was longer than that of 271.0 days for triplets ($P < .01$) or that of 272.0 days for quadruplets ($P < .05$). Whereas, gestation for twins (281.7 days) was significantly longer than for triplets ($P < .01$). Gestation lengths of 280.6 and 281.4 days for twins reported by Gordon et al. (1962) and Vincent and Mills (1970), respectively, plus 269.2 days for triplets reported by Turman et al. (1971) are consistent with present results. In contrast, the gestation lengths of 275.0 and 277.4 days reported for twins by Bellows et al. (1970) and Turman et al. (1971), respectively, are somewhat shorter than those obtained in the present study. A similar inconsistency was noted between present data (Table V) and those results presented by Gordon et al. (1962) and Vincent and Mills (1970) who reported gestation lengths for triplet births of 262.8 and 279.0 days, respectively.

Single birth calves were found to be heavier than multiple birth calves ($P < .01$). Twins were heavier than triplets ($P < .05$) and both twins and triplets were heavier than quadruplets ($P < .01$). Other studies (Gordon et al., 1962; Bellows et al., 1970; Vincent and Mills, 1970; Turman et al., 1971) report similar trends in differences between birth weights of different types of births. However, those weights reported do not agree with the present findings. Non-significant differences were noted in birth weights of calves from artificial insemination to those of natural service. It is not possible to explain the differences in calf birth weights between artificial insemination and natural service becasue the birth weights are confounded with bull effects (Table VI).

## Problems Related to Multiple Births

Retained placentas appeared to be more of a problem in this study than has been reported in similar studies. They occurred in 6 (54.5%) of 11 cows producing multiple births while cows producing single births required no assistance in placenta elimination (Table V). Gordon et al. (1962) indicated 18.2% retained placentas, while Vincent and Mills (1970) noted 36% and Turman et al. (1971) reported that 47.8% of those cows producing multiples required assistance. These researchers observed a higher incidence of retained placentas in births larger than twins.

In the present study trends for a greater incidence of retained placentas, were found in the heavier litters rather than among those litters of larger numbers (Table VII). In Treatments III and IV which

TABLE VI

AVERAGE BIRTH WEIGHTS BY NATURAL SERVICE[a] AND ARTIFICIAL INSEMINATION[b] OF ALL CALVES CALVED AS SINGLES OR IN MULTIPLE SETS BY COWS TREATED WITH PMS[c] AND HCG[d]

| | | Birth Weight | | | |
|---|---|---|---|---|---|
| | | Male | | Female | |
| Type of birth | No. of sets | Natural (lbs) | A.I. (lbs) | Natural (lbs) | A.I. (lbs) |
| | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. |
| Singles | 8 | 82.5 ± 3.6 (4) | 94.0 ± 7.3 (1) | 70.5 ± 5.1 (2) | 80.0 ± 7.3 (1) |
| | | (8)[e] | | | |
| Twins | 6 | 53.0 ± 5.1 (2) | 53.4 ± 2.7 (7) | ---- (0) | 62.7 ± 4.2 (3) |
| | | (12)[e] | | | |
| Triplets | 4 | 50.0 ± 7.3 (1) | 57.8 ± 3.6 (4) | 43.0 ± 5.1 (2) | 50.9 ± 3.3 (5) |
| | | (12)[e] | | | |
| Quadruplets | 1 | 32.0 ± 5.1 (2) | ---- (0) | 33.0 ± 5.1 (2) | ---- (0) |
| | | (4)[e] | | | |

[a]Angus range bulls maintained at the Fort Reno Research Station were used for natural services.

[b]Frozen semen (1 ml per insemination) from an Angus bull of proven fertility obtained from American Breeders Service was used for A.I.

TABLE VI (Continued)

[c]PMS injections subcutaneous, 1,080 I.U. on day 5 and 1,440 on day 17 of either a natural or synchronized estrus.

[d]HCG injections intravenous, 2,500 I.U. on day of first estrus or on day 3 of first post-PMS estrus.

[e]Number in parentheses indicates number of animals in each group.

TABLE VII

MULTIPLE BIRTH CALVING DATA FOR THOSE COWS WITH RETAINED PLACENTAS VS. THOSE WITHOUT RETAINED PLACENTAS

| | | Birth Associated With Retained Placentas | | | | Births Not Associated With Retained Placentas | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sex ratio | Litter wt. | Calf wt. | | Sex ratio | Litter wt. | Calf wt. | |
| Type of birth | No. of sets | M:F[a] | (lbs) | Male (lbs) | Female (lbs) | M:F[a] | (lbs) | Male (lbs) | Female (lbs) |
| | | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. | | Mean ± S.E. | Mean ± S.E. | Mean ± S.E. |
| Twins | 6 | 5:1 | 127.3 ± 9.1 (3)[b] | 61.6 ± 3.3 | 74.0 ± 7.4 | 4:2 | 116.0 ± 9.1 (3) | 58.5 ± 4.3 | 57.0 ± 3.7 |
| Triplets | 4 | 4:5 | 161.7 ± 9.1 (3) | 57.8 ± 3.7 | 50.8 ± 3.3 | 1:2 | 136.0 ± 9.1 (3) | 50.0 ± 7.4 | 43.0 ± 5.2 |
| Quadruplets | 1 | --- | ---- | ---- | ---- | 2:2 | 130.0 ± 15.8 (1) | 32.0 ± 5.2 | 33.0 ± 5.2 |
| Total | 11 | 9:6 | 144.5 ± 6.4 | 59.9 ± 2.5 | 54.7 ± 3.0 | 7:6 | 122.8 ± 7.1 | 49.7 ± 3.0 | 44.3 ± 2.6 |

[a]Sex ratio expressed as male:female.

[b]Number in parentheses indicates number of cows that either did or did not have retained placentas.

utilized artificial insemination, all of the cows (4) producing multiple births required assistance compared to one of three (33%) and one of four (25%) in Treatments I and II, respectively, which used natural service.

Those litters associated with retained placentas had a mean male: female ratio of 3:2 whereas in those litters not related to placental retention the mean sex ratio was 7 males: 6 females. Comberg and Velten (1962) reported that with male twins, fetal membranes were retained 73% of the time; with mixed twins 42% and with female twins only 20%. Dabash (1964) suggests that due to the greater weight of male calves the cow producing multiple births with a sex ratio dominant for males may be predisposed to placental retention. Data presented in Table VII generally supports this author's conclusion.

Two calves from two sets of multiple births did require assistance at time of parturition. This is in disagreement with other studies in which dystocia has not been associated with multiple births (Hammond, 1949; Turman _et al._, 1971).

Data is not yet available on post-weaning performance of multiple birth calves and numbers of freemartins resulting from heterosexual female births. However, present data indicates the overall sex ratio for the 12 sets of multiple births was 46.7% (14) males and 53.3% (16) females (9 of which occurred in heterosexual births). Those reproductive tracts taken from all (2) dead post-natal heifers from heterosexual birth females were found to be typical freemartins. Those heifers born to single births (1) or homosexual multiple births (2) appeared to have normal reproductive tracts. Laster _et al._ (1971a) indicated that all surviving heifers (17) from heterosexual multiple births (17) were typical freemartins, however, he did not autopsy such heifers that died at, or soon after, parturition.

In this study 15 cows (23.1%) either did not show signs of estrus or failed to stand for the bull on the first estrus after gonadotropin treatment. Various other studies have indicated that silent heats often accompany PMS injections (Avery et al., 1962; Gordon et al., 1962; Hafez et al., 1964). Gordon et al. (1962) found 70 (14.4%) of 486 cows injected with various levels of PMS had silent heats. They attributed a larger incidence of the problem to lower PMS levels or the injection of PMS either too late or poorly timed in the estrual cycle. These researchers also concluded that lactating cows supporting more than one calf displayed a higher rate of silent heats than cows with single calves or non-lactating cows. However, Turman et al. (1971), using a higher dose level of PMS than that used in the present study, observed estrus in 93.8% of 81 non-synchronized PMS treated cows.

## Pregnancy Determination

In order that each cow be given every opportunity to conceive after the second PMS injection no attempt was made to determine ovulation by palpation. Cows that had apparently conceived at the post-PMS estrus were rectally palpated after a period of 110 to 130 days for either a negative or positive diagnosis of pregnancy. Care was taken to avoid excessive handling for determination of fetal number. Utmost care of handling of the cows during gestation was used to prevent unnecessary fetal mortality. As reported by Gordon et al. (1962) extensive prenatal manipulation by rectal palpation may have caused 32.5% of those cows with multiple births in utero to resorb or abort. Laster et al. (1971b) associated low conception rates and high fetal mortality with high lumbar laparotomy.

## Supplemental Feeding

In the present study, all cows were supplemented as indicated in Materials and Methods section during the last two months of pregnancy, and, although good survival rates and few abortions were observed, it was not possible to determine the effect of supplemental feeding on these results. However, increased feeding may not limit placental retention due to the result of heavier litters.

The need for establishing maintenance requirements for cows with plural fetuses is clear. For example, in 11 multiple births, cows averaged a parturient weight loss of 96.5 lbs or about 100 lbs from fall to time of parturition over the average weight losses for single birth cows. Consequently, maintenance requirements based on weight losses from fall to time of parturition for the cow supporting one fetus may not be adequate for cows with more than one fetus.

Hammond (1959) and Gordon _et al_. (1962) suggest fewer third-trimester abortions, smaller number of retained placentas and higher survival rates when supplemental feed is given during the last two months of pregnancy to cows supporting multiple fetuses.

## CHAPTER V

### Summary

Four treatment groups of multiparous beef cows were treated with subcutaneous injections of 1,080 I.U. of PMS on day 5 and 1,440 I.U. of PMS on day 17 of the estrual cycle followed by an intravenous injection of 2,500 I.U. of HCG on day of estrus or 3 days post-PMS. The PMS injections were time from either a non-synchronized or synchronized estrus. Estrus was synchronized by individual feeding of CAP (6-chloro-$\Delta^6$-17-acetoxyprogesterone) 18 days, 10 mg per head per day for 16 days and 5 mg the last two days. On the first estrus after the second PMS injection cows were bred to a natural service or to artificial insemination or were inseminated the third and fourth days after second PMS injection disregarding occurrence of estrus.

The first treatment group composed of 15 cows received PMS injections timed from a natural estrus followed by an injection of HCG given on day of estrus and breeding by natural service. The 16 cows in the second treatment group were given PMS timed from a synchronized estrus followed by HCG on day of estrus and breeding by natural service. In the 16 cows of the third treatment PMS injections were timed from a synchronized estrus and HCG was injected at time of artificial insemination on day of estrus after second PMS. Eighteen cows were given PMS on the same basis as the previous group with HCG administered on day 2 after second PMS and artificial insemination carried out on day 3 and 4 dis-

regarding the occurrence of estrus in the fourth treatment.

A total of 65 cows were treated, 21 (32.3%) conceived at the first estrus following the second PMS injection and produced 9 singles and 12 multiple births (7 sets of twins, 4 sets of triplets and 1 set of quadruplets).

Conception rate to first estrus after second PMS was higher in Treatment I than II ($P < .05$), III ($P < .01$) or IV ($P < .01$). The first treatment group resulted in a higher conception rate than Treatments III and IV ($P < .05$) for the entire 60 to 90 day breeding season following PMS injection. The four treatments yielded 4, 4, 2 and 2 sets of multiple births, respectively. Angus-Holstein crossbreds responded with a higher conception rate at the first post-PMS estrus than Hereford cows ($P < .01$).

Of those cows showing estrus following second PMS injection the majority of conceptions (79.0%) and multiple births (81.8%) occurred in cows conceiving on days 2, 3 or 4 after second PMS. Survival rates were: singles 100%, twins 91.7%, triplets 75% and quadruplets were 50%. The differences in livability of single calves and the various multiple births was significant in the case of quadruplets ($P < .05$), and approached significance for twins and triplets ($P < .1$). The calving rate for live calves at two weeks was 142.9% based on 21 cows pregnant after post-PMS estrus, 113.0% based on 54 cows wintered or 93.8% for 65 cows originally treated.

The gestation length of 283.6 days for single births was significantly longer than the 271.0 days for triplets ($P < .01$) or the 272.0 days for quadruplets ($P < .05$). Twins were carried for a longer gestation (281.7 days) than triplets ($P < .01$). Single birth calves were found to be heavier than multiple birth calves ($P < .01$). Twins were significantly heavier than triplets ($P < .05$), while twins and triplets were heavier than quadruplets ($P < .01$).

Retained placentas occurred in 6 (54.5%) of 11 cows producing multiple births. This problem generally persisted in the heavier litters with a higher male:female ratio. Two multiple birth calvings were associated with dystocia.

Silent heats also presented a problem in this study. Fifteen (23.1%) cows either did not show any signs of estrus or failed to stand for the bull on the post-PMS estrus.

The need to overcome the various problems related to multiple births combined with the encouraging results obtained from this study should stimulate the continuance of research in this area.

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VITA

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