A STUDY OF THE EFFECTS OF VARIOUS HORMONE TREATMENTS ON THE REPRODUCTIVE ACTIVITY OF PREPUBERAL AND POST-PUBERAL BEEF HEIFERS AND LACTATING BEEF COWS

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INTRODUCTION

The modification of reproductive activity in the beef female by various exogenous hormones has been widely studied during the past two decades. It has provided control mechanisms, whereby the physiologist could more intensively study ovarian activity. One very important aspect of these studies has been the development of techniques for synchronizing the estrual activity of a cow herd.

A practical program for estrus synchronization could greatly facilitate the widespread use of artificial insemination in beef herds, thereby extending the use of proven sires. However, treatments which result in a synchronization of estrus give rise to other problems. The most important of these problems is reduced fertility. The exact nature of this decline in fertility at the induced estrus with certain treatments is not known, and requires further study.

An important problem in the beef industry is the period of lactational anestrous following parturition. Attempts have been made to stimulate sexual activity during this period, and thereby shorten the calving interval. Certain hormone treatments have stimulated sexual activity but, generally, the response has been variable and fertility has been low.

This study was carried out to determine the effects of different hormonal treatments on beef females of varying ages. Oral progestogens and placental gonadotropins were employed in an attempt to stimulate the development of precocious puberty. Oral progestogens were used in sexually mature breeding females to synchronize estrus and study subsequent fertility levels. The effect of various combinations of gonadal hormones was studied in post-partum beef cows.

REVIEW OF LITERATURE

Investigations With Prepuberal Animals

The ovaries of sexually immature animals of some species have long been known to be capable of supporting the development and release of viable ova. In a study of ovarian development in calves, Casida et al. (1935) reported that follicular development was well underway a few days after birth and appeared to be augmented with age. Casida (1934) had observed that the presence or absence of vesicular follicles appeared to be the limiting factor governing the ovary's ability to respond to exogenous gonadotropins. This type of ovarian structure is descriptive of that which Nalbandov (1964) classifies as a tertiary follicle. Casida (1934) studied the ovaries of 190 veal calves which had been slaughtered at ages from one to twelve weeks and reported only 3 calves lacked vesicular follicles.

Vesicular follicles are present at birth in the lamb and foal, but do not appear in the sexually immature pig until 10-14 weeks of age (Casida, 1934). Histological studies were made of the ovaries of 16 purebred gilts by Casida (1935) to determine when the process of formation of tertiary follicles began. Vesicular follicles were just beginning to be formed at 83 days of age. A gilt 36 days of age was not responsive to gonadotropins, again indicating that the existence of vesicular follicles was a prerequisite for ovarian response to an injection of exogenous gonad-stimulating hormones. Induced ovulations in immature rats and mice were obtained only after antrum formation (vesiculation) which occurred at 12-14 days of age in mice and 11 days of age in rats (Zarrow and Wilson, 1961).

The exact mechanism which stimulates the development of tertiary follicles from primary follicles is unknown. Casida et al. (1935) suggested this might be due to an increasing gonad-stimulating activity on the part of the pituitary long before puberty. In contrast to this view are the results obtained by Hollandbeck et al. (1956) which indicated the onset of puberty coincided with the lowest titer of available pituitary gonadotropic hormone. They hypothesized the drop in gonadotropic hormone levels from birth to puberty may be due to an actual decrease in the rate of hormone secretion, but it was more likely due to a shift in the proportion of follicle stimulating hormone (FSH) and luteinizing hormone (LH) making up the gonadotropic complex. Workers in the past few years have come to accept the concept that FSH and LH exist as two separate entities and not as one complex.

The exact age at which farm animals reach sexual maturity is quite variable and dependent on many factors. Wiltbank et al. (1959) studied 125 Hereford heifers to determine the approximate age and weight at which calves exhibited their first estrus. Their average age and weight at puberty was 434 days and 562 pounds, respectively.

Gain from weaning to puberty was found to be more highly associated with age at puberty than was weaning weight. The average daily gain of heifers (Angus, Brahman, and Zebu cross) from weaning until one year of age and from weaning to 18 months of age was observed to influence the age of puberty (Reynolds et al., 1963). Other studies have illustrated the importance of adequate nutritional levels on the occurrence of first estrus in farm animals. Sorenson et al. (1959) studied the influence of plane of nutrition on the development and function of the reproductive organs of dairy heifers up to 80 weeks of age. Feeding levels were designed to furnish 60, 100, and 140 percent of the total digestible nutrients (TDN) recommended for growing dairy heifers. First estrus was detected at 72.0, 49.1, and 37.4 weeks of age, respectively, for the preceding feeding regimes. Studies with swine also indicate a high energy intake is desirable for early puberty and high ovulation rates (Haines et al., 1955; Robertson et al., 1951).

Other factors affecting age at puberty in dairy heifers were presented by Hawk et al. (1954). Spring born heifers reached first estrus at a significantly ($P < .01$) earlier age than those born during each of the other seasons. Although no explanation was given, one would suspect the abundance of spring forage available the following year would raise the energy intake and, thereby hasten the onset of puberty. In this study a highly significant negative correlation (-.33) was reported between age at puberty and breeding efficiency. These findings supported their initial hypothesis that breeding efficiency may be lowered by extreme delays in the occurrence of first estrus.

Numerous workers have tested the effects of gonadotropins on the ovaries of the immature female. Casida <u>et al</u>. (1943) noted in calves ranging in age from seventeen to one hundred seventeen days that treatment with a pituitary gonadotropin stimulated the ovaries to exhibit a measurable gross response depending on the presence of vesicular follicles. The increase in size of the ovaries during treatment bore only a slight association with the number of follicles present at the beginning of treatment. They believed the ovarian response to the gonadotropic treatment is probably more affected by some basic condition which had produced or maintained those follicles in the first place. They also reported that artificially matured and ovulated follicles of prepuberal calves were fertilizable, but the fertilization rate was extremely low (2 of 19). Casida (1934) observed that injection of unfractionated sheep pituitary extract, which has primarily a follicle-stimulating effect, produced marked follicular maturation in prepuberal calves when administered in large doses. Small doses of the partially purified follicle-stimulating substance injected into 3- to 5-month old lambs resulted in follicular maturation, ovulation and formation of a corpus luteum. The preceding results are in direct contrast of those of Hertz and Hisaw (1934) who observed the occurrence of ovulation only after a combination of treatments involving both follicle stimulating hormone and luteinizing hormone. This indicated that the ovulatory process may depend on a synergistic balance between the two gonadotropic hormones. Mansour (1959), while studying the hormonal control of ovulation in ewe lambs, was able to consistently achieve ovulation in animals primed with pregnant mare's serum (PMS) followed by chorionic gonadotropin (HCG). The absence of ovulation from animals treated with PMS suggests the possible absence or inadequacy of necessary ovulating factors. In a study by Rowland (1944), follicular maturation was produced in immature rats by PMS with ovulations occurring after injection of chorionic gonadotropin (HCG). Limited success in stimulating ovulation was observed when PMS alone was administered.

Marden (1953) and Black et al. (1953) were able to obtain large numbers of follicles but very few mature corpora lutea in calves injected with follicle stimulating substances. Marden found the development of a corpus luteum could occur as early as the third week of postnatal life. Black et al. (1953), in a more extensive study, reported a general fertilization failure thought to be associated with either an inadequate or improper maturation of the follicles and ova, or with a possible inability of the juvenile uterus to induce maturation and full fertilizing capacity in the sperm. They hypothesized it might also be due to impaired sperm transport in the juvenile reproductive tract. Casida (1935) tested both horse pituitary powder and pregnant mare's serum and reported no typical follicular maturation with the pituitary powder (2.5 grams in aqueous suspension) but produced three ovulations in eight days in a 113-day old gilt administered 15 grams of PMS. Follicles similar in size to normal mature follicles were more easily ovulated than those that were larger.

Fox et al. (1964) observed an influence of age on the response of the immature rabbit ovary to PMS. With 200 IU PMS, there was an increased sensitivity with age to a peak followed by a decline in response. Howe *et al*. (1962) reported two factors that affected response to exogenous gonadotropic hormones. The first of these in older heifers was the stage of the estrous cycle at the time of treatment. The second factor was a hypothesized complex interaction between the pituitary and the ovaries and possibly the adrenals following the injection of two or more hormones. In their studies Howe *et al*. (1962) reported that injections of pregnant mare's serum and chorionic gonadotropin resulted in hyperfollicular development. Progesterone injections ranging from 2.5 to 100 mg. per treatment reduced substantially the number of small follicles with a corresponding increase in the moderate and large follicles and some ovulation. The addition of estradiol was later found to suppress follicular growth (Howe *et al*., 1964). Estradiol and progesterone may have inhibited the follicle stimulating action of PMS, or estradiol may have acted directly on the ovary to inhibit the development of the follicle. Suppression may have been due to the interaction of exogenous hormones and the endocrine glands *in situ*.

The response of the ovaries of immature animals to gonadotropins would, no doubt, be subject to modifications by many factors. These may include; (1) an inherent species-specific, maturation rate, (2) the effects of other endocrine glands throughout their development, (3) the influence of endogenous gonadal hormones, and (4) the various inductive mechanisms, particularly of nervous system origin (Bodemer *et al*., 1959). Quinn and Zarrow (1964) noted PMS induced ovulation in the immature rat appeared to involve a neuroendocrine mechanism which can be inhibited by the action of certain drugs (morphine and chlorpromazine) on the central nervous system.

It was postulated by Byrnes and Meyer (1951) that as the animal becomes older the pituitary becomes increasingly refractive to estrogen. This refractiveness was believed to be an important factor in the attainment of sexual maturity. Apparently more estrogen is required to limit the release of FSH and invoke the release of LH. Possibly early in life not enough follicular maturation occurs before ovulating hormone is released, therefore, the ovum is not released. This work disagrees in theory with that of other workers who believe the lack of viable ova is associated with an insignificant production and release of ovulating hormone (Hertz and Hisaw, 1934; Mansour, 1959).

Grant (1936) reported oestrus behavior in sheep will not occur unless a waning corpus luteum is present in the ovaries. This assumes that in some way luteal tissue from the previous cycle conditions the follicles which are approaching maturity to undergo a preovulatory enlargement and luteinization in the presence of the correct anterior pituitary FSH-LH balance in the blood. This raises the question as to how the initial corpus luteum is formed at the onset of sexual maturity to elicit ovulation where no previous luteal activity has occurred. Cole and Miller (1933) and Marden (1953) reported that although the outward manifestations of heat were lacking the reproductive organs were not entirely quiescent throughout anestrous. Marden (1953) noted in most animals a series of cycles ensue before puberty in which follicular enlargement occurred, but was not followed by ovulation. These nonovulatory cycles occur until luteinization appears with the formation of the initial corpus luteum. It may be that the granulosa cells of the follicles approaching maturity become capable of exerting a mild luteal action even before ovulation has occurred.

Marden (1952), to test the theory of the waning corpus luteum, subcutaneously injected an FSH active substance twice daily for periods of from seventy two to one hundred and twenty hours in the presence of a regressing corpus luteum. Those cows receiving FSH without subsequent LH had 4 new ovulations out of 130 follicles stimulated (3 percent). Those cows receiving both FSH and LH had 126 unovulated follicles and 199 fresh ovulations (61 percent). Silent ovulations occurred in a large number of instances. These results agree with those of Rowson (1951) who reported that in cows treated with pregnant mare's serum, 52 percent ovulated when the corpus luteum was present as compared to a 14 percent ovulation rate in cows with the corpus luteum removed.

Synchronization of Estrus

Considerable research time has been devoted to experiments dealing with control of the estrual cycle. Numerous compounds have been tested in an attempt to more accurately predict the time of onset of estrual behavior and occurrence of ovulation. Such attempts hope to provide to the producer a means of more closely controlling the breeding season and extend the application of artificial insemination, especially in beef cattle production systems.

Reports indicate thus far that synchronization is not difficult to obtain with exogenous progesterones, or certain other hormones, but with certain limitations that may tend to cause the producer to criticize their use. There is a tendency for some decrease in fertility when the treated animals are mated at the induced estrus. The exact nature of the cause of reduced conception is not known.

The amount of literature dealing with synchronization and subsequent fertility is quite voluminous. There are many different aspects which must be considered, such as type of hormonal agent, method of administration, dosage level, mode of action, and response in terms of estrual activity and fertility after drug administration. To cover it completely is nearly impossible; therefore, only that dealing with cattle will be discussed.

Various means have been used to administer the drugs. The progesterone and estrogenic compounds were at first injected intramuscularly to study their physiological effects. The development of oral progestational agents has made the use of hormones of more widespread value. Certain compounds have been equally effective whether given orally or injected.

Progesterone injections have been known for some time to inhibit estrus. Christian and Casida (1948) tested the effect of daily intramuscular injections of 25 and 50 mg. for 14 days. Fifty mg. was found to completely suppress heat and ovulation during treatment with estrus occurring 5-6 days after treatment. Injection of 25 mg. prevented estrus but did not completely inhibit the occurrence of ovulation. Use of 50 mg. or 25 mg. progesterone injections for 14 days followed by insemination at second post-treatment estrus resulted in conception rates of 57.9 percent for the 50 mg. group and 72.2 percent for the 25 mg. treated group. A study of the subsequent fertility of heifers injected with 50 to 100 mg. of progesterone was conducted by Willett (1950). Estrus was observed to occur an average of 5 days following the injeations of progesterone. Use of an inexperienced inseminator resulted in decreased conception rates of 39 percent (5 of 13 heifers) as compared to a 67 percent (6 of 9) conception rate by an experienced inseminator.

Using a limited number of beef heifers, Ulberg and Patterson (1954) injected 3 levels of progesterone (12.5 mg., 25.0 mg., and 50.0 mg.) for 14 or 19 days and found the overall fertilization rate was 77.0 percent, with a decrease in the fertilization rate as the progesterone dosage increased. Daily injections of 50 to 100 mg. of progesterone resulted in a 12.5 percent conception rate from first post-treatment service and a 65.2 percent conception rate for second post-treatment service (Trimberger and Hansel, 1955). From these and other results, Ulberg (1955) speculated that the harmful effect of progesterone is temporary and probably the inability to remain pregnant is due to embryonic death and not to a fertilization failure.

Nellor and Cole (1954) tested the effects of crystalline progesterone implants (540 and 1120 mg.) and found estrus and ovulation were inhibited for at least 16 days after a single injection. In a later study Nellor and Cole (1956) reported the 1120 mg. dose prevented estrus and ovulation, but was ineffective in synchronizing estrus because only one heifer cycled immediately following treatment while the remainder showed heat from 29 47 days later. Injection of 560 mg. progesterone followed 15 days later by a single injection of 2140 IU of equine gonadotropin stimulated 16 of 19 to exhibit estrus 1-4 days after the gonadotropin injection. Administration of this level of gonadotropin should be stimulus enough to elicit estrus without previous priming with progesterone. The conception rate subsequent to this treatment was 17 percent. This poor conception rate was found to be due to the presence of many infertile ova.

Ray et al. (1961) reported very inconsistent results with exogenous progesterone injections followed by gonadotropins. Injections of 560 mg. of progesterone followed 14-15 days later by 750-2250 IU of PMS resulted in very aberrant exhibitions of heat (2-38 days post-treatment) and only one pregnancy in eight inseminated animals. They also noted what they concluded to be a subfunctional uterine endometrium. Lowered fertility observed subsequent to progesterone treatment was believed to be partially due to this underdeveloped endometrium. In a trial with intact rats, Barnes and Meyer (1964) reported that the viability of the embryo varied inversely with the dose of MAP. Estrone injections simultaneously with MAP stimulated implantation. Apparently the block placed on luteinizing hormone release was detrimental in that LH stimulates the production of estrogen which is required for uterine preparation before implantation.

Johnson et al. (1958) initiated a study using a reposital type progesterone (100 mg.) injected intramuscularly on either the 2, 3, 4, 6 and 9th day following breeding to improve reproductive efficiency. It was not intended to synchronize estrus but to promote the formation of a more optimal uterine environment. The reposital progesterone resulted in a 67.9 percent conception at first service as contrasted to 42.0 percent in the control group. Injection of exogenous progesterone had been shown to prevent the release of luteinizing hormone (Zimbelman et al., 1961; Loy et al., 1960), thus causing a decrease in the potential ability of the corpus luteum to provide progesterone necessary for the development of a sufficient uterine endometrium to maintain pregnancy. Apparently Johnson et al. (1958) provided this needed progesterone.

Other compounds have been used in an attempt to synchronize estrus. Anderson et al. (1962) injected daily a mixture consisting of 98.5 percent 17-ethynyl-17-hydroxy-estr5(10)-3n-3-one (Norethynodrel) and 1.5 percent ethynyl-3, 17-estradiol-3-methyl ether (Enovid) for 10 days. This mixture, referred to as NE, was administered to two groups at the rate of 0.042 mg. and 0.065 mg. per pound of body weight, respectively. The daily injected groups (1 and 2) returned to estrus 3-7 days post-treatment and a 44 percent conception rate was obtained. Its oral effectiveness was tested in group 3 and group 4 at the rate of 0.4 mg. and 0.8 mg. per pound of body weight per day. However, this compound was not active orally as indicated by the lack of synchronization and the 75 percent conception rate.

Other work involving the use of estrogenic substances has been done in low fertility cows (Reynolds et al., 1956). Administration of 10 mg. of diethylstilbestrol daily in the ration from 24 days before breeding to slaughter at 34 days post-breeding apparently did not increase conception rates. Only 29 percent of 13 treated animals had conceived and were carrying a normal embryo at slaughter. Wickersham and Schultz (1964) found diethystilbestrol (DES) treated dairy heifers had a lower breeding efficiency than nontreated heifers. No effect on milk production was observed after DES was administered.

Fertility following an injection of 2 mg. of estradiol 96 hours after termination of progesterone injections (25 mg. per day for 14 days) was compared to that obtained when 25 and 50 mg. daily injections of progesterone were given for 14 days (Ulberg and Lindley, 1960). The conception rate at first service for the controls was 50.9 percent of 106 heifers, 31.2 percent of 64 given 25 mg., 17.2 percent of 40 given 50 mg., and 38.1 percent of 42 given 25 mg. progesterone plus 2 mg. estradiol. Estradiol improved conception at first service somewhat when compared to progesterone treatments alone but still was considerably below the control results.

Wiltbank (1962), using two hundred cycling heifers, studied synchronization and fertility after administration of combinations of various levels of progesterone (20 and 40 mg.) and estradiol (0, 20, 40, or 80 mcg.). Seventy-three percent of the heifers receiving 20 mg. injections of progesterone for 24 days were in estrus during the 2nd, 3rd, and 4th day following treatment while 88 percent of those receiving 40 mg. of progesterone were in heat during the 4th, 5th, and 6th days. Ninety-eight percent of those getting 40 mg. of progesterone plus estradiol were in heat during a four-day period. Conception rates ranged from 13-56 percent with the highest rate obtained in the 40 mg. progesterone plus 80 mcg. estradiol group. The conception rate of the control heifers was 60 percent indicating some decrease in fertility associated with treatment.

The posterior pituitary substance, oxytocin, has been studied by some researchers to determine its effect on reproductive activity (Armstrong and Hansel, 1959; Hansel _et al_., 1961). Armstrong and Hansel (1959) reported daily injections of oxytocin from 15 days post-estrus for a complete cycle induced estrus at an average of 10.6 days after the previous estrus. The purpose of this treatment was to explore the possibility that oxytocin might enhance gonadotropic secretion. Instead the occurrence of estrus was somewhat delayed with an estrual cycle interval of 25.6 days. The time of the next subsequent heat period was, however, 20.4 days. Hansel et al. (1961) used a combination of 130 U.S.P. units of oxytocin and 50 mg. of progesterone concurrently for seven days. Most of the heifers subjected to this treatment came in estrus 6-8 days following the last progesterone injection rather than 3-5 days after the last injection as anticipated. Of 26 animals treated as previously described, 50 percent conceived and calved in a 16-day period.

Probably the most widely used oral compound has been 6- -methyl-17-acetoxyprogesterone (MAP). This substance is very effective in synchronizing estrus but some reduction in fertility after its use has been noted (Zimbelman, 1961; Nestel et al., 1963). Hansel and Malven (1960) fed MAP at a level of 968 mg. for 10 days followed by 500 mg. for another 10 days per animal per day. No estrus was observed during treatment but only 8 of 22 cows settled when artificially mated three days after the treatment period. Nellor et al. (1960) reported that levels above 0.4 mg. MAP per pound body weight per day for 15-20 days resulted in complete inhibition of follicular development during the treatment period. Oral administration of 0.2 mg. MAP per pound body weight per day suppressed estrus but ovulation was not completely inhibited in all animals.

Zimbelman (1961), comparing individual and group feeding of 0.5 mg. MAP per pound of body weight, found 12 heifers (75 percent) in heat between 48 and 84 hours after the last feeding. The conception rate at first service for the untreated, individually fed, and group-fed animals was 6 of 8 (75 percent), 2 of 8 (25 percent), and 6 of 8 (75 percent), respectively. Eighty-one percent of the cows in the two treated groups conceived with one or two services. The preceding study is comparable to that of Nelms and Combs (1960) who observed complete suppression of estrus in 33 cows with calves that were group-fed 220 mg. MAP per day. Heat in these animals occurred on the second and third day post-treatment in all cases, and the resulting pregnancy rate was a creditable 66 percent. Group feeding 60 two-year-old heifers 250 mg. MAP per head per day resulted in 40 percent pregnant at first service. This conception rate was, however, found to be similar to the rate obtained in 60 control females bred during the first 21 days of breeding. Even though conception in the treated heifers was low, their calving dates were observed to be more grouped than those of the control group. Collins <u>et al</u>. (1961) also reported complete inhibition of estrual behavior in 15 beef heifers during a twice-a-day feeding trial of MAP at the rate of 0.5 mg. per pound of body weight. Fourteen heifers were in estrus 2 to 8 days after the end of treatment. Nestel <u>et al</u>. (1963) reported 85 percent of 38 animals exhibited an induced estrus after the cessation of MAP feeding, but only 24 percent of these heifers conceived to artificial insemination. Sorenson (1962) fed 180 mg. MAP daily in cottonseed meal for 18 days and observed 75 percent of 97 heifers exhibited a synchronized estrus. Using both artificial insemination and natural mating he obtained the following conception rates: control, 60.4 percent; artificial inseminated synchronized group, 33.3 percent; and naturally serviced synchronized group, 53.1 percent. In a later trial with 54 heifers treated 18 days, conception at first service was 29.6 percent. An explanation for the 20 percent decrease in fertility with artificial insemination was not readily available.

Although several different dosages of MAP have been studied, Zimbelman (1963) concluded that 180 mg. per day was sufficient to prevent any ovulatory activity. Zimbelman (1963) and Johnson and Ulberg (1965) noted that the progesterone compounds produce an antiovulatory effect, with follicular development occurring but the matured ova not being released. They believed the progesterones did not prevent the production and release of the FSH-like gonadotropins but did inhibit the release of ovulating hormone.

Wiltbank (1964) divided one hundred cycling beef heifers into two groups to study the use of dihydroxyprogesterone acetopheide (DA) to synchronize estrus. In the first phase he fed 400 mg. of DA daily for 9 days plus an injection of estradiol valerate on the second day to one group while the others served as controls. Approximately 25 days after the first synchronized estrual period the treatments were reversed. Synchronization for these two trials was 86 percent and 82 percent, respectively, with most of the heifers in heat in a 36-hour period. Pregnancy examinations 35 days after breeding during the second phase indicated 50 percent of the cycling animals and 32 percent of the synchronized animals were pregnant. The difference in conception rates of treated and control heifers was believed to be due to greater embryonic mortality in the heifers fed DA (Parker et al., 1965).

Van Blake et al. (1963) experimented with 6-chloro-Δ^6-dehydro-acetoxyprogesterone (CAP) and found that four of four treated cows conceived after being fed 0.05 mg. per pound for 15 days. Using larger numbers in another phase of this trial, 16 of 23 conceived when bred at the induced estrus after being fed 12 mg. per day for 18 days. More extensive research needs to be done before the true value of this product can be determined.

Post-Partum Interval

Cattlemen have long been faced with the problem of delayed rebreeding of cows after parturition. This period, known as the post-partum interval, is one of the more variable aspects of the reproductive cycle of bovine females. Suspended reproductive activity because of long periods of ovarian inactivity are very critical problems to the beef producer. Where the breeding season is limited long post-partum intervals present the possibility of having dry cows in the following season because of a nonreturn to estrus in time for breeding. Burris and Priode (1958) regressed the percentage of cows failing to calve on previous calving date and found for each delay of 20 days in the previous calving date, 6.1 percent do not calve.

Sexual activity, as denoted by the psychic manifestation of heat, appears to be suppressed somehow by lactation which has been referred to as the natural birth spacing mechanism (Ferin et al., 1964). Post-partum ovarian quiescence has been hypothesized to be due to a deficiency of gonadotropins or a low ovarian sensitivity or possibly a combination of both. Saiduddin and Foote (1964) suggested the post-partum interval might possibly be a pituitary recovery period subsequent to the period of extensive inhibition during pregnancy. They observed a significant ($P<.05$) increase in mean relative pituitary potency from day 5 to day 30 following parturition.

Several reports pertaining to the interval from calving to subsequent reproductive activity such as uterine involution, first ovulation, and first estrus, are present in the literature. Lasley and Bogart (1943) made 711 observations on range beef cattle and calculated the mean length of the interval from parturition to first estrus to be 80.2 ± 1.30 days. This figure can be contrasted to results obtained by Buch et al. (1955) who found the interval from parturition to first estrus to be only 33 days in dairy cattle. They also reported the average period required for full involution of the uterus was 47 days, with primiparous cows involuting earlier than pluriparous cows. They observed estrual behavior in all of their females before involution was complete. These findings are unique in that most workers report the interval from calving to first estrus averages from 15 days to 20 days longer than the interval from parturition to involution of the estrus. Foote et al. (1960a), using records kept on 13 pairs of twin beef heifers (9 fraternal and 4 identical) with 61 service periods, determined the time to uterine involution, first ovulation, and first estrus after calving were 43.7, 38.4, and 58.6 days, respectively. Casida and

Venzke (1936) had earlier reported the time from parturition to first ovulation in dairy cattle was 40.7 days.

In other work involving beef cattle, Lindley et al. (1958), drawing from the records of 3,606 gestations of Hereford cows, reported the average interval from calving to first estrus to be 75.5 days which is slightly longer than the time period noted by Warnick (1955). Warnick observed that first estrus after parturition in Angus cows occurred in 59.2 days as compared to Herefords which required 62.7 days. This breed difference was not significant nor was age of the cow or sex of the calf found to have any significant influence on post-partum interval.

Factors affecting the onset of reproductive activity after calving has been a topic of interest to several workers. Norwood (1963) concluded post-partum interval was significantly affected by parity, season and retained placenta. He found involution intervals averaged five days longer in pluriparous cows than in primiparous cows (40.25 vs 35.25 days). Herman and Edmondson (1950) did not observe a seasonal effect on the interval from calving to first estrus which they noted was 57 days with a standard deviation of 28 days. Average daily milk production and the occurrence of first estrus were also found not to be related to one another. However, other workers have reported a relationship between frequency of milk removal and return to sexual activity (Clapp, 1937; Wiltbank and Cook, 1958). Clapp (1937) stated cows milked two times daily came into estrus an average of 23 days sooner than did cows milked four times daily. Wiltbank and Cook

(1958) found the interval from parturition to first estrus to be 84 days for nursed cows as compared to 54 days for cows milked twice daily. Somehow the release of oxytocin apparently inhibited the production of sufficient amounts of estrogen to bring the cows into estrus.

Another factor which is thought to have a significant influence on post-partum reproductive phenomena is energy level before and after calving (Wiltbank et al., 1962). They concluded that in spring calving cows the level of feeding before calving had more influence on the occurrence of estrus after calving while level of feeding after calving was more important in obtaining higher conception rates than level of feeding before calving. Increasing the energy intake from a low to a high level on cows that had not shown estrus in 90 days stimulated 8 of 10 to exhibit estrus in an average of 49 days. In females not raised to a higher energy level, none of the 10 showed estrus. Low-low levels (4.5 lbs. TDN before and 8.0 lbs. TDN after calving) apparently caused a high percentage of the cows to fail to exhibit estrus and increased the services per conception to 3.00 as compared to 1.55 in the high-high level (9.0 lbs. TDN before and 16.0 lbs. TDN after calving. Turman et al. (1965) reported rebreeding of two-year-old heifers may be a problem even when maintained on a liberal plane of nutrition, but low planes of nutrition are likely to cause prolonged delays before conception. Comparing three levels of winter feeding they observed the following average post-partum intervals for the high, moderate and low levels of feeding were 65, 70, and 78-85 days, respectively.

Many researchers have attempted by various hormonal treatments to shorten the period of lactational anestrous following calving. Numerous types of drugs have been administered to stimulate sexual activity. Attempts to obtain an earlier first estrus by drug induced synchronization has not been successful in all instances. The gonadal hormones, estrogen and progesterone, as well as diethylstilbestrol diproprionate, have had limited success. In the early work with stilbestrol, Casida and Wisnicky (1950) injected 20 mg. within 9 hours after calving and noted a slight nonsignificant decrease in post-partum interval to first estrus (63.1 vs. 57.7). Silent first ovulation was observed to have occurred in 68 percent of the cows.

Foote and Saiddudin (1964a) studied the effect of single and multiple 800 mg. acetylcholine injections 12 days after calving and obtained negative results. Treatment did not significantly affect the uterine involution rate but the intervals from calving to first ovulation, first estrus and conception were significantly longer. If post-partum anestrous is due to a lack of gonadotropins, apparently acetylcholine is not sufficient stimulus to elicit increased release of the triggering mechanism for more production.

The effect of oxytocin on interval variation from parturition to uterine involution, first ovulation and first estrus was studied by Cameron and Fosgate (1964). This substance is known to have a stimulatory effect on the contracture of the myometrial layer of the uterus. An increase in muscle tonus in the uterus would seemingly shorten the period to post-partum reproductive activity. The average intervals for the treated and untreated groups, respectively, were as follows--to uterine involution, 41.0 and 42.7 days; to ovulation, 27.5 and 24.7 days; and to first estrus, 41.3 and 40.9 days. Cameron and Fosgate (1964) stated oxytocin could also exert its influence through inhibiting gonadotropic secretion, but this hypothesis did not appear to hold true in this study as indicated by the small nonsignificant differences reported between treatments.

Fosgate et al. (1962) studied the physiological effects of 22 daily intramuscular injections of 100 mg. 17-alpha-hydroxyprogesterone-N-caproate on post-partum reproductive activity and observed treatment significantly ($P < .01$) increased the time from parturition to uterine involution and first estrus. The treated and untreated animals exhibited involution and first estrus in 42.0 vs 27.5 days and 70.0 vs 47.9 days, respectively. Conception rates for the two groups were similar.

Zimbelman (1963) attempted to synchronize post-partum beef cows by initiation of MAP treatment prior to the occurrence of first post-partum ovulation. He observed a significant decrease in variability but was unable to decrease the time interval from calving to first post-partum ovulation. He reported 19 of 22 treated post-partum cows had synchronized ovulations as determined by rectal palpations. Palpation accuracy was observed to be limited. Zimbelman noted that ovulation may be synchronized in nursing cows which had not yet begun to cycle if the time at which ovulation was normally expected was being approached. Possibly the mechanism sustaining the anestrous period is of neural origin and not subject to control by exogenous hormones. Foote et al. (1960b) used a progesterone injection at the rate of 1 mg. per lb. 14 days after calving to study post-partum response to hormone administrations. Uterine involution was not influenced by the progestational substance (41.7 vs 41.4 days) but treatment exerted a negative influence on time of first ovulation (61.1 vs 41.7 days) and first estrus (83.0 vs 65.6 days) for the treated and control group, respectively.

Estrogen injections within two weeks after calving have been successful in shortening the number of days required for the onset of reproductive activity (Foote and Walker, 1961; Foote and Hunter, 1964). The average interval from parturition to first ovulation reported by Foote and Walker (1961) was 16 days shorter for the cows treated with 10 mg. of β-estradiol than for the untreated cows. Treatment did not significantly decrease the variation of the interval to first ovulation in the treated primiparous cows as it did in the treated pluriparous cows. Foote and Hunter (1964a) injected 10 mg. estradiol-17β intravenously 15 days after calving and found the average interval from parturition to first estrus, first ovulation and second ovulation were all significantly ($P < .01$) shorter in the treated than in the control group. These results indicate that corpus luteum formation was normal and regular cyclic conditions can be stimulated to occur in post-partum individuals. By some intricate mechanism, possibly by blocking the release of the substance referred to as luteotrophic hormone (Simmons and Hansel, 1964), estrogen causes the regression of the corpus luteum of lactation.

The actual existence of this luteotrophic substance has not been verified, except in certain laboratory animals.

Niswender *et al.* (1964) reported injections of various levels of estradiol-17β (0, 30, 40, 80, 160, 320, and 640 mcg.) twice daily from day 6 to either day 12 or day 18 of the estrous cycle caused a reduction in progesterone concentration and total progesterone content. This was determined by ovariectomizing 100 yearling heifers at day 6 (controls), day 12 and day 18 of the estrous cycle. They also studied the effect of twice daily injections of estradiol-17β on corpus luteum weight. Only the 640 mcg. level reduced corpus luteum size when ovariectomy occurred on day 12. Ovariectomy after estrogen treatment from day 6 to day 18 indicated all measures of corpus luteum activity were significantly ($P < .05$) reduced below that of the control values. This study indicates the possible mode of action of estrogens. Apparently they somehow inhibit luteotropic activity resulting in a regression of luteal tissue present.

Other studies of the influence of estradiol on post-partum reproduction have been done (Quevedo *et al.*, 1965; Foote *et al.*, 1965). Using three different herds of Holstein cattle which included 58 pluriparous and 46 primiparous cows, Quevedo *et al.* (1965) reported the results of treatment of one-half of each age group with 10 mg. estradiol-17β intravenously 12 days post-calving. An increasing number of parities and treatment both tended to increase the length of the interval from parturition to uterine involution. Average interval to first estrus was decreased in the treated animals, but the observed difference was not significant.

Foote et al. (1965) treated 40 Hereford cows either on the 12th or 17th day post-calving or on both the 12th and 17th day post-calving with 10 mg. estradiol intravenously and observed no significant difference among group averages for the interval from calving to uterine involution, first ovulation, and first estrus. Greater variation in the intervals to first ovulation and first estrus was reported for the group receiving two injections of estradiol-17β five days apart.

Some work has been done in beef cattle using a combination of the ovarian hormones. Foote et al. (1960c) studied the effects of a single dose of progesterone 30 days after calving as well as an injection of progesterone plus 10 mg. estradiol 20 days after progesterone. The average number of days to first estrus were essentially the same in the treated groups as they were in the untreated group. Evidently large single doses of progesterone are not capable of triggering the release of the gonadotropins. Injection of 50 mg. of progesterone and 10 mg. of estrogen, either singly or in combination, reduced the interval from parturition to uterine involution, first ovulation, and first estrus (Foote, 1962; Foote and Saiduddin, 1964b; Foote and Hunter, 1964b). Although a single injection of progesterone was reported in earlier studies to be ineffective in reducing these time periods, multiple injections used in the studies by Foote and his coworkers were successful.

The results obtained with the estrogen injections appeared to substantiate those obtained by Niswender et al. (1964) who noted corpus luteum regression with high levels. Norwood (1963) found estradiol, progesterone, and MAP did not significantly reduce involution interval in primiparous cows. Injection of .05 mg. β - estradiol, however, did significantly increase the interval from calving to uterine involution in pluriparous cows when injections were made on alternate days. Quevedo et al. (1965) had observed this lengthening of post-partum interval to involution following single injections of estradiol.

Summary of Review of Literature

The ability of prepuberal animals to respond to gonadotropins appears to depend on the presence of vesicular follicles. This type of ovarian structure is present at birth in the calf and the lamb but not until 14 weeks of age in the pig. Injection of the placental gonadotropins, PMS and HCG, have produced ovulation of viable ova in both lambs and calves. To obtain luteal tissue development and ovulation apparently both a follicle stimulating hormone and a luteinizing hormone are essential.

Numerous studies have explored the use of both injectable progesterone and orally active synthetic progesterone analogs in suppressing estrus and ovulation. Very reliable results allowing one to predict the time when heat will occur after cessation of treatment have been obtained with these compounds. Quite variable results have been obtained with regard to conception rate after treatment.

Lactational anestrous is another problem the animal physiologist has attempted to solve through the application of various hormone treatments. Estradiol-17β has achieved some success in decreasing the time from parturition to coincident estrus and ovulation. Again fertility at these induced periods of estrus has been a problem.

GENERAL PROCEDURE

The data in this study were collected over a period of two years. The results pertain to the use of various hormones in combinations for differing ages of beef females. Certain trials required specific sets of procedural practices. However, since many procedures were employed, in general, in all trials they will be discussed as a group in this section.

The animals used in these studies were part of Project 1219 or the purebred breeding herd (Project 1256) maintained at the Fort Reno Livestock Research Station at El Reno, Oklahoma. The sexually immature heifers employed to study prepuberal responses were Herefords, Angus and Angus-Hereford crossbreds. Natural matings in the breeding herd were made to Hereford and Angus bulls. One trial involving breeding at first or second estrus after treatment required the use of artificial insemination to semen of Red Angus bulls. The breeding season usually began May 1 and extended through July. Bulls were removed from the breeding pastures on August 1. Out-of-season breeding records, and all records pertaining to estrual behavior in the prepuberal heifers were obtained by using vasectomized bulls.

The cattle were grazed on native grass pasture throughout the year. Supplemental grain and cottonseed meal was provided to maintain desired winter gain or as a means of administering the oral progestogen. Specific trials calling for differences in energy level will be discussed later under the procedures of that particular experimental period.

The 6-methyl-17-acetoxyprogesterone[1] (MAP) was administered in the daily feed allotment. The animals were individually fed in all except one trial in which group feeding was practiced. The MAP was received as a premix in soybean meal at the rate of 33 mg. MAP per gram of soybean meal. The recommended dosage rate, 180 mg. MAP per day (Zimbelman, 1963) was administered in two ways. In the prepuberal heifers, which were individually fed in all trials, the amount of premix (5.14 gms.) required to supply 180 mg. was weighed into individual packets and the contents of these packets were sprinkled over the heifers' daily allotment of feed. The individually fed cows were, for the most part, fed 3 lbs. of milo plus 2 lbs. of cottonseed meal in which the MAP had been mixed previously. The group fed heifers received 3 lbs. of milo and 1.5 lbs. of cottonseed meal containing the recommended amount of MAP. In the group feeding trial the ration was placed once daily in open troughs with adequate trough space such that all heifers could eat without crowding. The heifers were fed in the morning and only a limited number of the animals failed to consume their portion of the ration.

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[1]Repromix, The Upjohn Company, Kalamazoo, Michigan.

The pregnant mare's serum[2] (PMS) used was a commercial preparation containing 50 IU per ml. Twenty ml. of PMS were injected subcutaneously in the neck region.

The chorionic gonadotropin[3] (HCG) was a powdered commercial preparation containing 500 IU per ml. This material was injected intravenously at the rate of 1000 IU per animal.

The estradiol-17β[4] (ECP) used in the study of involution of the bovine uterus was an oil soluble 17 (beta) cyclopentylpropionate ester of alpha estradiol and provided 1 mg. of estradiol-17β per ml. Injections of ECP were given intramuscularly in the rump area.

Rectal palpations were made in prepuberal heifers to determine the amount of ovarian response to treatment which had occurred. The ovaries were manipulated to obtain some estimate of the degree of follicular development and to determine if ovulation and subsequent corpus luteum formation had occurred. Rectal palpations were made weekly in post-parturient cows to study the phenomenon of involution of the uterus. These weekly palpations were continued until involution of the uterus had occurred.

An estimate of the milk production of the cows was also obtained. The procedure for obtaining this estimate involved separating the calves from their mothers the evening before milk production estimates

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[2]Gonadin, Cutter Laboratories, Berkeley, California.

[3]Libigen, Savage Laboratories, Houston, Texas.

[4]ECP, The Upjohn Company, Kalamazoo, Michigan.

were obtained. The following day the calves were weighed to the nearest half-pound, then allowed to suckle their mothers. After nursing was completed the calves were reweighed and the weight before nursing was subtracted from the weight after nursing. This difference was assumed to be the amount of milk obtained. The calves were again penned away from the mothers during the day and the procedure repeated that evening. The total of these two figures provides an estimate of the cows daily milk production. The process was repeated twice, one week apart, to obtain an estimate of the cows' milk production.

TRIAL I

Materials and Methods

Fifteen Angus, Hereford, and Angus-Hereford crossbred heifers were allotted to three treatments on the basis of weight and age in the summer of 1964, with the major emphasis placed on weight as indicated by the results of Sorenson <u>et al</u>. (1959). The range in age and weight for these heifers was 208-242 days and 360-540 lbs., respectively. None of the heifers had attained puberty as indicated by the observation of estrus. Rectal palpations carried out before treatment failed to detect palpatable corpora lutea indicating that little ovarian activity had occurred.

All heifers were individually fed 1.5 lbs. CSM and .5 lb. ground milo per head per day. The treatments were as follows: Group 1 (MAP) received 180 mg. MAP per heifer per day daily for 15 days; Group 2 (MAP +PMS+HCG) received 180 mg. MAP per heifer per day daily for 15 days and were injected subcutaneously on the last day of MAP feeding with 1000 IU of PMS followed three days later by 1000 IU of HCG intravaneously; and Group 3 (PMS+HCG+MAP) received a subcutaneous injection of 1000 IU of PMS followed 3 days later by an intravenous injection of 1000 IU of HCG. Ten days after the HCG injection, 180 mg. MAP per heifer per day was fed for 15 days. Individual packages containing 180 mg. MAP were mixed into each heifer's feed allotment at time of feeding to make certain each heifer received her daily treatment dosage.

All heifers were checked with vasectomized bulls to determine occurrence of estrus prior to, during or following treatment. Rectal palpations were carried out on heifers 3 days after the MAP feeding period or 3 days after injection of PMS. Palpation of heifers of this size is extremely difficult making diagnosis of ovarian activity uncertain and in certain instances impossible. The observations made by rectal palpation were follicular development, occurrence of ovulation, and corpus luteum formation as a result of treatment, and coincident ovarian activity as an indication of the establishment of a regular cyclic pattern of estrus behavior.

A fourth group (PMS+HCG) of 5 Angus and Angus-Hereford crossbred heifers ranging in age from 205-223 days and in weight from 320-355 lbs. were injected subcutaneously with 1000 IU of PMS and 3 days later with 1000 IU of HCG intravenously. No attempt was made to palpate the heifers in Group 4 because of their small size.

The animals used in the prepuberal studies are listed in Appendix Table XXIII.

Results and Discussion

The purpose of this study was to study the effectiveness of MAP and the placental gonadotropins, PMS and HCG, in triggering the mechanism to stimulate the onset of puberty. The use of PMS and HCG has been known to cause estrus and ovulation, but whether subsequent estrual periods occur after these treatments has not been reported. The function of MAP could be to regress all luteal tissue present or it might possibly serve as a block on pituitary hormone release. The results of Trial I are summarized in Table I.

Estrus was not detected in any of the heifers in group 1 which received only MAP daily for 15 days during the treatment period or soon after the removal of MAP from the ration. Two heifers of this group apparently were in estrus but not detected since they conceived to the service of the fertile, although supposedly vasectomized, bull which had been inadvertently used. Although pregnancies at this young age were undesirable, the presence of the fertile bull did provide data that would not have been observed otherwise. The bred heifers with no record of estrual behavior being observed indicated that the use of a marking harness was an unreliable means of detecting estrus in this trial.

Using a gestation length of 275 days to calculate an estimated breeding date for the two pregnant heifers it was found that conception had apparently occurred very soon after the removal from MAP treatment. Although estrus was not observed it is obvious that the heifers were in estrus, did ovulate a fertilizable ova, and were capable of conceiving and carrying the fetus to term. Both of the calves were alive at birth, each weighed 50 lbs. with one heifer requiring a cesarotomy.

One of the three nonpregnant heifers in group 1 exhibited an estrual period approximately 40 days after the MAP treatment but did not return to estrus. The other two nonpregnant heifers of this group did not cycle. Palpation three days after treatment was

TABLE I

EFFECT OF 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) AND PLACENTAL GONADOTROPINS (PMS AND HCG), SINGLY OR IN COMBINATION, AND IN DIFFERENT SEQUENCES ON ESTRUAL BEHAVIOR IN PREPUBERAL BEEF HEIFERS

					No. Observed in Estrus After					
Group	Treatment	No.	Ave. Age	Ave. Wt.	PMS	HCG	MAP	No. Days From PMS Treatment To Estrus	No. Observed In a 2nd Post Treat. Estrus	Ave. Int. From 1st to 2nd Estrus
1	MAP	5	224	400	0	-	-[a]	-	-	-
2	MAP-PMS-HCG	5	232	401	4	-	-	3	2	7
3	PMS-HCG-MAP	5	227	432	5	-	1	2	1	27
4	PMS-HCG	5	216	333	4	1	-	3	3	15

[a]Two heifers found to be pregnant had apparently conceived shortly after removal from treatment without being observed in estrus.

successful in only two of the 5 heifers, because of the extreme small size of the other 3 heifers. The ovaries of the 2 heifers palpated were smooth and apparently of normal dimensions.

Howe et al. (1962) reported that progesterone injections administered to immature dairy heifers reduced the number of follicles present. Whenever the progesterone treatment was followed by PMS and HCG only 26 percent of the heifers ovulated. The use of gonad-stimulating substances after the progesterone treatment period apparently stimulated a measurable amount of ovarian response before slaughter of the animals. The progestational agent used in Trial 1, MAP, although not triggering the observable exhibition of estrus after treatment did apparently prevent ovulation until after the removal of treatment. Zimbelman (1963) concluded MAP accomplished this by preventing the release of ovulating hormone.

The heifers of group 2 receiving MAP for 15 days followed by the PMS and HCG injections responded to the treatments with estrus occurring in 4 of the 5 heifers after PMS, but failed to develop a cyclic pattern of heat expression. The injection of PMS was followed by estrus approximately 60 hours post-treatment in these 4 heifers. A corpus luteum was palpated 3 days after PMS in 3 of 4 heifers reported in estrus. The HCG was given at this time but apparently was unnecessary because ovulation had already occurred. The fourth heifer was difficult to palpate thus a positive diagnosis was not possible. Two of these four heifers returned to estrus in a 5-9 day period indicating the corpus luteum was not functionally maintained or estrus would have been retarded. The presence of corpora lutea after PMS alone conflicts with the results obtained by Mansour (1959) in sheep. He concluded ovulation did not occur unless a luteinizing substance in addition to PMS injection contained enough luteinizing hormone to stimulate ovulation.

The intravenous injection of 1000 IU of HCG was found by rectal palpation to have stimulated the formation of large masses of luteal tissue increasing the size of the ovaries to 2 or 3 times normal dimensions. The HCG administered in this manner appeared to have caused considerable luteinization of the follicles present without stimulating further ovulation. Later palpations of these heifers revealed a regression to normal size of the ovaries with no functional activity occurring. Howe et al. (1962) reported ovulation in 3 of 8 heifers treated with PMS and HCG with excessive follicular development occurring in all calves. Apparently the HCG source used in this study when injected intravenously acted so rapidly that luteinization of the follicle occurred before ovulation was possible. The usual observation reported after a combination of injections including PMS and HCG was follicular development followed by ovulation (Hertz and Hisaw, 1934; Mansour, 1959; Rowland, 1944).

Of the 2 heifers in group 2 that were observed in estrus twice, one was marked by the vasectomized bull as being in estrus 22 days after the previous heat period. This was the only heifer to exhibit two estrual periods at what could be considered at normal time interval. Palpation 7 months later indicated this heifer was pregnant to the service of the fertile teaser bull. After calving a breeding date was estimated and found to approximate this latter estrual date. Conception in this case prevented further cyclic estrual activity. Another heifer on this treatment was also found to be pregnant and was calculated to have conceived approximately 40 days after the PMS injection. This heifer had been in estrus 3 days after the PMS injection but did not have later recorded heat dates, although it is obvious that at least one occurred. Possibly she had had a short estrual period and mated to the vasectomized bull during the night. Marking harnesses were used but this again indicates their unreliability.

The one heifer in this group which did not exhibit estrus failed to ovulate. The other two nonpregnant heifers failed to establish a regular pattern of cyclic activity as evidenced by a lack of estrual behavior subsequent to the estrus induced by PMS.

The results obtained in heifers of group 3 indicated that the injection of the placental gonadotropins (PMS and HCG) prior to the 15-day feeding period of MAP was not as successful in stimulating ovarian activity following MAP treatment as was observed when the gonadotropins were administered after feeding. The injection of 1000 IU of PMS again stimulated estrual behavior (5 of 5). Palpation after the intravenous injection of 1000 IU of HCG revealed 3 of 4 heifers ovulated with excessive luteinization of other unovulated follicles occurring in all four heifers. The HCG again appeared to cause a luteinization without ovulation. The small size of one heifer of group 3 prevented making any observations by palpation.

Only one heifer in group 3 exhibited estrus following treatment with MAP for 15 days beginning 10 days after the HCG injection. This heifer had reached a weight of 550 pounds although only 9 months of age. Wiltbank _et al_. (1965b) reported Angus-Hereford crossbred heifers reach puberty at 550 pounds and 13.2 months of age. This heifer, if weight is the most important criterion determining puberty, could conceivably have reached puberty. However, such was not indicated because she failed to return to estrus until 60 days later. The other four heifers in this group did not exhibit estrus other than after the PMS treatment prior to MAP administration. Palpation approximately 30 days after cessation of MAP treatment revealed a corpus luteum on the ovary of one heifer later found to be pregnant. Calculation based on date of calving indicated mating to the vasectomized bull had occurred approximately 20 days after the removal of MAP from the ration. No studies have been reported which could be compared to this treatment. Production of an artificial corpus luteum with PMS and HCG was apparently accomplished, but regression of this structure with MAP seemingly returned the heifers to their previous anestrous condition.

A fourth group of 5 heifers were treated with PMS and HCG without MAP to study whether or not the artificial corpus luteum produced would regress naturally and stimulate later estrual cycles to occur. When only PMS and HCG were administered 4 heifers exhibited estrus 3 days after the PMS injection and the fifth heifer was in estrus 6 days following the HCG treatment. Three of 4 heifers responding to PMS returned to estrus, two of them within 5 to 7 days after the first heat period. Another heifer returned to estrus 33 days later and conceived to the service of the fertile teaser bull. This heifer was 8.5 months of age at the time of conception. One other heifer of this group was not observed in heat but conceived approximately 60 days after the PMS-HCG induced estrual period recorded for her. The two remaining heifers of this group did not return to estrus subsequent to the induced estrual period. The heifers in this group were not palpated because of their size. Treatment of prepuberal animals with a combination of PMS and HCG has repeatedly been observed to stimulate follicular development and ovulatory activity (Marden, 1953; Mansour, 1959; Howe et al., 1962). However, establishment of a regular pattern of cyclic activity has not been reported. The presence of the fertile teaser bull confounded the results of this trial with regard to the onset of cyclic activity but apparently cyclic behavior was not triggered as evidenced by a failure to observe estrus in the nonpregnant heifers.

The results of this trial are in agreement with reports in the literature that prepuberal calves do respond to treatment with exogenous hormones, especially the placental gonadotropins, PMS and HCG. Two of 5 heifers treated with MAP alone apparently exhibited estrus after treatment although these periods must have been a short duration. No heat date had been recorded, but they conceived at some estrual date shortly after removal from treatment. Treatment with PMS both before and after MAP feeding stimulated the exhibition of estrus. The use of HCG after PMS stimulated some ovulations but in many instances appeared to have luteinized the existing follicles without stimulating ovulation. Incorporation of MAP into the ration after the PMS and HCG injections resulted in only one induced estrus of five treated animals. The injection of PMS and HCG without MAP resulted in all heifers exhibiting estrus after treatment with 3 heifers returning to estrus at later dates but not when normally expected. Only one heifer was in estrus twice after treatment at what could be called a normal interval. This resulted from treatment first with MAP for 15 days followed by PMS and HCG.

Seven of 20 heifers used in this study conceived during the course of this trial. The average age at conception for these heifers was approximately 8 months with a range from 7 to 9 months of age. Six heifers gave birth to live calves with an average birth weight of 51 pounds. One heifer required a cesarean section; however, the other 6 heifers although requiring considerable assistance calved normally.

The results obtained in trials of this type where the observation of estrus is the major criterion of response can be subject to considerable error. Heifers of this age are known to have subthreshold heat cycles of very short duration. The marking harnesses available did not do a satisfactory job. Some heifers in estrus late in the evening or during the dark hours are not detected. Heat detection depends on the herdsman observing the bull or other heifers riding. Undetected heats are known to occur only if pregnancy results as it did in this trial where unrecorded matings had occurred.

TRIAL II

Materials and Methods

Approximately 2 months after the Trial I treatments were imposed, 14 of the 15 heifers used in groups 1, 2, and 3 of Trial I were reassigned to another set of treatments. One animal used previously whose size was approaching that observed at the onset of puberty by Wiltbank et al. (1965b) was not used in Trial II. The heifers were allotted to 2 groups on the basis of previous treatment and weight. Group I received a single subcutaneous injection of 1000 IU PMS followed 6 days later by 180 mg. MAP daily for 15 days. Group 2 heifers received only the subcutaneous injection of 1000 IU PMS with no subsequent feeding of MAP.

The heifers were individually fed 1.5 lbs. cottonseed meal and .5 lb. ground milo of supplemental feed per head per day. To insure that all heifers received their daily treatment, the progestational material (MAP) needed per day was weighed into individual packets and poured over the feed allotment of the treated heifers. Vasectomized bulls were used to detect estrus. Rectal palpations were made 3 days after the PMS injection and 2 days following cessation of MAP treatment.

Results and Discussion

This trial was initiated to determine if PMS alone was sufficient to stimulate estrual behavior followed by the development of a regular pattern of cyclic behavior. These heifers were older and nearer an age at which puberty normally occurs. Imposition of treatments at this age might possibly be more successful in animals nearing puberty than in animals 90-100 days younger. The purpose of administering the MAP treatment after the PMS injection was to determine what ovarian activity might occur should the progestogen regress the luteal tissue formed. It is of importance to know whether MAP will trigger the mechanism for follicular development and ovulation or return the animals to the anestrous state which persists before the onset of puberty.

The data pertaining to 2 heifers in group 1 and 3 heifers of group 2 were omitted when it was determined that the heifers were pregnant as a result of conception following previous treatment. The results pertaining to the nonpregnant heifers in this trial are summarized in Table II.

Only one of the five nonpregnant heifers in group 1 exhibited estrus after the PMS treatment. This heifer did not, however, come in heat following the 15-day period during which MAP was administered in the ration. Three of the remaining four heifers exhibited estrus within 3 days following the MAP treatment period. One heifer of this group of three was noted to have another estrual period. This occurred 19 days later, which would be considered a normal cycle interval. No further estrual behavior to indicate establishment of

TABLE II

EFFECT OF 1000 IU PREGNANT MARE SERUM (PMS) ALONE OR IN COMBINATION WITH 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) FOR 15 DAYS ON REPRODUCTIVE ACTIVITY IN PREPUBERAL BEEF HEIFERS

Group	Treatment	No.	Average Age (Days)	Number in Estrus Following PMS	Interval From PMS To Estrus (Days)	Number in Estrus After MAP	Interval From MAP To Estrus (Days)	No. Observed in Second Post-Treatment Estrus	Interval Between Estrual Periods
1	PMS - MAP	5	297	1	3	3	2.3	1	19
2	PMS	4	305	2	2.5	-	-	0	-

a regular cyclic pattern was observed in any of this group of heifers. This one heifer exhibiting two periods of estrus after MAP treatment was 11 months of age at the time of second post-treatment heat and could have conceivably reached puberty without treatment; however, the lack of subsequent cycles would not indicate the onset of puberty had occurred. Wiltbank et al. (1965b) had, however, reported Angus heifers wintered to gain 1.0 pound per day had an average age at puberty of 11.2 months.

The failure of more of the heifers in group 1 to exhibit estrus following the PMS treatment was not expected. Only one previous treatment probably would not be enough to sensitize the ovaries and make them refractive to subsequent treatments.

Rectal palpation after PMS revealed ovulation had not occurred. This observation differs from those made in Trial I where ovulation was diagnosed as having occurred, but does agree with reports in the literature by Mansour (1959) and Hertz and Hisaw (1934). They concluded ovulation requires a combination of injections involving a follicle stimulating substance followed by a luteinizing substance. Howe et al. (1962) used a combination of PMS and HCG to obtain ovulation in sexually immature heifers.

Of the four nonpregnant heifers in Group 2 only two were observed in estrus following the PMS treatment. Heat was manifested in these 2 heifers from 1 to 4 days after the PMS treatment. Neither of these two heifers were observed in heat at any subsequent time nor were the other 2 heifers in this group. Rectal palpations in this group led to the conclusion the ovaries were not stimulated by the PMS as indicated by their outer margins being smooth.

The greater response to MAP treatment than in Trial I might possibly indicate that as heifers approach puberty they are more responsive to a progestational agent, but would seem to contradict the response to PMS unless the ovaries were refractory to PMS. Whereas response to PMS was exhibited by almost all of the animals in Trial I, very few responded when PMS was injected a second time at the onset of Trial II. Since the dosage level used was the same in both trials, some mechanism inhibited the ovaries from responding the same when treated the second time. Nalbandov (1964) reported animals treated continuously with a protein hormone from a different species gradually lose their ability to respond to it. Since the animals had received only one previous injection of PMS this antihormone reaction would not be expected to have occurred. The results of this trial would indicate such a buildup of antihormones had occurred. The present state of being pregnant in some of the heifers prevented the exhibition of any response to PMS treatment. The exact cause for all the nonpregnant heifers not responding was not understood.

The MAP treatment after PMS possibly permitted the regression of the luteal tissue formed after PMS administration, but was not sufficient stimulus to elicit the establishment of cyclic estrual behavior. One heifer did have one estrous cycle of normal length, but could not be considered as having reached puberty since a cyclic pattern of estrual behavior was not established.

TRIAL III

Materials and Methods

Seventeen Angus-Hereford crossbred heifers born in early 1964 were individually fed 180 mg. MAP daily in 2 pounds of cottonseed meal for 15 days. These heifers ranged in age and weight from 301 to 366 days and 400 to 555 pounds, respectively. Their average age and weight were 324.9 days and 477.6 pounds, which are near the age and weight at which puberty or the exhibition of first estrus occurs as reported by Wiltbank et al. (1965b).

Harnessed vasectomized bulls were used to detect estrus prior to, during and following treatment in these heifers. All heifers that had not exhibited estrual behavior within 30 days after the cessation of MAP treatment received a single subcutaneous injection of 1000 IU of PMS.

Rectal palpations were made 1 day prior to the beginning of treatment and 6 days following the end of the MAP treatment period to determine ovarian activity.

Results and Discussion

This study was initiated to study the possibility of stimulating the onset of puberty in heifers nearing the age at which first estrus normally occurs. Earlier studies have shown MAP treatment was ineffective in triggering the development of a regular cyclic pattern of estrual activity in heifers 8-10 months of age. Its potential in stimulating cyclic activity in 10-12 month old heifers could be much different.

The results of this trial are summarized in Table III. Of the 17 heifers receiving 180 mg. MAP daily seven (41.2 percent) exhibited an induced estrus. Those seven heifers were in heat 2-5 days following the removal of MAP from the ration. Two other heifers came in heat on the nineteenth and twenty-second day after the last feeding of MAP with the length of time after feeding before estrus was shown indicating the possibility of occurrence of silent ovulation soon after the MAP treatment ceased. A corpus luteum was detected in one of these heifers 6 days after treatment providing proof of ovulation occurring without estrus. Six of the nine heifers returned to estrus at a time which could be considered normal for the regular cycle to have occurred. One of the 3 remaining heifers did not exhibit estrus until 90 days later; whereas, the other two heifers were not observed in heat again throughout the observation period. Not all of the 6 heifers that did have two normally spaced estrual periods after treatment developed a regular cycle. Four exhibited an estrual period after a normal interval, but two others lapsed into an anestrous condition. All heifers of this group which exhibited some estrual behavior eventually were observed in anestrous, but recycled at some later date. These irregular cycles indicate the possibility of regular cyclic ovarian activity, but with estrogen levels not high enough to stimulate estrus. It could also be poor detection associated with the unreliability of the marking harness.

TABLE III

DEVELOPMENT OF ESTRUAL BEHAVIOR AFTER A 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE TREATMENT FOR 15 DAYS ALONE, OR FOLLOWED BY 1000 IU PMS IN ELEVEN-MONTH-OLD HEIFERS

Treatment[a]	No.	Ave. Age (Days)	Average Weight	No. Observed in Induced Estrus	Days From Treatment To First Estrus	No. Ovulated 6 Days Post-Treatment[b]	No. Observed in Second Post-Treatment Estrus	No. Observed in Third Post-Treatment Est.
MAP	17	324.9	477.6	7	3.7	5	6	4
MAP - PMS	8	327	475	7	2.1	-	2	0

[a]PMS was administered 30 days post MAP treatment in all heifers not observed in estrus following the 18-day treatment period.

[b]One known silent ovulation.

Rectal palpation of all heifers 6 days after the cessation of MAP treatment revealed for the most part that those animals which had exhibited an induced estrus had also ovulated. Apparently the corpus luteum was normal and functional because a number of these animals completed a normal cycle and exhibited heat again. Two heifers in estrus late on day 5 after treatment did not have a corpus luteum when palpated on day 6.

Eight heifers which had not exhibited estrus within 30 days post-treatment received a subcutaneous injection of 1000 IU PMS. Seven showed heat within a 3-day period, with six of 7 heifers in estrus on the second day after the injection. One heifer of this group was not observed in estrus. Only two heifers returned at a time resembling a normal interval after the PMS induced estrus.

The occurrence of cyclic estrual activity after the induced estrus where palpation indicated a corpus luteum was present provides some support for the theory of the essentiality of a waning corpus luteum (Grant, 1936). He reported estrus behavior will not occur unless a waning corpus luteum is present in the ovaries. Marden (1952) also reported the presence of luteal tissue in the ovaries improved the chance of occurrence of estrual activity. The failure of PMS to stimulate more cyclic estrus behavior than occurred can perhaps be attributed to the lack of luteal tissue. PMS has a partial luteinizing effect, but is more effective as a follicle-stimulating substance. Mansour (1959) and Hertz and Hisaw (1934) were able to produce ovulation only with a combination of a follicle-stimulating and luteinizing hormones. If PMS did not stimulate enough luteal tissue formation the overall failure of the heifers to return to estrus would substantiate Grant's (1936) claim that a waning corpus luteum is required for subsequent cyclic estrual behavior.

The results of this trial indicates that oral progestogens can be effective in stimulating the mechanism for onset of puberty in calves if the heifers are near an age where puberty normally occurs. The response to the oral progestogen would be a short term effect in that a cyclic pattern of estrual activity will not be established. The use of PMS was also effective in causing the exhibition of estrus, but since only two of eight heifers returned, it could not be considered a satisfactory procedure for stimulating the onset of cyclic estrual activity in this age of heifers.

TRIAL IV

Materials and Methods

This study was initiated in the spring, 1964, using 38 two-year-old Hereford heifers that had calved in February and March. The heifers used in this study had been divided into two groups the preceding fall and maintained at different levels of nutrition prior to calving. Nineteen heifers were fed a high level (H) defined as the amount of supplemental feed necessary to maintain their fall weight through calving. The other 19 heifers were fed a low-moderate level (LM) which was the amount of supplemental feed during gestation to allow a 15 percent decrease in their fall weight. Following calving all heifers were placed on the high level of feeding. The heifers grazed native grass pasture during the wintering period with access to a complete mineral mixture fed free choice. The heifers were individually fed the supplemental feed which was ground milo and cottonseed meal fortified with synthetic vitamin A to provide 15,000 IU per heifer per day. Each heifer received 2 lb. cottonseed meal per day and milo was adjusted on the basis of weight changes in the heifers of each lot as determined by weighing the heifers every 28 days. An effort was made to standardize amount of fill by holding the heifers away from feed and water overnight in drylot.

All heifers at calving were scored for calving difficulty on a scale from 1-5 as follows:

1.-Calved naturally without aid.

2.-Some assistance required.

3.-Difficult parturition but calf pulled by hand.

4.-Very difficult parturition with mechanical assistance required.

5.-Cesarean section operation.

The primiparous heifers used in this trial were alternately assigned to either a treated or control group at the time of calving. An attempt was made to allot the cows such that the average calving date for the two groups would be approximately equal. The heifers of both groups were pastured together and individually fed the same quantity of supplemental feed. The treated group was fed 180 mg. MAP per heifer per day for 18 days beginning on the twenty-fifth day after parturition. The MAP was measured into individual packets containing 180 mg. and the contents of one package were poured over each treated cow's feed allotment, insuring that all treated cows received the prescribed dose recommended by Zimbelman (1963). Treatment was on an individual heifer basis beginning on day 25 after calving.

Fertile Hereford bulls were maintained with the cows following calving with mating permitted at the first post-partum estrus. These bulls remained with the cows throughout the breeding season and were removed August 1. Pregnancy was diagnosed on the basis of rectal palpations made in early October. The heifers calved

February to April 1965. The 1965 calving dates were used to confirm the 1964 breeding dates and to calculate calving intervals.

Student's "t" test, as illustrated by Steel and Torrie (1960), was used to determine if a significant mean difference in the length of the post-partum interval to first estrus, time from calving to subsequent conception, and the calving interval might exist between the treated and control group.

Results and Discussion

This study was initiated to determine the effect of 180 mg. MAP per head per day in stimulating the onset of post-partum reproductive activity in lactating beef cows. It was hypothesized that MAP would cause a regression of the corpus luteum present during lactation, and whenever the progestational substance was removed the release of gonadotropins would be uninhibited and follicular growth and ovulation would occur. Should MAP stimulate an earlier return to reproductive activity following calving, the calving interval would be shortened, thus improving the reproductive efficiency of the cow.

The results pertaining to degree of synchronization are presented in Table IV with the distribution of matings for the cows illustrated in Figure 1.

None of the 18 treated heifers were observed in estrus before or during the 18-day treatment period which began 25 days after calving. Four heifers (22 percent) were observed in estrus within three to six days following the removal of MAP from the diet which would indicate some synchronization had been possibly achieved. Two of the four synchronized heifers were known to conceive to natural service at this induced estrus. Six additional heifers were observed in estrus at a time when the second subsequent post-treatment heat period would be expected to occur, but neither of the two nonpregnant heifers which had earlier exhibited an induced estrus were observed in heat at this time. Four of the 6 heifers in heat from day 16 to day 28 conceived. By 28 days post-treatment six of 18 (33 percent) treated heifers had conceived and subsequently produced live calves at parturition. The remaining heifers in the treated group returned to cyclic activity at varying intervals following treatment. Only one heifer of the treated group failed to produce a live calf. All untreated heifers produced calves after normal gestation intervals. It would appear from the results of this trial that an oral progestogen is relatively ineffective in synchronizing estrual activity in noncycling post-partum beef heifers, but had no detrimental effect on subsequent fertility.

TABLE IV

DEGREE OF SYNCHRONIZATION OBTAINED FOLLOWING ADMINISTRATION OF 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) PER DAY FOR 18 DAYS TO NON-CYCLING LACTATING BEEF HEIFERS BEGINNING 25 DAYS AFTER PARTURITION

Group	No.	No. in Estrus by 6 Days Post-Treatment	No. Conceiving	No. in Estrus 16-28 Days Post-Treatment	No. Conceiving
MAP	18	4	2	6	4

The heifers used in this experiment were not palpated, thus nothing was determined about corpus luteum regression, follicular development or occurrence of silent ovulations. Rectal palpations

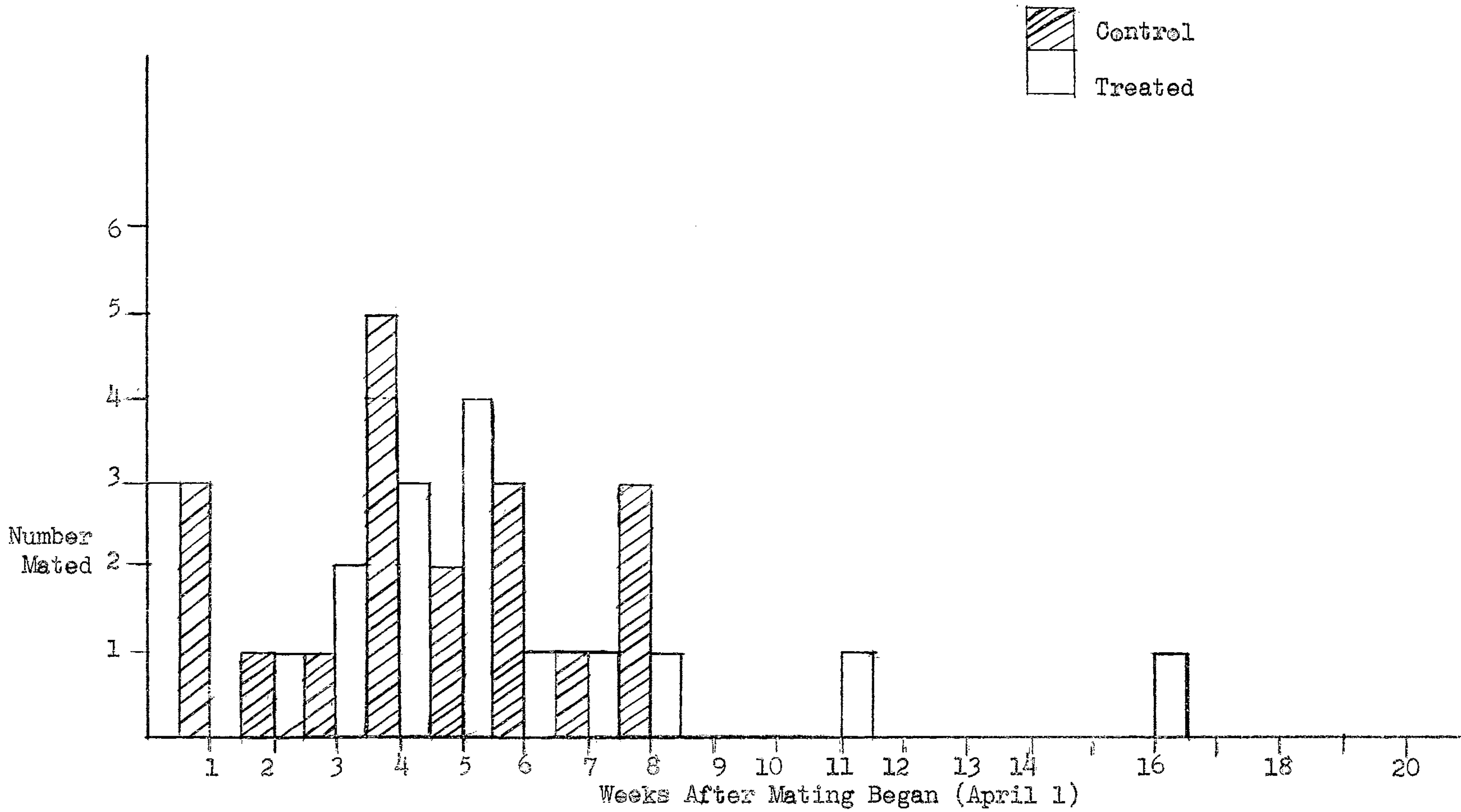

Figure 1. Distribution of Matings in Post-Partum Two-Year-Old Hereford Heifers Following 180 mg. MAP Per Day for 18 Days Beginning on Day 25 Post-Partum.

were not carried out in an attempt to eliminate this possible cause of early embryonic death loss. This decision was strengthened by the report by Zimbelman (1963) that there was considerable error associated with rectal palpations made in post-partum cows. It was not known whether the four heifers in estrus following removal of MAP from the ration failed to conceive because of an anovulatory estrus or for some other reason. However, it would appear that some of those heifers which exhibited estrus did ovulate normally since six of ten conceived.

Various workers have attempted to synchronize the occurrence of estrus in noncycling post-parturient cows. In general, their efforts also have not met with much success. Zimbelman (1963) did, however, report that 19 of 22 treated post-partum were considered synchronized on the basis of occurrence of ovulation between 2 and 7 days after the last feeding. Administration of 180 mg. MAP per head per day began either 9-15 days or 16-22 days post-partum in an effort to begin treatment before any ovulatory activity occurred. Syncrhonization was reported as having occurred if ovulation was noted by rectal palpation. However, with this technique considerable error was believed to occur because of the uncertainty of differentiating between a follicle and a corpus luteum in post-partum cows.

Foote et al. (1960b) reported no synchronization in Angus and Shorthorn cows treated 14 days post-calving with a single implant of 1 mg. per pound body weight of progesterone. First estrus post-partum after this treatment ranged in occurrence from 35-154 days.

Foote and Hunter (1964b) also reported very little synchronization was achieved by injecting 50 mg. progesterone daily from day 12 to day 23 post-calving. The cows averaged 41 days from parturition to first estrus with a range of 26-54 days. This same progesterone treatment plus 10 mg. estradiol 17-β on day 25 or the estradiol treatment alone did produce greater synchronization. The progesterone plus estradiol treatment resulted in an average date of occurrence of first estrus of 27 days (range 26-43 days); whereas, the average date of occurrence of estrus in those treated with estradiol alone was 27 days with a range of 26-37 days and a standard deviation of 2.5 days.

Ten of the 18 treated cows (55.6 percent were observed in heat within 27 days after removal of treatment which would be a post-partum interval of 69 days or less. Of 18 control cows, 16 (88.9 percent) were observed in estrus on or prior to day 69 post-partum. The remaining eight treated cows had extended periods of anestrus (70-169 days) before reproductive activity was observed. This compares to a range of 71 to 92 days for the remaining control cows.

The other measures of reproductive performance studied were the interval from calving to first post-partum estrus, percent conception at the first post-partum estrus, and the calving interval. These results are given in Table V and VI. In Table V the means for the subgroups of level of nutrition are listed and in Table VI the two different planes of nutrition are combined to give an overall mean for the MAP treated and the control group. The important features of these two tables are the values associated with post-partum interval, days from calving to conception, percent conception at the first post-partum estrus, and the calving interval.

Since allotment to the treatment or the control group was on the basis of calving date, the average pretreatment calving dates are very comparable. Average calving difficulty score was slightly higher for the cows maintained at the higher plane of nutrition, indicating higher feeding regimes prior to calving tend to be associated with an increase, though nonsignificant, in the difficulty of parturition. The average birth weight of the calves produced by these Hereford heifers is presented in Table V. The high level groups had average birth weights of 70.9 and 69.3 lbs.; whereas, the average weights for the low-moderate level cows were 63.9 and 66.4 lbs. The difference was small and seemingly should not be great enough to explain the difference in average overall calving difficulty scores for the high and low-moderate groups of 1.9 and 1.6, respectively. While the average difference in birth weight seems to offer the most logical explanation for the difference in calving difficulty it should be noted that the low-moderate group with the lowest average birth weight had an average calving difficulty score similar to that of the high groups. There is also the possibility that the heifers on the high level deposited enough internal fat in the area around the birth canal to hinder parturition, but this could not be determined.

The heifers maintained at the high level of nutrition lost more weight at calving than desired. They were theoretically fed to gain enough weight between the end of the breeding season and beginning of the calving season such that body weight after calving would be similar to their previous fall body weight. The low-moderate group was fed so body weight after calving would represent a 15 percent loss in

TABLE V

A COMPARISON OF THE REPRODUCTIVE PERFORMANCE OF TWO-YEAR-OLD HEREFORD HEIFERS MAINTAINED AT TWO LEVELS OF WINTER FEEDING BEFORE CALVING AND TREATED WITH 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) FOR 18 DAYS BEGINNING ON DAY 25 POST-PARTUM

Item	MAP Group		Control	
Plane of Nutrition	High	Low Moderate	High	Low Moderate
Number	10	8	9	11
1964 Ave. Calving Date	59.6	51.9	60.8	60.5
Ave. Birth Weight	70.9	63.9	63.9	66.4
Ave. Calving Difficulty Score	2.0	1.8	1.8	1.5
Weight Change at 1st Calving	-86.5	-137.5	-52.2	-131.4
% Fall Weight Loss	10.2	16.0	5.9	15.3
Ave. Date 1st Estrus	135.9	125.8	117.9	119.3
Post-Partum Interval	76.3	73.1	57.1	58.7
Days Calving to Conception	95.6	77.0	68.4	67.7
% Conception at 1st PPE*	55.6	75.0	66.7	54.7
1965 Ave. Calving Date	70.4	43.9	50.3	48.8
Calving Interval	375.4	358.0	355.3	354.3

TABLE VI

THE INFLUENCE OF 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) PER DAY FOR 18 DAYS BEGINNING DAY 25 POST-PARTUM ON THE REPRODUCTIVE PERFORMANCE OF TWO-YEAR-OLD HEREFORD HEIFERS

Item	MAP	SD	Control	SD
Number	18		20	
1964 Ave. Calving Date	56.2		60.6	
Ave. Birth Weight	67.5		67.6	
Ave. Calving Difficulty Score	1.9	1.4	1.6	1.1
Ave. Date of 1st Estrus	131.4		118.6	
Post-Partum Interval	74.9**	30.6	58.0**	15.8
Days Calving to Conception	86.8	34.5	71.4	22.3
% Conception at 1st PPE*	64.7		60.0	
1965 Ave. Calving Date	57.9		49.5	
Calving Interval	367.9	13.5	354.8	28.5

*PPE - Post-Partum Estrus

**($P < .05$)

body weight following calving. The goal in the low-moderate group was realized for both the MAP and control group (16 and 15.3 percent loss, respectively) but the high level treated and control group lost 10.2 and 5.9 percent, respectively, of their fall weight (Table V).

The average date of occurrence of first post-partum estrus was day 75 with a standard deviation of 30.6 days. Treatment began on day 25 and was terminated 18 days later. The earliest that estrus could have occurred would have been day 42. The length of time from calving to the exhibition of first estrus was not found to be affected by plane of nutrition but a significant ($P < .05$) mean difference due to MAP treatment did occur. The oral ingestion of 180 mg. MAP increased the post-partum interval from calving to first estrus by 17 days (Table VI). The reason for this difference was not known. Zimbelman (1963) reported MAP feeding had no significant effect on the average interval from calving or end of treatment to either post-treatment ovulation or conception within trials.

The period from calving to conception also was longer for the MAP treated cows by approximately 15 days, but the difference was not significant (Table VI). The increase in the interval to first estrus following calving would be reflected in this period. Considerable variation between levels of nutrition in the treated cows existed (Table V), with the interval from calving to conception longer in the group maintained at the high level. The standard deviation for the MAP treated group was 34.5 days.

The conception rate at the first post-partum estrus was comparable for the two groups being 64.7 percent for the MAP group and 60 percent for the controls. The within group plane of nutrition subgroups varied considerably and this together with small numbers of observations reduced the possibility of obtaining a significant difference (Table V). The conception rate in the MAP low-moderate energy group was higher than the high energy group, but the reverse was true in the control group. Conception rate was apparently not influenced by treatment in this trial.

The mean calving interval, or the time from the birth of one calf to the subsequent birth of another, was 12 days longer in the treated group than in the controls with considerable within treatment variation. The use of MAP, although increasing the interval from calving to first estrus did not cause a significant increase in calving interval. This was probably because conception rate at first post-partum estrus was not extremely high and considerable variation existed within both treatment groups with regard to calving interval.

The results of this trial indicate that oral ingestion of the progestogen compound, 6-methyl-17-acetoxyprogesterone, did not produce an alteration of ovarian activity in post-partum cows. Presumably a progestogen blocks the release of luteinizing hormones. If such is true, the maintenance of luteal activity in the ovary is mediated by some other source or substance. The extension of post-partum reproductive quiescence in this trial by feeding 180 mg. MAP was not expected. Apparently the heifer requires a set period of time to recover from the after effects of pregnancy and rebuild the uterus before starting another gestation period. The use of progestogens somehow inhibited this recovery, thus extending the period of anestrous following calving.

TRIAL V

Materials and Methods

This trial was initiated in the fall, 1963, using 60 weaner Hereford heifers maintained at the Fort Reno Livestock Experiment Station. The average fall age and weight for these heifers was approximately 250 days and 480 lbs., respectively.

All heifers were fed at a moderate level (0.5 lb. gain per day) from November 1 until March 1. On March 1, the heifers were divided on the basis of weight and sire into two comparable groups of 30 heifers each. One group remained on the moderate level of feeding to theoretically gain 0.5 lb. per day and the other group of 30 heifers were raised to a higher plane of nutrition to obtain an average daily gain of 1.0 lb. per day. The heifers were maintained at these two different levels of nutrition, designated moderate energy (ME) and high energy (HE), until shortly before the breeding season began on May 1.

All heifers were individually fed during the wintering period. Beginning in mid-October, they received a daily allotment of 1.5 lb. of cottonseed meal fortified with synthetic Vitamin A to provide 15,000 IU per heifer per day fed every other day. Approximately November 1, one lb. milo per head per day was added to the diet, and was gradually increased to 3 lbs. per head per day.

Beginning March 1, the heifers of the high energy lot were increased to 5 lbs. of milo per head per day which was further increased to 6 lbs. per day 15 days later. The vitamin A fortified cottonseed meal was continued at the level of 1.5 lb. per head per day throughout the winter feeding period.

The 30 heifers maintained at each energy level were allotted on the basis of weight into two comparable groups of 15 heifers each. The treatment design is presented in Table VII. Estrus in one group of 15 heifers from the high energy group (Lot I) and one group of 15 heifers from the moderate energy group (Lot II) was synchronized at a time to permit them to be artificially inseminated at the first induced estrus. Estrus in the remaining group of heifers on each energy level (Lots III and IV) was synchronized at a time to permit insemination at the same time as Lots I and II but at their second post-treatment estrus. Only nine of the 60 heifers had not shown at least one estrual period previous to the MAP treatment.

TABLE VII

ARRANGEMENT OF TREATMENTS USED TO COMPARE FERTILITY FOLLOWING ARTIFICIAL INSEMINATION AT FIRST ESTRUS VERSUS SECOND ESTRUS IN HEREFORD HEIFERS MAINTAINED AT TWO DIFFERENT ENERGY LEVELS

Energy Level	Bred At First Estrus	Bred At Second Estrus
High	Lot I (15)	Lot III (15)
Moderate	Lot II (15)	Lot IV (15)

Synchronization was accomplished in all heifers by feeding 180 mg. 6-methyl-17-acetoxyprogesterone (MAP) per heifer per day for 18 days. The MAP was mixed with the cottonseed meal at the rate of 3.64 grams of the pharmaceutical premix (Repromix) to 1 lb. of cottonseed meal. Each heifer was individually fed once daily her allotment of MAP. In order that all heifers could be inseminated during the same period, time of feeding MAP was staggered. The heifers in Lots III and IV, which were to be bred at the second post-treatment estrus, were fed MAP for 18 days starting March 25 and ending April 11. The heifers in Lot I and Lot II were fed MAP beginning April 15 and ending May 2. Artificial insemination of all lots was begun May 1, and carried out for six days.

Vasectomized bulls wearing grease-marking harnesses were maintained with these heifers prior to, during and after treatment. The error associated with detecting estrus by using harnessed vasectomized bulls has been discussed previously (Trial I). The fertile vasectomized bull previously mentioned in Trial I was also inadvertently used in these groups of heifers and unfortunately settled some of the heifers.

All first service matings following the MAP feeding periods were by means of artificial insemination. Artificial insemination was practiced only at the one service period. The heifers were pasture-mated at all later heat periods to fertile bulls maintained with the herd until August 3. The semen of all bulls used had been evaluated for motility and adequate concentration, and all bulls were believed to be of good fertility.

All artificial inseminations were made using frozen semen from a single ejaculate from a Red Angus bull. This bull was in the bull stud of the American Breeders Service and had an excellent fertility record. The services of an experienced inseminator with a very creditable record for services per conception on virgin heifers were obtained to minimize the possibility that any decrease in conception rate would be due to an inexperienced inseminator. The pickup bulls used for subsequent natural matings were black Angus that had been progeny tested to a level to indicate they did not carry the recessive red gene.

Difference in conception rate were tested by using a chi-square test for two independent samples as illustrated by Siegel (1956). Bartlett's test for homogeneity of variance (Steel and Torrie, 1960) was used to test the effect of MAP treatment on the variation in time of occurrence of mating during the breeding season.

Results and Discussion

Sixty yearling Hereford heifers were fed 180 mg. MAP per head per day for 180 days to study its effectiveness in synchronizing estrus. A comparison was made of conception rates to artificial insemination carried out at the first or second post-treatment estrual period. Two planes of nutrition were fed during the two months immediately preceding the breeding season to study the response to MAP and subsequent conception rate of heifers receiving a high versus a moderate energy intake.

The average daily gains prior to and during the treatment periods of the various lots of heifers were close to what was desired and are reported in Table VIII. The heifers of all lots gained approximately 0.4 lbs. per day during the wintering period prior to March 1. The heifers of Lots II and IV were continued on a moderate energy level (ME) but average daily gain did increase slightly above the desired 0.5 lbs. per day when grazing conditions improved in mid-April. As a result, the average daily gain in Lots II and IV was 0.53 and 0.63 lbs. per day, respectively, during the two-month period preceding the breeding season. The heifers of Lots I and III were raised to a high energy intake (HE) for the two months preceding the breeding season. The average daily gain during this period was 0.94 and 1.15 lbs. per day for Lot I and Lot III, respectively.

Nine heifers (15 percent) were observed in estrus while receiving MAP (Table VIII). Estrus during treatment was observed in only one heifer of the two groups mated at the induced estrus. This heifer was in the moderate energy group (Lot II), and was not observed in heat and inseminated at the induced period. She did, however, return to estrus 19 days post-treatment, and conceived but later aborted. Four heifers in each of Lots III and IV exhibited estrus during treatment. All four heifers in Lot III did, however, exhibit estrus again two days after the last MAP feeding. Two conceived to service by the fertile vasectomized bull at this induced period while the other two heifers conceived to artificial insemination at the second post-treatment estrus. Two of the four heifers observed in estrus during treatment in the moderate energy group (Lot IV) were observed to have an induced estrus two and three days after the last MAP feeding. All four heifers of Lot IV were observed in heat at

TABLE VIII

DESCRIPTION OF THE 60 HEREFORD HEIFERS FED 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD PER DAY FOR 18 DAYS AND OBSERVED FOR OCCURRENCE OF ESTRUS AND CONCEPTION TO ARTIFICIAL INSEMINATION AT FIRST OR SECOND POST-TREATMENT ESTRUS

				No. Exhibiting Estrus		Plane	Average Daily Gain	
Group	N	Average Age	Average Weight	Prior to Treatment	During Treatment	of Nutrition	Nov. 1 to March 1	March 1 to March 22
Lot I	15	399.7	545.1	15	0	HE	0.35	0.94
Lot II	15	394.2	545.3	13	1	ME	0.42	0.53
Lot III	15	420.0	572.4	12	4	HE	0.40	1.15
Lot IV	15	414.6	549.6	11	4	ME	0.42	0.63

the time when second post-treatment estrus should have occurred. None of these four heifers conceived to artificial insemination at this heat period, but all conceived at the next subsequent heat period when natural mating occurred.

Three of the nine heifers in estrus during treatment did not exhibit a synchronized estrus after cessation of the MAP treatment, thus reducing the response to synchronization with the oral progestogen. Since all later conceived no detrimental effect on fertility was apparent. Silent ovulation could have possibly occurred, but was not known to have done so because of the lack of palpation data.

Zimbelman (1963) concluded 180 mg. MAP per day prevented ovulation. Most workers have reported a complete suppression of estrus during treatment period (Nelms and Combs, 1960; Hansel and Malven, 1960; Zimbelman, 1961; Anderson et al., 1962), however, they used a higher dosage level. Nelms and Combs (1960) used a dosage level of 220 mg. MAP per day while Hansel and Malven (1960) fed MAP at a level of 968 mg. for 10 days then reduced it to 500 mg. for another 10 days and inhibited all signs of heat during the treatment period. Zimbelman (1961) studied both group feeding and individual feeding of 0.5 mg. MAP per pound of body weight daily and reported complete suppression of estrual activity. Anderson et al. (1962) fed MAP at two levels (150 and 210 mg. per head per day for 20 days) and completely inhibited estrus during treatment. Collins et al. (1961) reported that 0.5 mg. MAP per pound of body weight daily for 20 days prevented estrus and ovulation in all but one animal during treatment.

The distribution of estrual behavior given in Table IX and presented in Figures 2 and 3 illustrate the degree of synchronization

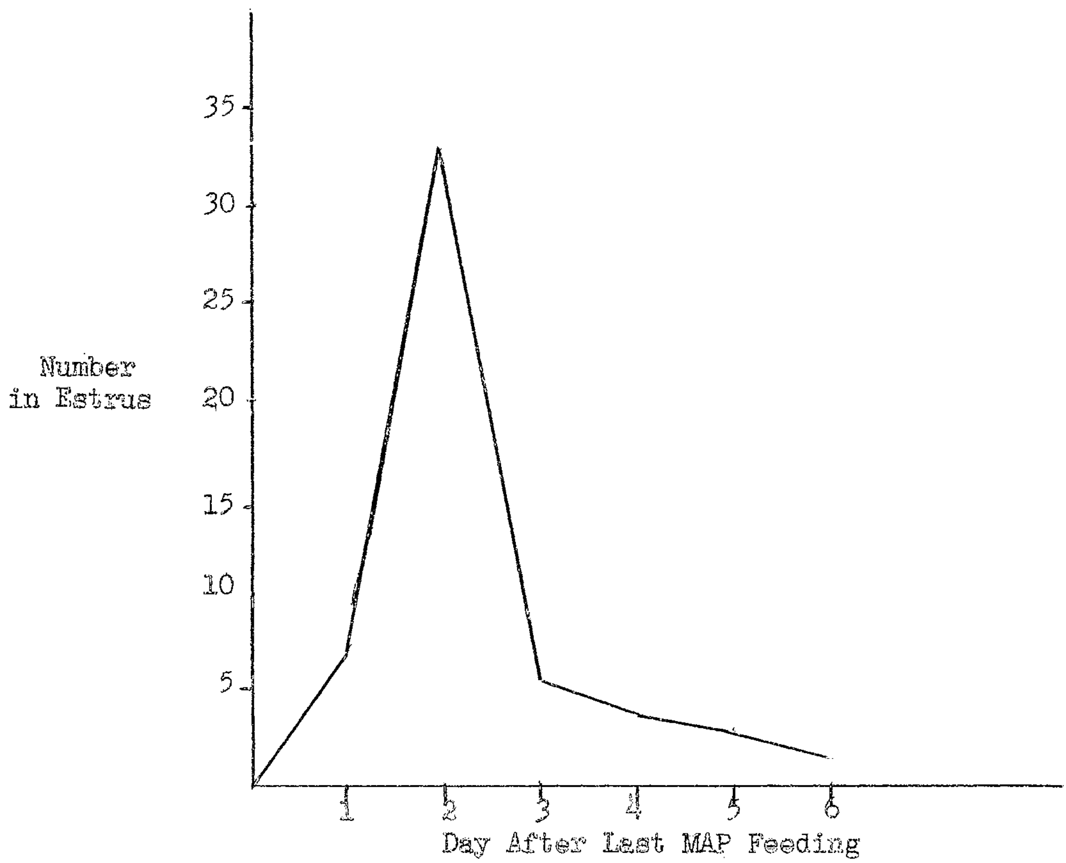

Figure 2. The Occurrence of Estrus in Hereford Heifers Following Treatment with 180 mg. 6-Methyl-17-Acetoxyprogesterone Per Head Per Day for 18 Days.

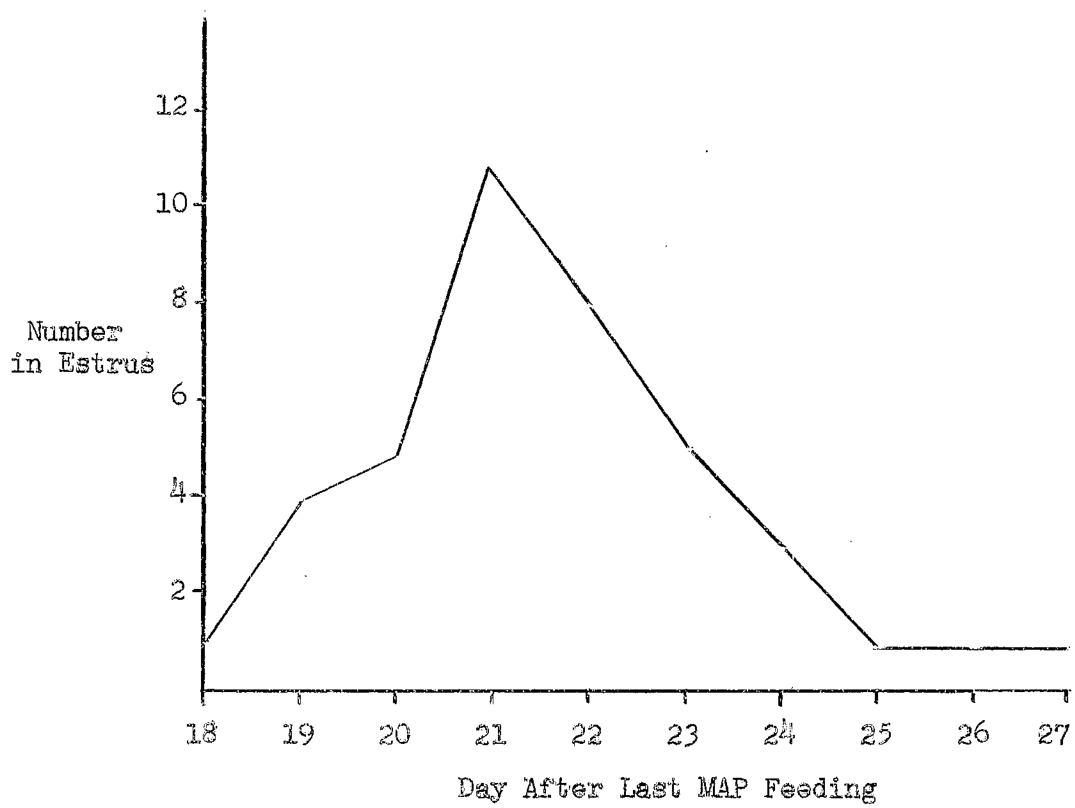

Figure 3. Distribution of the Occurrence of the Second Post-Treatment Estrus in Hereford Heifers Treated with 180 mg. 6-Methyl-17-Acetoxyprogesterone Per Head Per Day for 18 Days.

obtained at both first and second estrus following MAP treatment. Sixty percent of the 55 heifers exhibiting estrus did so on the second day following treatment. All heifers exhibiting a first post-treatment estrus did so within one to six days after the end of the treatment period. All heifers which exhibited a second estrus after MAP were observed to be in heat in a 10-day period beginning 18 days following removal of MAP from the ration. The distribution of occurrence of the second post-treatment estrus is given in Table IX and illustrated in Figure 3. Approximately 75 percent of the heifers were in estrus within a four-day period beginning 21 days after removal from MAP treatment. Included in this group are all heifers of Lots III and IV that were not bred at the induced estrus, plus those of Lots I and II that did not conceive to artificial insemination at the induced estrus.

TABLE IX

DISTRIBUTION OF ESTRUAL BEHAVIOR OF HEREFORD HEIFERS INDIVIDUALLY FED 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD PER DAY FOR 18 DAYS

Day After MAP	No. in Estrus	Percent in Estrus	Day After MAP	No. in Estrus	Percent in Estrus
1	7	12.7	18	1	2.5
2	33	60.0	19	4	10.0
3	6	10.9	20	5	12.5
5	4	7.3	21	11	27.5
6	2	3.6	23	5	20.0
			24	3	7.5
			25	1	2.5
			26	1	2.5
			27	1	2.5
Total	55	100.0		40	100.0

The intervals from last days of feeding of MAP to the occurrence of first estrus are given in Table X. The intervals were comparable in all four groups. The moderate energy group bred at the second post-treatment estrus exhibited a slightly longer mean interval from last feeding to occurrence of the induced estrus. Collins et al. (1962) reported a comparable mean interval from last feeding to estrus of 2.5 days after treatment with 0.5 mg. MAP per pound body weight daily for 20 days. The mean interval from last feeding to second post-treatment estrus was also comparable for all four groups except both moderate energy groups tended to require more time before the second induced estrus occurred (Table X).

TABLE X

THE AVERAGE INTERVAL TO FIRST AND SECOND POST-TREATMENT ESTRUS IN HEREFORD HEIFERS INDIVIDUALLY FED 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD PER DAY FOR 18 DAYS

Group	No.	Interval From MAP To First Estrus	No.	Interval From MAP To Second Estrus
Bred at First Estrus				
High Energy	15	2.3	9	19.6
Moderate Energy	12	2.3	8	22.5
Bred at Second Estrus				
High Energy	15	2.4	13	21.7
Moderate Energy	13	2.8	13	22.7

Two of the five heifers not showing an induced estrus were in Lot IV, while the other three heifers were in Lot II. Both heifers of Lot IV and one heifer of Lot II were observed in estrus when the second post-treatment estrual period would be expected to occur. This indicates the possibility of silent ovulation having occurred at the induced estrus. The two heifers of Lot II not observed in estrus at either the first or second periods after MAP were later found to be pregnant as a result of mating with the fertile teaser bull. On the basis of calving dates, one of these heifers apparently conceived shortly after MAP treatment. The other heifer conceived approximately 14 days after the last day on which artificial insemination was performed. Four additional heifers, scheduled to be artificially inseminated at the second estrus after treatment, conceived to natural matings with the vasectomized bull at their first post-treatment estrual period. These results again point out how the use of a fertile, although supposedly vasecomized bull affected the results of this trial.

The actual number of days during which artificial insemination was practiced in each group was significantly reduced ($P<.005$) in the groups bred at first estrus when compared to the two groups bred at the second post-treatment estrus. The variation in time of occurrence of estrus would be expected to increase each cycle after the induced estrus because of the normal range for the estrous cycle. Lasley and Bogart (1943) reported 79 percent of 781 cycles in range beef cows were between 17 and 23 days in length. The other 21 percent were either more than 23 days, or less than 17 days in length.

The results presented in Table X would indicate level of energy had very little influence on time of occurrence of estrus after treatment. The first induced estrus in the group bred at the first estrus occurred 2.3 days after cessation of treatment in both the high and moderate energy groups (Table X). In the groups bred at second estrus (Lots III and IV), there was also a comparable interval from cessation of treatment to the first induced estrus (2.4 days for the high energy group and 2.8 days for the low-moderate energy group). The interval from the end of MAP treatment to the occurrence of second estrus was similar for all groups (19.6, 22.5, 21.7, and 22.5 days). The high energy group bred at first estrus did have a three-day shorter mean interval. The difference in energy content between the two levels apparently was not extreme enough to produce a statistically significant influence on response to treatment if one truly exists (Table XI and Figure 4). Both moderate energy groups did exhibit a slight increase in the interval between cessation of treatment and occurrence of a second induced estrus. This difference was not statistically significant.

TABLE XI

THE INFLUENCE OF PLANE OF NUTRITION ON OCCURRENCE OF FIRST AND SECOND POST-TREATMENT ESTRUS IN HEREFORD HEIFERS INDIVIDUALLY FED 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD PER DAY FOR 18 DAYS

First Estrus			Second Estrus		
	No. in Estrus			No. in Estrus	
Day After MAP	High Energy	Moderate Energy	Day After MAP	High Energy	Moderate Energy
1	6	1	18	1	0
2	17	16	19	3	1
3	2	4	20	3	2
4	2	2	21	4	7
5	2	1	22	5	3
6	1	1	23	1	4
			24	2	1
			25	1	0
			26	0	1
			27	0	1
Total	30	25		20	20

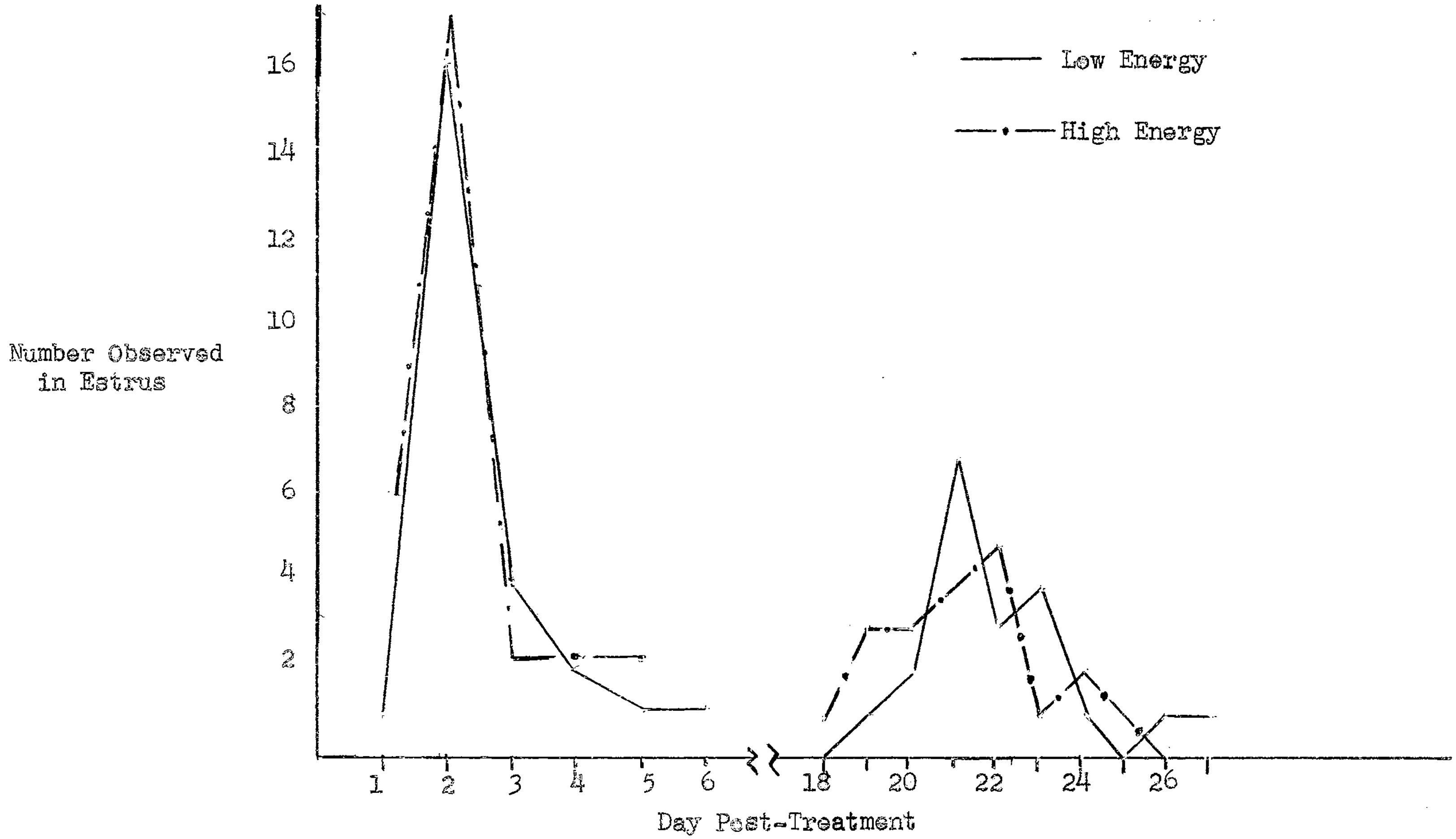

Figure 4. The Occurrence of Estrual Behavior in Hereford Heifers Maintained at Two Different Energy Levels then Synchronized with 180 mg. 6-Methyl-17-Acetoxyprogesterone Per Head Per Day for 18 Days.

The preceding results relative to synchronization of estrus are comparable to those reported in the literature. Nelms and Combs (1960) reported 220 mg. MAP per head per day for 15 days caused heat to occur on the second and third day post-treatment in all of 33 cows; whereas, Collins et al. (1961) observed 14 of 15 beef heifers were in estrus within 2 to 8 days following MAP treatment at a level of 0.5 mg. per pound of body weight for 20 days. Sorenson (1962) noted 75 percent of 97 heifers exhibited a synchronized estrus, somewhat lower than the 92 percent of 60 heifers reported in heat in this trial. Nestel et al. (1963) observed 85 percent of 38 heifers exhibited an induced estrus following MAP treatment.

The conception rates at first service, number of repeat matings, services per conception and number of open heifers are presented in Table XII.

TABLE XII

THE FIRST SERVICE CONCEPTION RATE, SERVICES PER CONCEPTION AND NUMBER OF OPEN HEIFERS FOLLOWING ARTIFICIAL INSEMINATION AT THE FIRST OR SECOND POST-TREATMENT ESTRUS PERIOD AFTER TREATMENT WITH 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD PER DAY FOR 18 DAYS

Group	No. Bred Artificially	No. Conceived	Percent Conceived	No. Repeat Matings	Svcs./ Conception	No. Open
Bred at First Estrus						
High Energy	12	4	33.3	10	1.7	2
Moderate Energy	10	4	40.0	11	1.5	1
Bred at Second Estrus						
High Energy	13	7	53.8	9	1.5	0
Moderate Energy	11	1	9.1	15	1.9	1

The number conceiving when artificially inseminated at the first induced estrus was 8 of 22 heifers bred (36.4 percent). The difference between the two energy levels was not significant. Of the twelve heifers in the high energy group which were artificially inseminated, four conceived (33.3 percent); whereas, four settled of ten (40 percent) artificially bred in the moderate energy group. One heifer in the moderate energy group did conceive to natural service at the first induced estrus. This raised the first service conception rate for this group to five of eleven (45.5 percent).

The lower first service conception rate for the high energy group was reflected somewhat in the slight increase noted in services per conception. The high energy group required 1.7 services per conception as compared to the moderate energy group which averaged 1.5 services for conception to occur. Six of the eight heifers in the high energy group (Lot I) failing to conceive to artificial insemination at the induced estrus later conceived to their first natural mating by fertile black Angus bulls; furthermore, three additional heifers settled to later natural mating by the pickup bulls and two heifers were open at the end of the breeding season. A total of 22 matings were required by the 13 heifers of Lot I that settled. Additional matings to two open heifers were not counted. Twenty-one matings were required to settle the 14 cows in the moderate energy group bred first at the induced estrus. Two required three services; whereas, six settled at first natural service after artificial insemination. The large number of second services in Lot I, in addition to the fact that three heifers in Lot II were not inseminated caused the increase in number of services per conception for Lot I. The high energy group had two open cows with known breeding records, while the moderate energy group had only one nonpregnant cow.

The first service conception rate in the two lots artificially inseminated at the second post-treatment estrual period was eight of 24 heifers (33.3 percent). The major cause of low first service conception rates was only one conception of 11 heifers (9.1 percent) bred in the moderate energy group. The high energy group had a 53.8 percent conception rate with seven conceiving of 13 heifers bred. Whereas, conception rate was higher in moderate energy groups than the high energy group bred at the first induced estrus, the reverse was true in the groups bred at the second post-MAP estrual period. The explanation for the cause of the extremely low first service conception rate in the moderate energy group was not readily available. This very low conception rate was contrary to most reports in the literature.

The extremely low first service conception rate of the moderate energy group artificially inseminated at the second post-treatment estrus was reflected in the services per conception for the high and moderate energy groups of 1.5 and 1.9, respectively. Nine of the 10 heifers in the moderate energy group still open after artificial insemination did, however, conceive to the first natural service after the second post-treatment estrual period insemination. The high energy group required 22 matings to settle all 15 cows, the only group which had all cows produce a live calf.

The conception rate to artificial insemination in this trial was found to be very low, even when practiced at the second post-treatment estrus. Of 22 heifers artificially bred at the first induced estrus, eight conceived (36.4 percent). However, when 24 heifers were inseminated at second estrus only eight settled to first service (33.3 percent). The low percentage of conceptions at first service following MAP treatment has been observed by other workers (Sorenson, 1962; Nestel et al., 1963; Hansel and Malven, 1960). Conception rates at the first induced estrus following treatment have almost universally been reported as less than 50 percent. First service conceptions at second estrus have generally been higher.

The first service conception rate of 36.4 percent observed following artificial insemination at the first induced estrual period after MAP treatment in this trial was comparable to those reported by others. Hansel and Malven (1960) reported only eight of 22 cows conceived when artificially mated three days after the treatment period. Only 24 percent of 38 heifers conceived to artificial insemination in a trial reported by Nestel et al. (1963). Nelms and Combs (1960) observed that group feeding two-year-old heifers 250 mg. MAP per head per day resulted in 40 percent pregnant at first service. Anderson et al. (1962) reported conception rates after the 20-day feeding period were 55 percent for the treated group and 60 percent for the controls. Collins et al. (1961) reported 17 of 30 dairy heifers (56.7 percent) inseminated were pregnant after synchronization with 0.5 mg. MAP per pound of body weight fed 20 days. Sorenson (1962) observed 33.3 percent of the artificially inseminated synchronized group conceived at first service. In another trial Sorenson (1962) found 54 heifers treated 18 days with 180 mg. MAP had a first service conception rate of 29.6 percent.

Various workers have reported a rebound effect on fertility at the second post-treatment estrual period, following progesterone injections as well as progestogen treatment. Zimbelman (1961) both individually fed and group fed two groups of eight heifers. The first service conception rate was 25 percent (2 of 8) and 75 percent (6 of 8) for the individually fed and group fed lots, respectively. Of these two treated groups, 13 of 16 heifers (81 percent) conceived with one or two services. In another trial conducted by Zimbelman (1963) conception rate after 28 days in three groups of beef heifers fed 180 mg. MAP was 87, 85, and 83 percent. These conception results through two services were comparable to what was commonly expected.

There are several possible reasons for the low first service conception rates observed in this trial. One plausible explanation could be that the semen, although microscopically good, lacked the fertilizing capacity necessary for normal conception rates. The inseminator might have played an important role in decreasing fertility at artificial insemination. Such was not believed to be true because this technician was chosen on the basis of his past record in settling heifers. A control group where synchronization was not attempted would have been desirable for comparison to first and second induced estrus conceptions. It was decided not to have a control group because the objective of this trial was to compare conception rate at the first induced estrus to conception rate at the second post-treatment estrual period. The control group would have also reduced the numbers within all groups, which were to some extent limited even without a control group. One other important consideration which influenced the decision to not include a control group was that the services of the inseminator were available for only one week and only a small portion of a control group would be expected to exhibit estrus in that period.

In this trial a cervical infection was detected in six heifers by the inseminator. This was in all likelihood contracted at an earlier heat period from the vasectomized bulls. At least one heifer of each group was reported to have a cervical infection, with only one group having more than one infected heifer. The high energy group which was inseminated at the first induced estrus had three infected heifers. One settled to the vasectomized bull, one conceived at the next service period to the pickup bull and one remained open. The three heifers in the other groups all conceived to the first natural service by the pickup bulls. None of the heifers with known cervical infections conceived to artificial insemination. While it could not be determined, it is possible that a low grade infection was present in several additional heifers and could have affected conception rate to artificial insemination.

The heifers were bred for crossbred calves in an effort to insure that as many cows as possible would produce and rear a live calf. Crossbred calves are known to exhibit a greater liveability than straightbred calves. Wiltbank et al. (1965a) reported a two percent increase in calves born alive where straightbred dams were carrying crossbred calves versus straightbred dams carrying straightbred offspring.

The use of semen from red Angus bulls for artificial insemination and black Angus for natural matings resulted in two different colors of crossbred calves from the Hereford heifers. This made it possible to pinpoint borderline matings as to whether the heifer conceived to artificial insemination or to natural service.

The effect of synchronization on distribution of calving dates is shown in Figure 5. The 55 heifers exhibiting an induced estrus did so within six days following treatment; whereas, the 40 heifers that exhibited a second estrual period did so within a ten-day interval. Using the calving dates of those heifers known to have settled to first service and comparing the variances in day of calving of heifers bred at the first induced estrus versus those bred at the second estrus, the variation in calving date was not significantly different between the two groups. The small numbers conceiving at first service may have had a major influence against obtaining a significant difference. The results do indicate that lack of 100 percent conception and inherent differences in length of gestation will spread calving dates over a much longer interval than that required for breeding.

The effect of varying the energy intake two months before the breeding season was found not to have a statistically significant influence on the occurrence of estrus and on conception rate

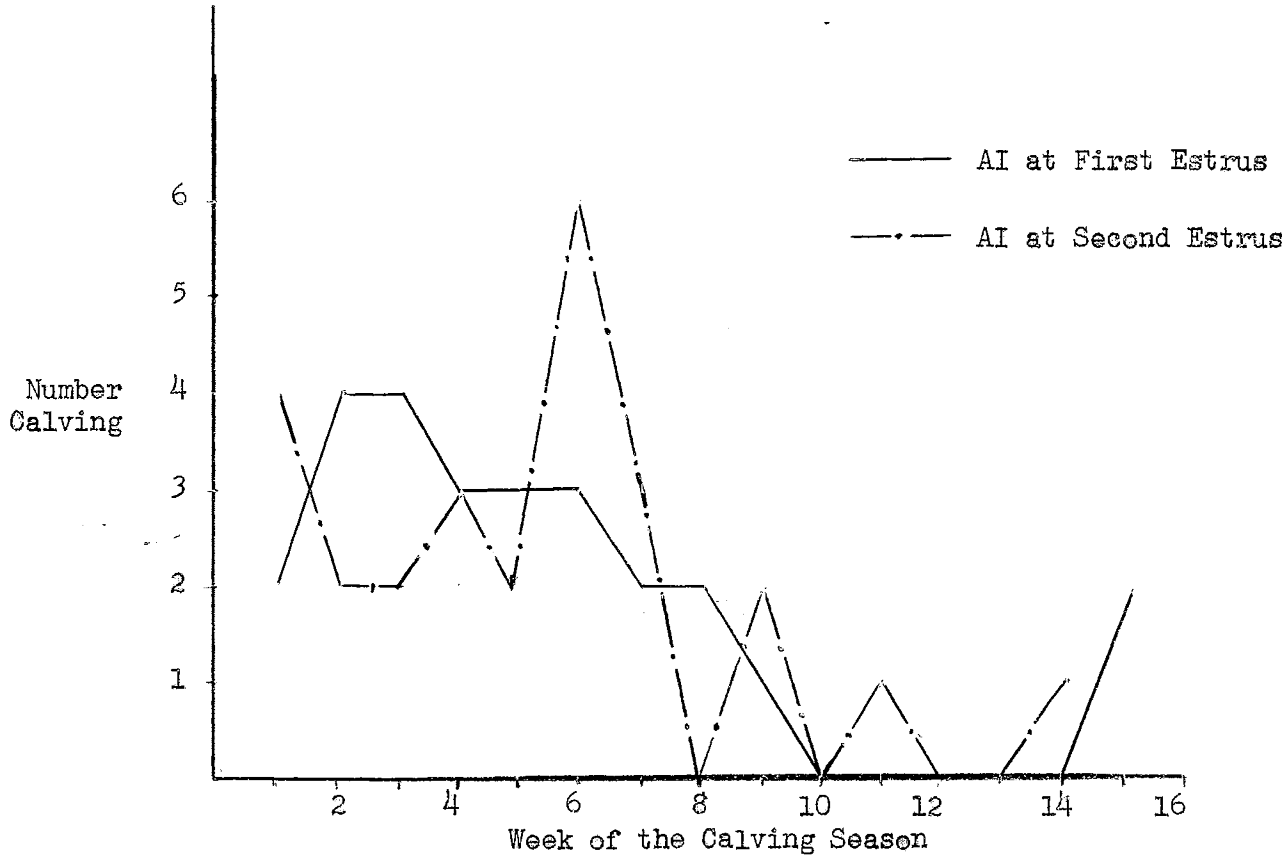

Figure 5. Calving Distribution of Hereford Heifers Fed 6-Methyl-17-Acetoxyprogesterone to Synchronize the Occurrence of Estrus and Artificially Inseminated at the First or Second Post-Treatment Estrus.

following MAP treatment. The conception rate for the various treatment groups are presented in Table XI. The numbers within a group were small and first service conception rates between lots were so similar that all differences were nonsignificant. Only the moderate energy heifers bred at second induced estrus had a difference approaching significance and this was because only one heifer of eleven inseminated conceived. However, nine of ten heifers naturally bred at the next subsequent estrus conceived. The results of this trial indicate that the two energy levels fed for two months before breeding did not have a statistically significant effect on the heifers' response to MAP and first service conception rate.

Nine heifers were recorded as not having been observed in estrus before the onset of treatment. Two heifers in this group were in the moderate energy lot inseminated at the induced estrus. They both were observed in heat two days post-treatment and one conceived to artificial insemination. The other heifer conceived to the first natural service. Three heifers in the high energy lot and four in the moderate energy lot also had not exhibited a prior estrus. These heifers in each group all exhibited an induced estrus. One heifer in the high energy group conceived to artificial insemination and the remaining two settled to their first natural mating. In the moderate energy group, one settled to the artificial mating and the other two conceived to the natural matings. The fourth heifer in this group was not observed in induced estrus but did exhibit a second post-treatment heat period. She did not conceive when bred artificially but did when mated later to a pickup bull. Silent ovulation apparently occurred at the induced estrus.

These results indicate if heifers have reached the approximate age and weight at which puberty should have occurred, their response will be similar to that of comparable heifers that are cycling.

TRIAL VI

Materials and Methods

The 84 heifers used in this study were part of the purebred Hereford and Angus herds (Project 1256) maintained at the Fort Reno Experiment Station. These heifers ranged from 12 to 15 months of age. The 23 Hereford heifers ranged in weight from 440 to 650 pounds while the range for the 61 Angus heifers was from 400 to 600 pounds. The heifers had been wintered on wheat pasture until February 1, 1965. They were then placed on native grass pasture supplemented by alfalfa hay. Beginning March 15 the heifers were group fed 1.5 pounds cottonseed meal and 3 pounds ground milo per head per day.

The 23 Hereford heifers and 61 Angus heifers were allotted within breed to one of the two treatment groups. Group 1 served as the controls and were group fed a ration of 1.5 pounds of cottonseed meal and 3 pounds of ground milo per head per day. Group 2 received the same daily ration plus 3.5 gms. of the progestational premix (Repromix) mixed with each pound of cottonseed meal. This amount provided 180 mg. MAP per head per day which was the dosage level recommended by Zimbelman (1963). Beginning March 26, 36 days before the mating season began, the MAP was fed once daily for 18 consecutive days. Ample trough space was provided so all heifers of a group could eat at one time.

It was necessary to consider several factors in assigning these heifers to treatment groups. The heifers were part of the breeding project and had previously been assigned to 21 different breeding groups; therefore, it was necessary to equalize the number treated and untreated within a particular breeding group. There were eight breeding groups of Hereford heifers and 13 Angus heifer breeding groups. Line of breeding was also considered and an effort was made to equalize between the two treatments the number of heifers from a particular line. In allotting heifers within each breed an attempt was made to balance the number which had shown estrus prior to treatment. Three Hereford heifers (13.6 percent) and 37 Angus heifers (60.7 percent) had been observed in estrus before the treatment period.

Vasectomized bulls, whose semen had been checked for presence of sperm, were maintained with the heifers prior to, during, and following the treatment period until the heifers were put into their predetermined breeding groups on May 1. Natural mating using previously assigned purebred Hereford and Angus bulls was begun 20 days post-treatment. The breeding season extended from May 1 to August 1 with each pasture checked twice daily to record matings. The vasectomized bulls were equipped with marking harnesses to aid in heat detections before the breeding season began. It was not feasible to use the harnesses during the breeding season; therefore, visual observations were the means of obtaining breeding records. The accuracy of these records might be questioned because of the possibility of night breeders and the short duration of estrus that heifers are known to have. Within 10 days after the onset of the breeding season 61 percent of the treated group and 54.8 percent of the controls had been observed in estrus. These figures indicate a fairly large number of the heifers were exhibiting estrus and being mated. Later palpation records indicated that a small number of heifers had mated but had not been observed in estrus.

A heifer in this trial was considered as having conceived if an estrual period was not recorded within 60 days following mating. Sixty-day nonreturn rate was believed to be the most reliable way to determine first service conception rate because palpations in the early stages of pregnancy are subject to considerable error. Also it was feared that palpation at this early date might increase the occurrence of early embryonic deaths. Rectal palpations were, however, made shortly after the end of the breeding season but only on heifers without a recorded mating or where mating had not occurred 60 days prior to the end of the mating period. All heifers in this trial were palpated October 1 to confirm pregnancy and breeding records.

Bartlett's test for homogeniety of variance as illustrated by Steel and Torrie (1960) was used to test the effect of MAP treatment on the variation in the time of occurrence of mating during the breeding season. Differences in conception rate were tested for by using a chi-square test for two independent samples as illustrated by Siegel (1956).

Results and Discussion

This study was initiated to test the effect of MAP on synchronization of estrus and subsequent fertility to natural service at the second post-treatment heat period in heifers group fed 180 mg. MAP per head per day for 18 consecutive days. The results pertaining to synchronization of estrus in the 42 heifers group fed MAP are reported in Table XIII and illustrated in Figure 6. All observations on one heifer of the treated group were omitted when palpation after failure to exhibit estrus revealed infantile reproductive tract. Twenty-seven percent (11) exhibited estrus during the feeding period. The number in estrus during treatment imposed by group feeding was higher than the 15 percent (9 of 60) observed in heat during individual feeding trials using MAP as the treatment material (Trial V).

TABLE XIII

DISTRIBUTION OF THE OCCURRENCE OF FIRST AND SECOND POST-TREATMENT ESTRUS IN HEREFORD AND ANGUS HEIFERS FOLLOWING GROUP FEEDING OF 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE PER HEAD DAILY FOR 18 DAYS

Day After Treat.	No. Exhibit-ing Estrus	Percent in Estrus	Day of 2nd Post-Treatment Estrus	No. Exhibit-ing Estrus	Percent in Estrus
1	0	0	20	3	7.3
2	4	9.8	21	1	2.4
3	12	29.3	22	7	17.1
4	9	22.0	23	10	24.4
5	7	17.1	24	2	4.9
6	1	2.4	25	1	2.4
			26	2	4.9
			27	3	7.3
Total	33	80.5		29	70.7

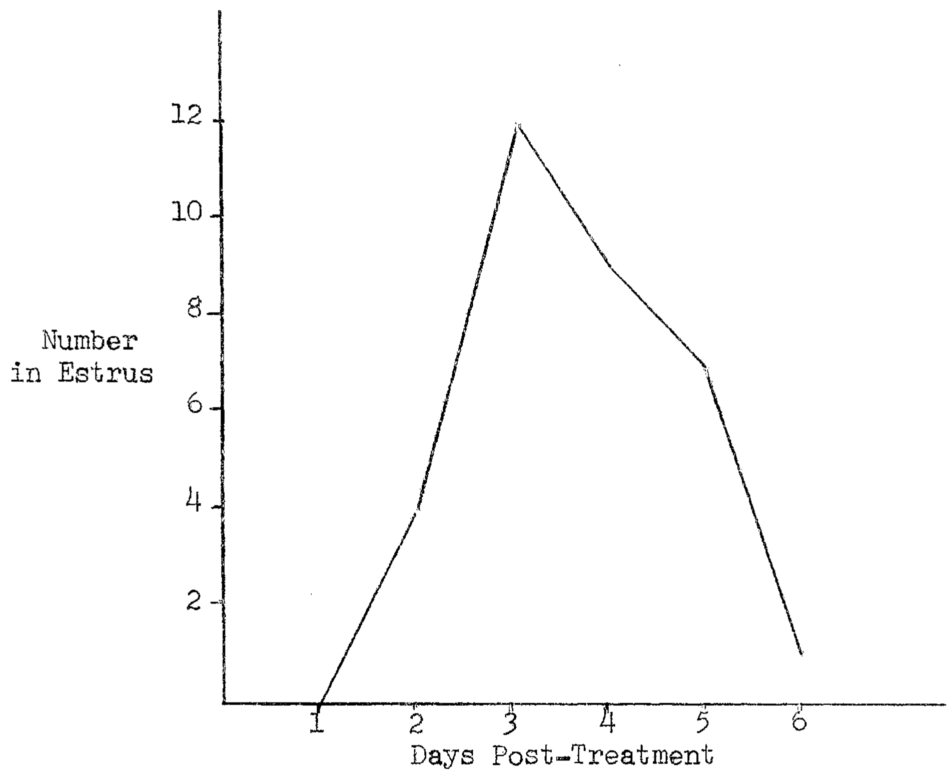

Figure 6. Occurrence of Estrus Following 6-Methyl-17-Actoxyprogesterone Treatment.

The time of occurrence of estrus during treatment varied from the onset of treatment to within four days prior to the cessation of treatment. Rectal palpations were not performed; therefore, it was unknown whether this estrus was accompanied by ovulation. Zimbelman (1963) stated the amount of 6-methyl-17-acetoxyprogesterone required to inhibit ovulation was greater than the amount needed to suppress the exhibition of estrus. The relatively high incidence of estrus during treatment indicates one limitation of a group-feeding program. It suggests that some heifers obtain less than their daily individual feed allotment and others more than the desired dose which could alter the response following cessation of treatment. Efforts should be made to see that each heifer consumes her feed allotment so that estrual behavior will be inhibited. Apparently the estrus during treatment was not an important factor since nine of the 11 heifers (81.8 percent) exhibiting estrus during the MAP treatment period in this trial returned to heat within five days post-treatment. Ten of the 11 heifers mated during the first week of the breeding season with six conceiving at this first service.

Zimbelman (1961) reported four of eight heifers were in estrus during the treatment period when fed 150 mg. MAP once or twice daily. He concluded from other trials, however, that 180 mg. MAP was completely effective in preventing estrus during the treatment period. This observation by Zimbelman in addition to the results of Trial V where heifers were individually fed 180 mg. MAP per head per day indicates group-fed heifers do not always receive the daily minimum dose required for retardation of estrus. Collins <u>et al</u>. (1961) reported group feeding 0.5 mg. MAP per pound of body weight inhibited estrus in 35 of 36 dairy heifers during the treatment period. This dosage is slightly higher than that given in this trial, which when expressed as milligrams per pound was approximately 0.3 mg.

Of the 41 normal heifers in the treated group, 33 (80.5 percent) were observed in estrus within a two- to six-day period following cessation of treatment (Table XIII). The mean interval from last feeding to occurrence of estrus was 3.7 days. The remaining eight heifers of the treated group were not observed in estrus in the 20-day period between the time of treatment and the onset of the breeding season. Six of these heifers did, however, exhibit estrus within the first four days of the breeding season indicating silent ovulation may have occurred at the induced heat period. It could also have been due to an error of detection as well as an actual failure to exhibit heat. The remaining two heifers in this group mated later in the breeding season. The occurrence of estrus following treatment is illustrated in Figure 6.

The 80.5 percent of the heifers in this trial exhibiting an induced estrus compares favorably with the 85 percent of 38 heifers exhibiting estrual behavior as reported by Nestel <u>et al</u>. (1963). Zimbelman (1963) reported that 93 percent of the heifers exhibiting estrus did so within four days after treatment with a mode of 2.0 days for beef heifers. Similar results were found in another study where 0.5 mg. MAP per pound of body weight was fed

(Collins et al., 1961). Nellor et al. (1960) reported a longer interval of from four to five days after 15 to 18 daily treatments with 0.4 mg. MAP per pound of body weight in beef heifers. This longer interval agrees with the results of this trial, whereby 68.4 percent of the 80.5 percent exhibiting estrus did so on the third, fourth, and fifth day after treatment. In Trial V where 60 Hereford heifers were individually fed the same dosage as in this trial, 84 percent of the heifers had exhibited estrus by the third day after the end of treatment with 60 percent of the heifers showing heat the second day (Table IX).

When these heifers were allowed to recycle and then placed in breeding pastures for mating at the second post-treatment estrus 75.9 percent of the 38 treated heifers which mated did so in an eight-day period beginning on the first day of the breeding season (Figure 7). The fact that 76.3 percent of the 38 heifers mating exhibited estrus in the first eight days of the breeding season indicated that, although some variation in cycle length existed, the animals still tended to be synchronized. Others have reported that the effect of synchronization was gradually lost as the time post-treatment increases. Thirty-six of the 38 heifers in the treated group had recorded matings in the first 21 days of the breeding season as compared to 34 of 37 controls exhibiting estrus during the first 21 days of the mating period. This distribution of matings is illustrated in Figure 7. The histogram indicates both the treated and the control groups tended to exhibit a grouping of matings soon after the start of the breeding season. The results

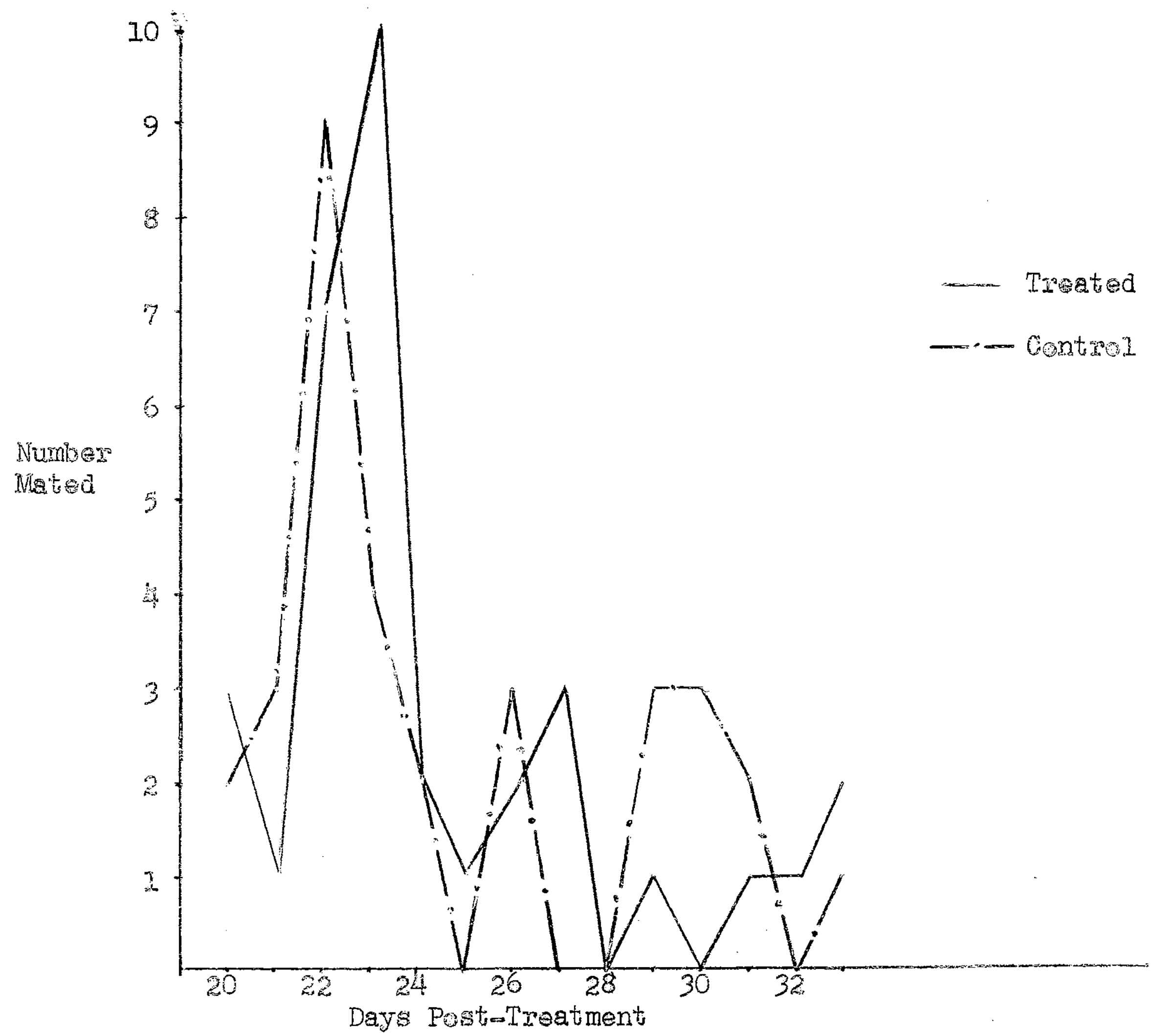

Figure 7. Distribution of Matings in 42 Untreated and 41 MAP Treated Heifers Beginning 20 Days Post-Treatment.

indicate the management practice of increasing energy intake by the addition of grain to the ration approximately 45 days (March 15) prior to the breeding season had some stimulatory effect on reproductive activity. The variance in occurrence of second post-treatment estrus during the breeding season was significantly ($P < .005$) less for the MAP treated heifers than for the control heifers.

Two heifers in the treated group were not detected in heat during the breeding season, but rectal palpations after removal of the bulls revealed both were approximately 65-70 days pregnant. The other heifer in the treated group had a recorded mating only 15 days before the end of the breeding season; therefore, rectal palpations shortly after the end of the mating season were inconclusive. Palpation 60 days post-breeding revealed she had conceived. Five heifers in the control group were not observed in estrus. Three were later found to be pregnant with two diagnosed as open heifers.

Of the 33 heifers that exhibited an induced estrual period soon after treatment there were only two in which estrus was not detected during the breeding season. These heifers, however, were found to be 150 days pregnant when palpated 60 days after the breeding season. The size of the fetus and the cotyledons indicated the heifers were bred soon after May 1, when the second post-treatment estrual cycle was scheduled to occur. This emphasizes again one very large source of error present when mating records are being taken. It was not possible with twice daily observations to detect all heat periods and record all matings. In this trial alone five heifers without recorded matings were later diagnosed as pregnant.

Because 60-day nonreturn rate was the criterion of conception, it is important to consider the interval between first and second mating in these two groups. Any mating followed by another mating within 60 days was ruled not a first service conception. Any time two matings occurred more than 60 days apart it was assumed that conception had occurred but the embryo has subsequently died for some unknown reason. Thus, to arrive at first service conception rate, intervals under 60 days were categoriezed as no conception; whereas, those intervals whose estrual periods were more than 60 days apart were assumed to be first service conceptions. These intervals are presented in Table XIV.

TABLE XIV

NUMBERS OF NONTREATED HEIFERS AND HEIFERS TREATED WITH 6-METHYL-17-ACETOXYPROGESTERONE WITH A SECOND RECORDED MATING AT VARIOUS INTERVALS AFTER THE FIRST MATING

Group	7-16 days	17-23 days	24-30 days	31-40 days	41-50 days	51-60 days	61-70 days	71-80 days
Treated	0	4	2	3	0	1	1	1
Nontreated	6	4	1	3	1	3	0	1

Very short estrous cycles were observed in six of the control heifers between the first and second mating. These could well be the result of an error of detection with the bull mounting but mating not completed. If this were true, however, it would be expected in both groups. The occurrence of these short estrous cycles was not random across treatment, rather the short cycles occurred only in the control group.

Two heifers in the group fed the progestational compound returned to estrus at intervals exceeding 60 days after the first mating as did one heifer in the control lot. These heifers were considered to have conceived at first service with embryonic mortality believed to be the cause of the return to estrus. Rectal palpations after the breeding season revealed, however, that one heifer of each group was pregnant at the time she exhibited this estrus. Nalbandov (1958) reported approximately five percent of all cows exhibit estrual activity during pregnancy. The remaining heifer in the treated group which had a 68-day interval between first and second estrus was found to have conceived at the second estrus; therefore, embryonic mortality following conception at the first mating could have occurred. The known occurrence of embryonic mortality was very low in this trial, indicating no deleterious effect of treatment.

The influence of group feeding 180 mg. MAP per day for 18 days on fertility at second post-treatment estrus is presented in Table XV. Since both Hereford and Angus heifers were used, first service conception rates were broken down by breed. In both the treated and control groups conception rate within each breed was approximately equal. However, the difference in first service conception of 73.7 and 48.6 percent of the treated and control groups, respectively, was significant ($P < .005$). The 48.6 percent first service conception rate observed for the controls was unusually low. No explanation can be given as to why these heifers did not settle as readily to first service as might be expected.

TABLE XV

INFLUENCE OF GROUP FEEDING 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE ON FERTILITY OF OBSERVED MATINGS AT SECOND POST-TREATMENT ESTRUS AS INDICATED BY 60-DAY NONRETURNS

Group	No. Treated	No. Mated	60-Day Nonreturns	Percent Nonreturns
MAP	41	38	28	73.7*
Hereford	11	11	8	72.7
Angus	30	27	20	74.0
Control	42	37	18	48.6*
Hereford	11	11	5	45.4
Angus	31	26	13	50.0

*($P < .005$)

It was apparent from the data obtained in this study that completely accurate breeding records are not obtainable by twice daily observations of heifers spread throughout several breeding pastures. Rectal palpations 5 days after the termination of the breeding season revealed that two of three treated heifers and three of five nontreated heifers were pregnant. At the time rectal palpations were carried out approximately 60 days later it was estimated that the two treated heifers and two of the three control heifers had conceived approximately 140-150 days earlier, apparently at an unobserved estrus period. The estimate made at the time of palpation of the probably stage of gestation of the remaining control heifer indicated that conception had occurred 20 days, or approximately one cycle later than had the other two heifers.

It was believed that stage of gestation could be estimated with some degree of accuracy by rectal palpation. For this reason palpation records were combined with breeding records in preparation of Table XVI.

TABLE XVI

CONCEPTION RECORDS OF NONTREATED AND TREATED HEIFERS RECEIVING 6-METHYL-17-ACETOXYPROGESTERONE AS EVIDENCED BY RECTAL PALPATION

Group	No. Treated	First Service Conception	Per-cent	Later Service Conception	Total Concep-tions(%)	No. Open	Per-cent Open
MAP	41	26	63.4	11	90.2	4	9.8
Hereford	11	6	54.5	3	81.8	2	18.2
Angus	30	20	66.7	8	93.3	2	6.7
Control	42	22	52.4	14	85.7	6	14.3
Hereford	11	5	45.5	6	100.0	0	0.0
Angus	31	17	54.8	8	80.6	6	19.4

The conception results of Table XVI vary slightly from those of Table XV where first service conception was based on 60-day nonreturn rate. The palpation data revealed two Hereford heifers in the treated group that had been considered first service conceptions were not pregnant. First service conception rates using palpation data was 10.3 percent lower than when 60-day nonreturns were used (63.4 versus 73.7). Theoretically these heifers could have conceived at this first estrus, but later lost their calves and had not had an opportunity to return to estrual activity before the breeding season ended. Also in the treated group one Angus heifer without a previous breeding record was found to be pregnant. Of 41 treated heifers in the MAP group, 37 (90.2 percent) conceived during the 90-day breeding season compared to 36 of 42 control heifers (85.7 percent) conceiving. Although the difference was not statistically significant, treatment did tend to improve conception rate. Also more treated heifers were bred in a shorter period of time which would group calvings and reduce labor requirements at calving time.

Palpations also revealed that 60-day nonreturn rate was not completely accurate in estimating the percent first service conception rate in the control group. Two heifers in this group apparently exhibited false heats or may have been marked while not in estrus. The size of the fetus indicated the length of pregnancy corresponded more closely with the first estrual period rather than the second period. Use of 60-day nonreturn rate resulted in an estimate of 48.6 percent (18 of 37) for the first service conception rate. Palpation data indicated this figure should be 52.4 percent (22 of 42). The difference between these two estimates can be explained by the fact the 60-day nonreturn rate was based on observed matings; whereas, the estimates given in Table XV was a comparison between the stage of gestation as to when conception occurred and reported mating using all heifers. Heifers diagnosed as being pregnant 150 days were assumed to have conceived at their first estrus even if such was not observed.

The palpation remarks indicated 60-day nonreturn was fairly accurate, but that certain discrepancies can be found when this technique is employed. Examples of these discrepancies were noted in the treated group where one heifer was diagnosed by palpation as being open after exhibiting only one heat period. There is some likelihood here of embryonic death which occurred too late for return to estrual activity before termination of the mating season. One heifer in the treated group was believed to be carrying a shorter term fetus than the breeding records indicated. Use of breeding records alone led to the conclusion that 28 of 38 (73.7 percent) treated heifers that mated conceived at their first estrus; whereas, palpation records indicated 26 of 41 heifers (63.4 percent) conceived to first service. The 10 percent difference indicated breeding records were fairly accurate. A smaller difference was found in the control group. Breeding records indicated 18 of 37 (48.6 percent) untreated heifers that mated conceived; whereas, palpation records led to the conclusion that 22 of 42 heifers (52.4 percent) had settled at their first mating. It should also be pointed out that the use of rectal palpations should not be assumed to be 100 percent accurate. Some confusion as to exact date of conception would be expected to occur since the difference in fetal size and development in two females whose conception occurred 20 days apart might vary only a slight degree.

Sorenson (1962) reported 54 heifers treated 18 days with 180 mg. MAP had a 29.6 percent conception rate at first service. Anderson et al. (1962) observed a 55 percent conception rate in heifers fed MAP for 20 days as compered to a 60 percent conception rate in the control group. Zimbelman (1961), however, reported a first service conception rate of 75 percent in dairy heifers group fed 0.5 mg. MAP per pound of body weight daily for 20 days. In three trials where beef heifers were bred a second estrus, Zimbelman (1963) reported that 87, 85, and 83 percent of the heifers bred conceived to first service. He reported an overall conception rate of 76 percent where both beef and dairy heifers and beef cows were mated at the second post-treatment estrus. In the preceding Trial V where first service conception at the first induced estrus was studied, the results were not indicative of what was usually observed. The conception rate at first and second estrus was 36.4 and 33.3 percent, respectively. The reason for the unusually low conception rate at the second synchronized estrus was unknown. First service conception at second post-treatment estrus in the individually-fed heifers was considerably below that of the group-fed heifers (33.3 percent vs 73.7 percent). It is likely that a major portion of this difference might be associated with the use of artificial insemination (Sorenson, 1962). However, Zimbelman (1963) also used artificial insemination and reported high conception rates at the second post-treatment estrus (87, 85, and 83 percent).

Important consideration must be given the good fertility obtained from matings made at second post-treatment estrus in this study. While no comparison was made with fertility at the induced estrus in this study other workers have observed low conception rates when heifers were bred at the induced estrus (Nestel et al., 1963; Sorenson, 1962). Nestel et al. (1963) reported 24 percent conceived to artificial insemination and 32 percent conceived to natural service at the induced cycle. Sorenson observed 33.3 percent conceived to artificial insemination while 53.1 percent conceived to natural mating when both groups were bred at the first induced cycle. Collins et al. (1961) artificially inseminated 30 heifers after MAP feeding and found that 17 (56.7 percent) conceived. Artificial insemination of 60 two-year-old heifers on the third, fourth, and fifth days after treatment--regardless of expression of estrus--resulted in 40 percent pregnant (Nelms and Combs, 1960). Nellor and Cole (955) found conception rate was less than 20 percent with artificial insemination after progesterone implants followed by gonadotropin injections. Barnes and Meyer (1964) believed MAP increased the rate of embryonic deaths in rats by delaying implantation. If this same hypothesis were true in other species low conception rate was not due to ovum infertility, but to an improper uterine environment and increased embryonic deaths. Synchronization at second post-treatment estrus was observed to be maintained to a large extent, but the factors which might cause a failure to conceive at first estrus apparently do not play as large a role; therefore, conception occurs more readily.

The management practices employed in this trial differed markedly from the usual practices. The availability of many bulls with the heifers assigned to 21 different breeding groups made artificial insemination unnecessary. The use of many breeding pastures, however, increased the labor requirements with regard to amount of time spent checking the different pastures. If a producer were to use a progestational compound and maintain the treated females together, artificial insemination would be a necessity unless an unusually large bull battery were available.

Table IX illustrated a consequence of feeding MAP which must be considered before an extensive program incorporating its use can be employed. If breeding were practiced at the first estrus in a sizeable herd, the number of bulls needed would be quite large. Breeding at second estrus tended to spread the occurrence of estrual behavior over more days, but there were still over 25 percent of the females in heat during one day. If the number of females treated is of any magnitude, it would seem in most herds that the only answer would be the use of artificial insemination. However, with this type of program and assuming a 90 percent detection rate and 70 percent first service conception rate, the producer would need to furnish enough pickup bulls to breed approximately 35 percent of his herd still not settled.

One possible means of relieving the pressure of having large numbers of females in estrus in a short period of time would be to begin feeding the progestational material to a portion of the herd adding additional groups at 10-day increments. At the end of 18 days feeding each group of cows would be removed from treatment and be placed with the bulls. Since there are apparently no adverse side effects from feeding Repromix for long periods of time, it would be feasible, physiologically, to begin feeding all cows at one time. At the end of 18 days, 15 or 20 cows could be removed from treatment and natural mating begun. At seven-day intervals other groups of 20 could be removed and bred, thus preventing overuse of the bulls or necessitating the use of artificial insemination.

Breed regulations on purebred breeders using artificial insemination, the additional labor requirement, and the cost of the progestational material are factors that will influence the use by breeders of programs of synchronization. The advantage in conception rate obtained by MAP treatment in this trial was not of the magnitude to justify its use with natural mating.

TRIAL VII

Materials and Methods

This trial was initiated in the spring of 1965 using 90 commercial Hereford cows (Project 1219) maintained at the Fort Reno Livestock Experiment Station. Approximately one-third of these cows (34) were three-year-olds suckling their second calf, and the other two-thirds (56) were two-year-olds raising their first calf. The trial was initiated to study the effect of estradiol on three facets of post-partum reproductive activity; uterine involution, occurrence of first post-partum estrus, and response to an oral progestin, 6-methyl-17-acetoxyprogesterone (MAP).

Data on uterine involution were obtained on 26 pluriparous and 33 primiparous cows. As the cows calved they were paired and one of each pair assigned within age groups to the treated and the other to the untreated group. The treatment was a single 5 mg. dose of estradiol-17β (ECP) injected intramuscularly in the rump region 8 to 15 days after calving. Rectal palpations were begun two weeks after calving and were repeated weekly until involution was complete. Complete uterine involution in these cows was ascertained to have occurred when the following criteria reported by Buch et al. (1955) were observed:

(a) Return of the uterus to its normal location in the pelvic or near-pelvic region.

(b) Normal and approximately equal size of the uterine horns was noted.

(c) Attainment of normal uterine tone and consistency was realized.

Time of occurrence of first post-partum estrus was determined by means of vasectomized bulls maintained in the herd at all times following calving. A cow was recorded as exhibiting first estrus after parturition when she would permit the bull or other cows to mount.

Before the synchronization portion of the experiment was begun two estimates of milk production were obtained one week apart in late March and early April, at which time the calves ranged from four to 60 days of age. The procedure followed was to separate the calves from the cows overnight. The following morning the calves were weighed prior to and following the suckling period. They were again separated from their dams and the same weighing procedure employed eight hours later. The difference in the weight of the calf before and after nursing was used as an estimate of the cow's total daily milk production. The purpose was to provide an approximation as to whether the cow was producing a high or low daily yield of milk at the time of initiation of the progestogen treatment rather than to obtain a measure of actual milk production. These cows averaging nine total pounds or more at the two weigh periods each day were categorized as the high-producing cows; whereas, those producing less than nine pounds per day were recorded as low producers.

There are many errors involved in this procedure of obtaining milk production estimates. Such differences as incomplete removal of milk and urination or defecation by the calf between weighings could affect the estimate tremendously. The very young calves in this trial would not be expected to be as complete nor as rapid in nursing as the older calves. Also, unless carefully watched, the older calves tended to nurse cows other than their own dams, causing an overestimate of their dams' production. Another source of error impending on this procedure is that the dams tend to remain in the area where the calves are confined, thus decreasing water and food intake which reduces milk production. This would probably be reflected more in the high-producing than in the low-producing cows.

On April 8, 89 cows were allotted within age group and previous ECP treatment to one of three groups on the basis of calving date, level of milk production, whether or not involution had occurred, and whether they had exhibited a post-partum estrus. An attempt was made to balance across treatments the number of cows that had completely involuted, those that had exhibited an estrual period since parturition and those regarded as heavy milk producers.

The three treatments imposed starting April 8 were as follows: control, no treatment; MAP, 180 mg. 6-methyl-17-acetoxyprogesterone per head per day for 18 days; and MAP+ECP, 180 mg. MAP per head per day for 18 days plus a single intramuscular injection of 1 mg. estradiol-17β(ECP) on the second day of the MAP feeding period. The cows of the control and MAP groups received a placebo treatment of 1 ml. distilled water at the time the MAP+ECP group received the injection of 1 mg. ECP. All cows were individually fed the same ration with the daily allotment of MAP added on April 8 and withdrawn from the feed on April 25.

When these three treatments were combined with the previous treatment of one-half the herd with ECP there were six treatment groups as outlined in Table XVII. With regard to the effects of MAP there were two different control groups. One group had not received a prior treatment with ECP and was designated as the control-control group (C-C); whereas, the other control group had been pretreated with 5 mg. estradiol-17β (ECP) and was referred to as the ECP-control group (ECP-C).

TABLE XVII

ALLOTMENT OF POST-PARTUM HEREFORD COWS TO THREE TREATMENT GROUPS THREE WEEKS PRIOR TO BREEDING FOLLOWING A PREVIOUS ADMINISTRATION OF ESTRADIOL-17β AT A ZERO AND 5MG. LEVEL 8-15 DAYS POST-CALVING

Group	Treatment 8-15 Days Post-Calving	
	Control	ECP
Control	18[a]	12
6-methyl-17-acetoxyprogesterone	17	13
6-methyl-17-acetoxyprogesterone-Estradiol-17	16	13

[a]Number of animals per treatment group.

Two groups were individually fed 180 mg. 6-methyl-17-acetoxyprogesterone (MAP) per head per day for 18 days beginning April 8 and ending April 25. Again one group had not been treated with ECP previously (C-MAP); whereas, the other had been injected 8-15 days post-calving with 5 mg. ECP (ECP-MAP). The last two groups received a combination of treatments. Both received the same level of MAP as the preceding group, but also were injected with a single intramuscular 1 mg. ECP treatment on the second day of the MAP feeding period. These two groups then were designated as C-MAP+ECP and ECP-MAP+ECP depending on the previous treatment with 5 mg. ECP.

The breeding season began April 26 with hand mating to six mature Hereford bulls carried out at the first induced estrus in the progestogen-treated cows and at the naturally occurring estrual periods in the control groups (C-C and ECP-C). Beginning May 3 one Angus bull and four yearling Hereford bulls were used in a pasture mating program to complete the breeding program. All bulls had been semen tested and were believed to be fertile. The yearling bulls were assigned to four breeding pastures with approximately 15 cows per pasture. The pastures were observed twice daily and all known matings recorded. The bulls were not equipped with marking harnesses, thus only cows observed in heat had recorded matings. The bulls were removed from the mating pastures August 2. Rectal palpations were carried out September 29 to determine the number of cows which had conceived.

Differences in the time required for involution of the uterus and the occurrence of first estrus after parturition between treatments within age groups were tested using Student's "t" test as discussed by Steel and Torrie (1960). An age by treatment interaction regarding involution of the uterus was tested for using the Doolittle technique presented in Steel and Torrie (1960). Chi-square analysis as discussed by Siegel (1956) was used to test for significant differences in conception rate between the six treatment groups.

Results and Discussion

Interval from Parturition to Involution of Uterus

Palpation records from 26 pluriparous and 33 primiparous Hereford cows were studied to determine whether a single injection of estrogen shortly after parturition had a significant effect on shortening the interval from parturition to involution of the uterus. Treatment with 5 mg. estradiol-17 β (ECP) was found to result in a significant ($P < .01$) reduction in the mean interval from parturition to uterine involution in the pluriparous cows but not in the primiparous cows (Table XVIII).

TABLE XVIII

EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF 5 MG. ESTRADIOL-17 β (ECP) ADMINISTERED 8-15 DAYS POST-CALVING ON THE INTERVAL FROM PARTURITION TO INVOLUTION OF THE UTERUS IN TWO- AND THREE-YEAR-OLD POST-PARTUM HEREFORD COWS

Age Group	Treatment	No.	Mean Interval To Uterine Involution	Standard Deviation
3 years	ECP	11	39.6**	4.9
	Control	15	44.4	8.9
2 years	ECP	17	38.5	5.8
	Control	16	37.2	6.6

**$P < .01$

The time required for involution of the uterus was decreased five days (39.6 vs 44.4 days) in the treated cows giving birth to their second calf as compared to nontreated cows. However, injection of ECP into cows which had produced only one calf did not result in a significant difference. Instead, the mean interval was found to be approximately one day longer (38.5 vs 37.2 days) in the treated group as compared to the untreated group. Buch _et al_. (1955) reported primiparous cows involuted earlier than pluriparous cows. Norwood (1963) also reported post-partum interval to uterine involution was significantly affected by parity with pluriparous cows averaging five days longer to involution than primiparous cows (40.25 vs 35.25 days). Injection of estradiol-17β by Norwood (1963) did not cause a significant reduction in involution interval in primiparous cows. Foote _et al_. (1965) injected 10 mg. of estradiol and did not observe a significant difference among group averages for the interval from calving to uterine involution.

The reason for the significant mean difference in the pluriparous cows but not in the primiparous cows was difficult to explain. Calculation by the Doolittle technique of a treatment by age interaction revealed a significant ($P < .10$) interaction did occur. This interaction measures the failure of each age group to respond the same to the treatment with ECP. As age of cow increased, treatment decreased the time interval from parturition to involution of the uterus. One of the physiological functions of estrogen is to sensitize the myometrium of the uterus to the effects of oxytocin which in turn increases the magnitude and force of muscle contracture. It is possible that the increased contractions aid in the earlier development of uterine tone. The ECP treatment probably increased the contractility of the uterine muscle. The stimulus of suckling should provide sufficient oxytocin. The ECP treatment probably increased the contractility of the uterine muscles of the cows of both groups. However, the myometrial muscles of the primiparous cows, having been subjected only one time to the stretching necessary to provide room for the fetus, may return to normal tone quite rapidly even without estrogen treatment. The pluriparous cows were aided by ECP in attaining uterine tone, thus involution occurred sooner because of the increased contractility of the treated group over the controls. These results indicated estrogen therapy would be most beneficial in hastening uterine involution in cows that had produced more than one offspring.

Interval from Parturition to First Estrus

The results of mating records obtained by use of harnessed vasectomized bulls were analyzed to determine the effect of a single 5 mg. intramuscular injection 8-15 days post-calving on the interval from parturition to exhibition of first post-partum estrus. There were 14 three-year-old cows and 25 two-year-old cows injected with ECP. Of those treated, 12 and 22 of the three- and two-year-old groups, respectively, exhibited an induced estrual periods within one to seven days after administration of ECP. The average interval from treatment until the manifestation of estrus was 3.1 days. Breeding to fertile males was not permitted at this induced period, and the induced estrus ignored when calculating the post-partum interval.

The post-partum intervals to first estrus reported in Table XVIII were calculated by using only those cows which exhibited estrual activity before the MAP treatment period began on April 8. In this study 14 of 39 cows (36 percent) exhibited estrus before the synchronization trial was initiated; whereas, 18 of 40 untreated cows (45 percent) exhibited estrus during the same period. The analysis of the data revealed injection of ECP did tend to reduce the interval from calving to first estrus in the two-year-old cows (Table XIX).

TABLE XIX

EFFECT OF A SINGLE 5 MG. ESTRADIOL-17β (ECP) INTRAMUSCULAR INJECTION 8-15 DAYS POST-CALVING ON THE INTERVAL FROM PARTURITION TO THE EXHIBITION OF FIRST POST-PARTUM ESTRUS

Age Group	Treatment	No. Treated	No. Observed in Estrus	Mean Interval To First Estrus	Standard Deviation
			Prior to April 8		
3 years	ECP	14	5	29.8	12.3
	Control	16	11	44.5	22.5
2 years	ECP	25	9	36.6*	5.3
	Control	24	7	45.7	9.9

*$P < .05$.

The mean difference in time required after parturition to exhibition of first estrus was found to be significantly ($P < .05$) reduced by ECP treatment in the primiparous group (36.6 vs 45.7 days), but was not significantly influenced in the pluriparous group. The mean difference for those showing estrus was greater in the pluriparous group (29.8 days for the treated as compared to 44.5 days in the controls) but the variation within treatment groups was of much greater magnitude as indicated by the standard deviations of 12.3 and 22.5 days for the treated and control groups, respectively. The large standard deviation in the case of the control cows in the three-year-old group was probably the result of some of the control cows having a very short post-partum interval ranging from 16 to 30 days in length.

Apparently in this trial since similar numbers of the control and treated cows in the two-year-old group returned to estrual activity before MAP treatment was initiated, and since the cows within the group returned at fairly comparable intervals a significant difference was realized. Of the 25 ECP-treated two-year-olds, nine (36 percent) returned to estrus before the MAP feeding period was initiated. Their average interval from parturition to first estrus was 36.6 days with a standard deviation of 5.3 days. The interval for the untreated group was 45.7 days with a standard deviation of 9.9 days. In the 24 control two-year-old cows, seven (29.2 percent) were observed in estrus before MAP feeding began. The ECP possibly had some stimulatory effect on ovarian activity in the two-year-old cows, but apparently did not in the three-year-old cows. With regard to the three-year-old cows, only five of 14 treated cows (35.7 percent) exhibited estrus before MAP treatment; whereas, 11 of 16 control cows (68.8 percent) returned to estrus before MAP treatment began. Although the average interval was only 29.8 days for the treated group and 44.5 days for the control, it would not be possible to conclude treatment reduced the interval from calving to first estrus when 33 percent fewer cows ever exhibited estrus.

The average interval from parturition to first estrus in this phase of Trial VII are biased downward. All cows in this portion of the study did not have time to come into estrus naturally since it was necessary to initiate the progestogen treatment on April 8. Thus, any cow previously in heat was inhibited from exhibiting estrus until the 180 mg. MAP treatment was removed from the ration. Of the 39 treated cows, only 14 (35.9 percent) exhibited estrus as compared to 18 of 40 control cows (45 percent) showing heat prior to start of MAP treatment. The limited data available suggests that treatment with 5 mg. ECP tends to reduce the interval from calving to first estrus. The physiological mechanism for this reduction in post-partum interval to first estrus was possibly the effect of estrogen in limiting of the functional life of the corpus luteum. Niswender _et al_. (1964) reported high doses (640 mcg.) caused a decline in the functional activity and size of the corpus luteum. The estrogenic substance, ECP, apparently was instrumental in regressing the corpus luteum.

Foote (1962) observed that 10 mg. injections of estradiol-17β reduced the interval from parturition to first estrus by over 20 days (49 ± 14.2 versus 27 ± 2.5 days) in pluriparous Hereford cows. Foote _et al_. (1964a) noted similar results in 20 beef cows treated with 10 mg. estradiol-17β injected intravenously. The average interval from parturition to estrus was significantly ($P < .01$) shorter in the treated cows than in the untreated cows. Norwood (1963) reported that the post-partum interval is significantly influenced by parity and season. The same conclusion with regard to parity can be drawn from the results of this trial. No explanation is readily available to surmise why parity influenced the response of certain cows to estradiol-17β therapy.

In this trial no attempt was made to determine the average interval from parturition to the occurrence of first ovulation. When involution was designated as completed, further palpations ceased. Up to the time of involution, palpation of the ovaries was carried out whenever possible. In some instances the ovaries were well down in the body cavity and impossible to reach. Silent ovulation was believed to have occurred in 17 of the 79 cows (21.5 percent) on which first estrual data were collected. Zimbelman (1963) reported palpation of post-partum cows for the occurrence of ovulation and certain limitations. Difficulty in differentiating between small follicles and corpora lutea limited its usefulness.

Synchronization of Estrus in Post-Partum Cows

In this portion of the trial the post-partum cows which had previously been allotted on the basis of calving date, level of milk production, and occurrence of uterine involution and first post-partum estrus were treated with MAP and ECP to study the role of exogenous hormones in stimulating the onset of sexual activity and their effect on fertility.

The effect of MAP, either alone or in combination with ECP, on the occurrence of estrus following treatment is presented in Table XX. If the period of day one to day six post-treatment is used as a measure of the degree of synchronization, estrual activity of the lactating beef cows in this trial was not synchronized by MAP. Approximately identical responses were observed in the control and MAP treated groups. If post-calving treatment prior to MAP administration was not considered, the percent of cows in estrus during the period 1-6 days after the last MAP feeding in the control, MAP and MAP+ECP groups, respectively, was 44.8, 46.7, and 44.8 percent.

TABLE XX

THE INFLUENCE OF 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP) AND ESTRADIOL-17β(ECP), ALONE OR IN COMBINATION, ON THE OCCURRENCE OF ESTRUAL BEHAVIOR IN TWO- AND THREE-YEAR-OLD HEREFORD COWS

Treatment Group	No.	Occurrence Of Estrus					
		Day After Last MAP Feeding					
		1-6	7-14	15-21	22-28	29-35	36-100
Control-Control	18	10	3	1	1	1	0
ECP-Control	11	3	3	0	1	0	1
Subtotal	29	13	6	1	2	1	1
Control-MAP	17	10	0	1	1	1	4
ECP-MAP	13	4	0	1	0	0	2
Subtotal	30	14	0	2	1	1	6
Control-MAP+ECP	16	7	0	1	2	0	4
ECP-MAP+ECP	13	6	1	0	2	0	1
Subtotal	29	13	1	1	4	0	5
Control-Post-Calv.	51	27	3	3	4	2	8
ECP-Post-Calv.	37	13	4	1	3	0	4

The treatment prior to MAP appears to be the major factor influencing the observed estrual response in the period immediately subsequent to MAP. There were 55.6, 58.8, and 43.8 percent of the cows observed in estrus 1-6 days after MAP feeding was terminated in the control, MAP, and MAP+ECP groups, respectively, when the post-calving ECP treatment was ommitted. In comparison, there were 27.3, 30.8, and 46.2 percent of the cows in estrus in the control, MAP, and MAP+ECP, respectively, that had received a single 5 mg. ECP injection within 8-15 days after calving. Of the 51 cows that had not been treated with ECP in the 8-15 day post-calving period, 27 (52.9 percent) were observed in estrus within six days after MAP was removed from the ration. Only 13 of 37 cows (35.1 percent) which had been pretreated with 5 mg. ECP were observed exhibiting estrual activity. The only group in which pretreatment with ECP had any apparent beneficial effect on time of first estrus was in the MAP+ECP lot. When this treatment was administered subsequent to the ECP pretreatment, the variance in occurrence of the first induced was significantly ($P < .005$) reduced as compared to cows not receiving the ECP pretreatment prior to the MAP+ECP combination of treatments.

The data reported in Table XX reveal no apparent effect of the MAP or MAP+ECP treatments <u>per se</u> on numbers of cows subsequently observed in estrus. The percent of cows on each treatment that had been in estrus by 100 days after the last MAP feeding were: controls, 82.8 percent; MAP, 80 percent; and MAP+ECP, 82.8 percent. However, when the data was recorded for each lot it was evident that there were differences related to whether or not the cows had received ECP in the period 8-15 days post-calving. The percent of cows in each group that had been observed in estrus by day 100 post-MAP were: control-control, 88.9 percent; ECP-control, 72.7 percent; control-MAP, 100 percent; ECP-MAP, 53.8 percent; control-MAP+ECP, 87.5 percent; and ECP-MAP+ECP, 76.9 percent. Of the 37 cows receiving the post-calving 5 mg. ECP treatment, 67.6 percent had been observed in heat by day 100, compared to 92.2 percent of the 51 control cows. Tests for homogeneity of variance revealed that the time to first observed estrus in cows receiving the MAP or serving as controls following the pretreatment with 5 mg. ECP was significantly ($P < .005$) increased over cows not pretreated with ECP then later serving as controls or treated with MAP.

Of the 30 cows receiving 180 mg. MAP per head per day without an additional treatment at the time of synchronization, 14 were observed exhibiting an induced estrus, or sexual desire within six days following the termination of MAP feeding. Of these 14 cows, involution of the uterus was known to have had occurred in 10 of the cows. Involution of the uterus had also occurred in 11 of the 16 cows which had not been observed exhibiting an induced estrus. Therefore, involution of the uterus was not believed to be a major factor in the occurrence of estrus following cessation of treatment with the oral progestogen (MAP) to induce estrus. In reference to the cows which had involuted before the MAP treatment, 10 had received the 5 mg. ECP injection 8-15 days post-calving; whereas, the remaining 11 cows had served as controls before the synchronization trial was initiated. The remaining cows that had not involuted were about equally distributed between the ECP-treated and control group.

More cows in both the control and ECP-treated groups failed to exhibit estrual activity before synchronization was attempted than failed to involute. In the 14 cows that exhibited an induced estrus after termination of MAP alone, seven (50 percent) had been observed in estrus before the first MAP was given. Only two of those that had an observed period of sexual desire had received a prior treatment with ECP 8-15 days after calving. Of the 16 cows that did not exhibit an induced estrus, 10 (65 percent) had not shown estrual activity before the MAP treatment was begun. Six of these nonestrual cows had received the post-calving injection of 5 mg. ECP. These results with regard to synchronization in noncycling cows are comparable to results presented elsewhere (Zimbelman, 1963; Hansel et al., 1961). The oral progestogens now available are very satisfactory compounds for synchronizing the occurrence of estrual activity in cycling animals, but are not as effective in controlling or inducing the expression of estrus in noncycling lactating beef cows.

Twenty-nine cows received a combination of injections involving both 180 mg. MAP per head per day and a single 1 mg. intramuscular injection of ECP on the second day of the MAP feeding period. Thirteen of these cows had previously received a 5 mg. ECP injection 8-15 days post-calving. Of these 29 treated cows, 16 failed to exhibit an induced estrus within six days after the termination of the MAP feeding period. Whereas, 11 of 13 cows in the group that had exhibited an induced estrus had involuted uteri and were observed in estrus before the MAP treatment began, only four of 16 of the cows that did not have an induced estrus had both involuted uteri and an observed heat before treatment. The two remaining heifers in the group exhibiting an induced estrus had both undergone involution of the uterus, but had not been observed in estrus before initiation of MAP feeding. In addition to the four cows in the noninduced group which had exhibited both estrual activity and involution, six cows had involuted but were not observed in estrus and six other cows both failed to either involute or show estrual activity before MAP feeding was initiated. Occurrence of both involution and estrual activity in these two groups appeared to be a major influencing factor on occurrence of estrual activity after MAP. Other factors are apparently exerting some influence because of the failure of four cows which had involuted and had been observed in a pretreatment estrus to exhibit an induced estrus. Errors of heat detection might have played a role.

In the groups treated with MAP and ECP to synchronize the occurrence of estrus, six of the 13 cows exhibiting an induced estrus had received a prior 5 mg. ECP injection. Of the 16 cows not observed exhibiting an induced estrus after the combined MAP and ECP treatment, seven had been injected 8-15 days post-calving with 5 mg. ECP. The prior treatment with ECP apparently did not have an influence on the occurrence of an induced estrus in the groups receiving a combination of MAP and ECP to synchronize estrus.

The age of cow was noted to have an effect on the occurrence of an induced estrus after the termination of the MAP feeding in all four groups. Only 13 of 36 treated two-year-old cows (36.1 percent) exhibited an induced estrus while 14 of 23 treated three-year-old cows (60.9 percent) were observed in estrus within 1-6 days following the termination of MAP feedings. Apparently the three-year-old cows were able to overcome any inhibitory influence lactation might have on post-partum sexual behavior.

The results obtained in this trial are somewhat comparable to those obtained in Trial IV. The untreated control cows in Trial IV had a significantly ($P < .05$) shorter post-partum interval than did the noncycling cows treated with 180 mg. MAP for 18 days during lactation. The results in this trial indicated that the control cows returned to estrus sooner in the period after the cessation of treatment than did those treated with 180 mg. MAP. Foote et al. (1960b) had observed that a 1 mg. injection of progesterone per pound of body weight 14 days after calving increased the interval to first estrus (83.0 versus 65.6 days). Foote (1962), using 80 pluriparous Hereford cows, found that daily injections of progesterone, progesterone plus a single injection of estradiol-17β, or a single injection of estradiol-17β alone would significantly reduce post-partum interval. The use of daily injections of progesterone apparently produces a response in lactating, noncycling beef females that oral progestogens will not produce.

Conception Rate

Immediately following the MAP feeding period, the breeding program was initiated. All cows were observed closely for estrus and hand mated to mature Hereford bulls for the first week following cessation of MAP feeding. Thereafter, the cows were pasture mated with twice daily checks to obtain breeding records. Rectal palpations made September 29 again indicated mating records using observed matings were not completely accurate. Several females without recorded matings were later found to be pregnant. Another factor of importance was the presence of a sterile yearling bull in the pasture mating group. All bulls had been semen tested and none with apparent semen deficiencies used. It appears that microscopic evaluation of semen was not completely effective in determining fertility. Breeding records later indicated one of the yearling Hereford bulls failed to settle any of the cows he serviced. For this reason conception data was reported only for the two-year-old cows. The cows in this trial were considered to have conceived at first service if the estimated length of gestation made at the time of the pregnancy check confirmed the breeding date available. If breeding records were not available, the cows were arbitrarily recorded as having conceived to first service if the estimated length of gestation was 130 or more days.

The conception data in the two-year-old cows are presented in Table XXI. Pretreatment with ECP resulted in a significant ($P < .05$) increase in the first service conception rate compared to the group pretreated as controls (69.6 vs 46.7 percent). The ECP and control pretreated groups had 16 of 23 cows and 14 of 30 cows, respectively, settle to first service. Apparently ECP had some stimulatory effect on the development of a suitable uterine environment capable of maintaining a fertile ovum.

The influence of pretreatment on the two control groups was noted to produce substantial differences in first service conception rate. The control group which had been pretreated with ECP had the highest first service conception rate of any group (85.7 percent) with six of seven cows conceiving to first mating. The difference in conception rate between the two control groups was highly significant ($P < .01$). The cause for this large difference was the extremely low first service conception rate in the control-control group (36.4 percent). A first service conception rate of 85.7 percent would be considered very good and could probably be attributed more to chance in this trial than to treatment. On the other hand, a 36.4 percent conception rate is relatively poor and would not be expected in a group of cows treated as these were. This conception rate could be related to some other unknown factor, especially when it was noted that total conception rate in this control-control group was only 63.6 percent.

TABLE XXI

THE CONCEPTIONS RESULTS OF TWO-YEAR-OLD HEREFORD COWS FOLLOWING TREATMENT WITH 180 MG. 6-METHYL-17-ACETOXYPROGESTERONE (MAP), ALONE OR IN COMBINATION WITH A SINGLE ONE MG. INJECTION OF ESTRADIOL-17β (ECP) AS DETERMINED BY RECTAL PALPATION[a]

Group	No. Cows	Conceiving to First Service No.	Conceiving to First Service Percent[b]	Conceiving During Breeding Season No.	Conceiving During Breeding Season Percent
Control-Control	11	4	36.4	7	63.6
ECP-Control	7	6	85.7**	7	100.0
Subtotal	18	10	55.6	14	77.8
Control-MAP	10	8	80.0**	10	100.0
ECP-MAP	8	4	50.0	8	100.0
Subtotal	18	12	66.7	18	100.0
Control-MAP+ECP	9	2	22.2	6	67.8
ECP-MAP+ECP	8	6	75.0*	8	100.0
Subtotal	17	8	47.1	14	82.4
ECP Pretreatment	23	16	69.6[c]*	23	100.0
Control Pretreatment	30	14	46.7	23	76.7

[a]Palpations were performed 90 days post-mating.
[b]All treatments compared to the control-control group.
[c]Statistically different from control-pretreatment.
*($P < .05$) **($P < .01$).

The conception data in the groups treated with 180 mg. MAP alone to synchronize the occurrence of estrus indicated pretreatment 8-15 days after calving with 5 mg. ECP resulted in a lower first service conception rate than was noted in the group serving as a control (50 percent versus 80 percent). It should be noted that this was the only group in which ECP pretreatment reduced first service conception response. Both groups treated with MAP alone had an improved first service conception rate over that of the control-control group (80 and 50 percent vs 36.4 percent, respectively). The control-MAP and ECP groups had eight of 10 cows and four of eight cows, respectively, conceiving to first service compared to only four of 11 of the control-control cows. The first service conception rate in the groups treated with 180 mg. MAP alone to synchronize the occurrence of post-treatment estrus was higher (66.7 percent) than that in the control and MAP+ECP group (55.6 and 47.1 percent, respectively). These results are slightly different from what is normally reported in the literature (Sorenson _et al_., 1962; Nestel _et al_., 1963; Anderson _et al_., 1962). Conception rate after synchronization with MAP was usually less than the controls when mating was permitted at the induced estrus.

When a combination of treatments involving both MAP and ECP were given to synchronize estrus, different results pertaining to conception occurred. The group receiving the ECP-MAP+ECP treatment had a first service conception of 75 percent (six of eight) which was statistically ($P < .05$) different from the control-control group (36.4 percent). The control-MAP+ECP group had a very low conception rate of 22.2 percent (two of nine). Three cows in this group failed to conceive throughout the breeding season. The breeding records on these cows were not particularly good because of the failure to observe the occurrence of mating in several of the cows. The pretreatment with ECP 8-15 days after calving in this instance was beneficial. An explanation for the severe drop in first service conception rate in this control-MAP+ECP group was not readily available.

Total conception throughout the breeding season was also presented in Table XXI. All three groups which had received the post-calving injection of 5 mg. ECP conceived and were pregnant at the time palpations were done. The control groups, however, had only one group in which total conception was 100 percent and this was the control-MAP group. All the 23 two-year-old cows which had received the ECP post-calving treatment conceived, compared to 23 of 30 control pretreated cows conceiving (76.7 percent). The difference in overall conception between the two post-calving main groups was statistically significant ($P < .01$). The control groups had a total of seven open cows at the time of pregnancy diagnosis. Four of these were in the control-control subgroup and three in the control-MAP+ECP subgroup.

The pretreatment with estradiol-17 β apparently greatly enhanced later conception. No open or nonpregnant cows were noted in the groups that had been previously treated with ECP. Conception at first service was much higher in all groups with the exception of the control-MAP group. Matings in all groups were extremely scattered throughout the breeding season and no pattern of return to estrual activity could be established. Synchronization occurred in some cows but hormone treatments to synchronize were much less effective than when administered to cycling, nonlactating females. The only stimulatory effect was the response in improved first service conception rate following a 5 mg. ECP treatment 8-15 days post-calving.

These results are somewhat contradictory to those of Foote (1962). He studied the effect of daily injections of progesterone, alone or in combination with 10 mg. estradiol-17β, and reported the number of services per conception were higher in the estrogen treated group than was true in the control (2.1 and 1.4, respectively). The number of services where progesterone was given alone was comparable to the controls, but progesterone plus estrogens increased the number of services per conception to a value comparable to that of the estrogen treated group. The results reported here are not directly comparable, but are such that, in this study, it can be concluded that pretreatment with ECP had a stimulatory effect on first service conception. They did note also that conceptions occurred sooner in these treated groups. Zimbelman (1963) reported synchronization of ovulation in 19 of 22 post-partum cows started on MAP 16-22 days after parturition. Sixteen of these 22 cows became pregnant at first ovulation. Use of 150 mg. MAP in this case produced much better synchronization results than did 180 mg. MAP did in the studies under report. Fosgate *et al*. (1962) used 17- hydroxyprogesterone-N-caproate and noted cows bred at the first estrus after treatment had a 70 percent conception rate. Estrus did not occur though until 70 days post-calving following 22 intramuscular injections of this compound.

Effect of Level of Milk Production

A study was made to determine the effect of high or low milk production on length of interval from parturition to involution of the uterus, occurrence of first estrus, and the response to MAP. The procedure, as discussed earlier, was to weigh the calf before and after suckling following an interval when the calf was penned away from the dam. The purpose again was not to obtain precise measures of milk production but to determine if the cow was a high or low producer. These cows producing nine lbs. per day or more were categorized as high producers. The results of this study are presented in Table XXII and Figures 8 and 9. The daily production in the two-year-old cows ranged from 4.0 to 12.0 lbs. per day; whereas, the production in the three-year-old cows ranged from 5.0 to 16.0 lbs. per day. The majority of the cows produced from eight to 11 lbs. per day.

The differences presented in Table XXII indicate that whether a cow was in the top half or bottom half in level of milk production had very little influence on the various aspects of post-partum reproduction. The difference in mean interval from calving to uterine involution was approximately 2.5 days in both age groups. However, this difference was in favor of the high producers in the two-year-old cows and the low producers in the three-year-old cows. The time from calving to involution in the two-year-olds was 39.3 and 36.8 days, and in the three-year-old cows it was 41.6 and 44.0 days for the low and high producers in both age groups, respectively. Similar circumstances were noted to have occurred with regard to the time from parturition to first estrus. The two-year-old cows required 48.5 days

TABLE XXII

THE INFLUENCE OF LEVEL OF MILK PRODUCTION ON INTERVAL FROM PARTURITION TO INVOLUTION OF THE UTERUS, OCCURRENCE OF FIRST ESTRUS RESPONSE TO MAP, AND FIRST SERVICE CONCEPTION RATE

Item	Age of Cows	No.	Level of Milk Production	
			Low Half	High Half
Post-Partum Interval to Uterine Involution (days)	2 years	30	39.3 ± 1.5	36.8 ± 1.6
	3 years	22	41.6 ± 1.4	44.0 ± 3.5
Post-Partum Interval to First Estrus (days)	2 years	26	48.6 ± 6.2	49.8 ± 6.9
	3 years	21	52.9 ± 6.6	51.7 ± 7.4
Cows Observed in Estrus 1-6 Days After Last MAP Feeding (%)				
Control	2 years	18	22.2	16.7
	3 years	11	27.2	27.2
MAP	2 years	18	22.2	16.7
	3 years	12	25.0	25.0
MAP+ECP	2 years	18	5.6	22.2
	3 years	11	27.2	36.3
Percent Conception (%)				
First Service	2 years	30	56.7	56.5
Total Conception	2 years	30	90.0	82.6

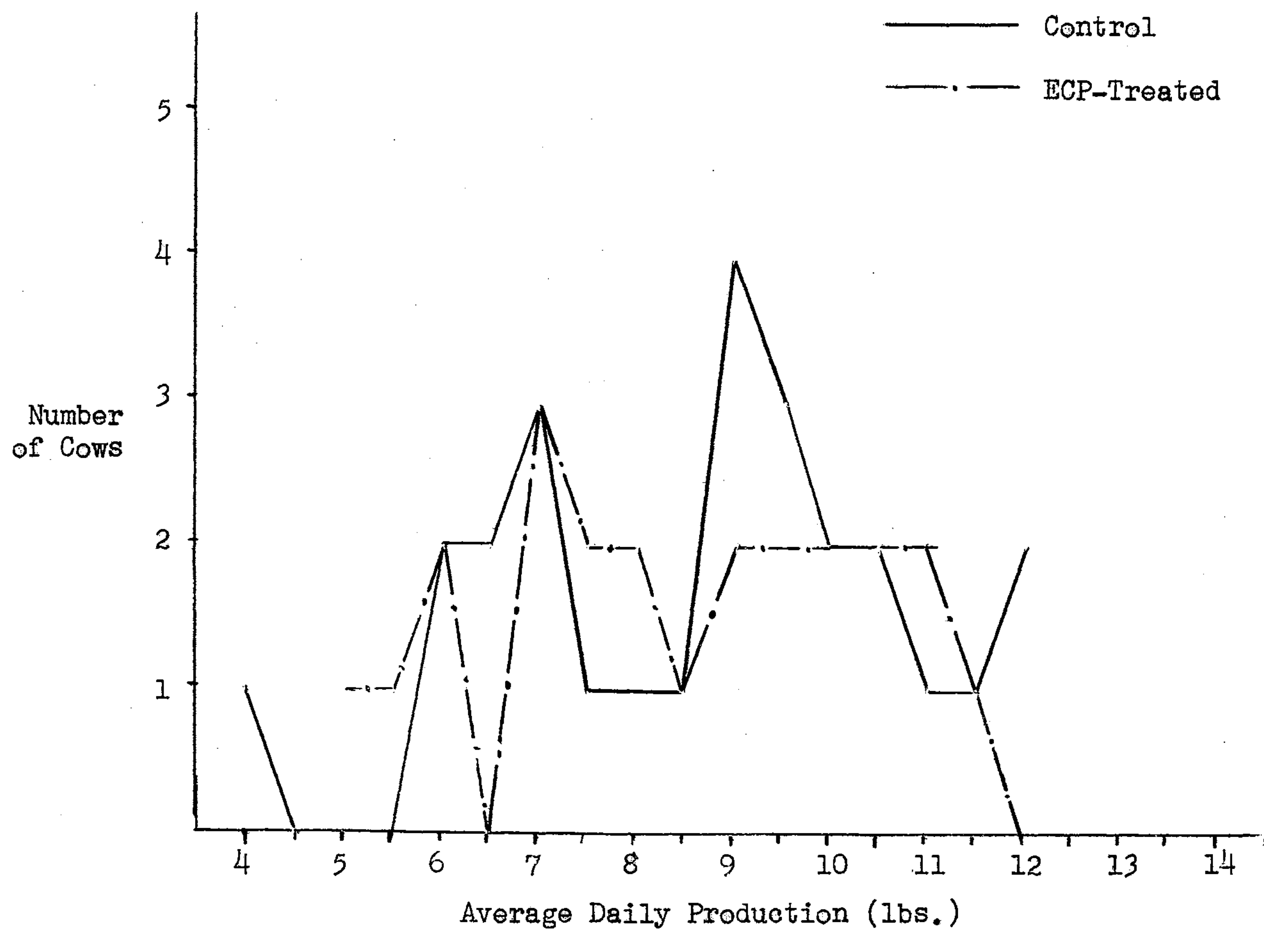

Figure 8. The Distribution of Average Daily Milk Production in Two-Year-Old Hereford Heifers Treated with 5 mg. Estradiol-17β(ECP) 8-15 Days Post-Calving.

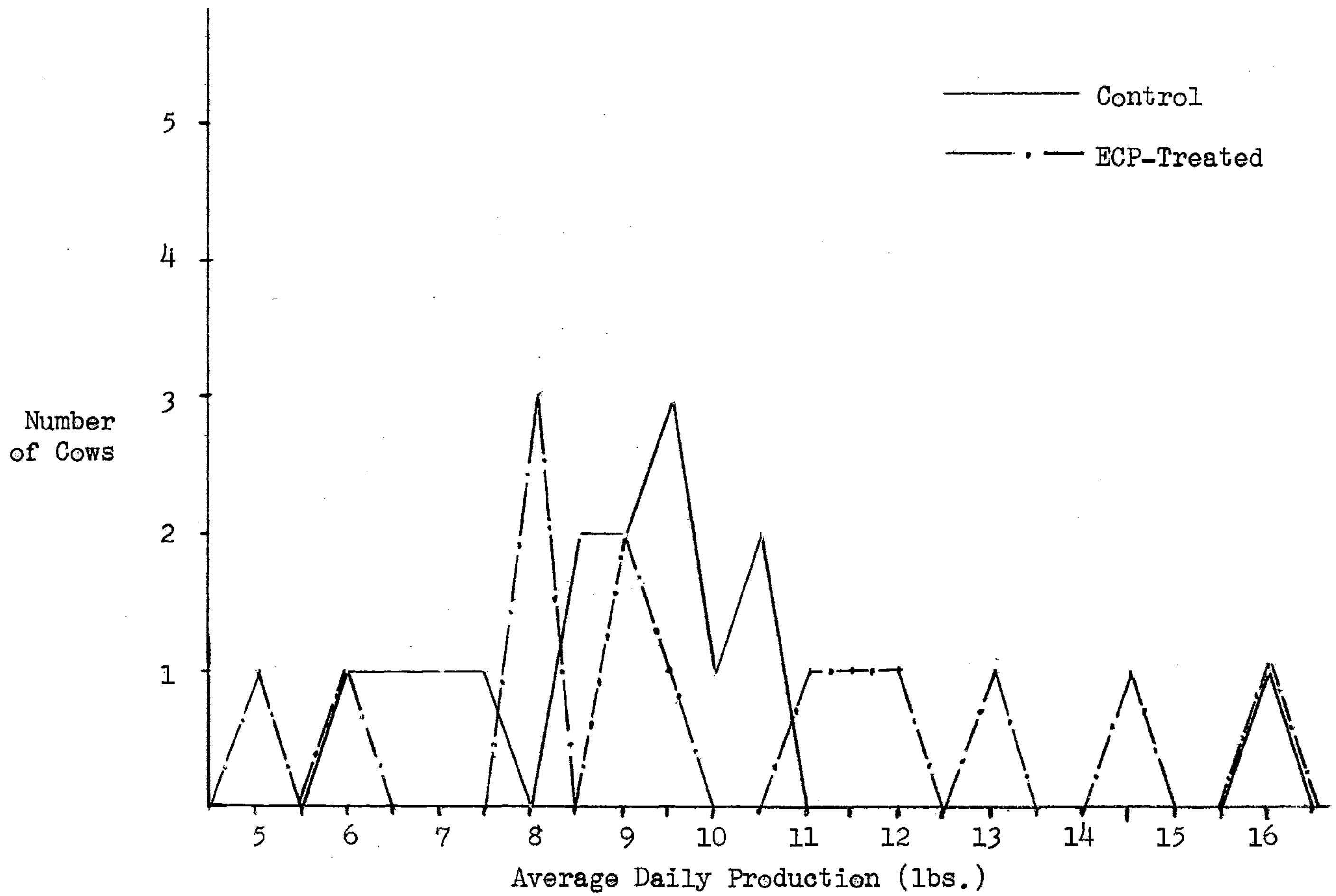

Figure 9. The Distribution of Average Daily Milk Production in Three-Year-Old Hereford Cows Treated with 5 mg. Estradiol-17β(ECP) 8-15 Days Post-Calving.

after calving for estrus to occur in the low level of milk production cows and 49.8 days in the cows producing more than 9 lbs. per day. The occurrence of estrus in the three-year-old cows producing more than 9 lbs. of milk per day required an interval of 51.7 days compared to 52.9 days for the low level cows. The occurrence of an induced estrus following treatment was apparently not associated with level of milk production to any sizeable degree. First service conception was also comparable between the two levels of milk production. First service conception in the two-year-old cows was 56.7 and 56.5 percent in the low and high level of production, respectively. Total conception during the breeding season for the two-year-old cows was 90.0 and 82.6 percent for the low and high producers, respectively. Conception data for the three-year-old cows was omitted because of use of an infertile bull. Level of milk production was concluded to have an insignificant association with the occurrence of reproductive activity after calving and was believed not to be associated with the degree of synchronization or conception rate of the cows after synchronization.

SUMMARY AND CONCLUSIONS

Various hormone treatments were administered to alter the reproductive activity of prepuberal and post-puberal heifers and lactating beef cows. Three trials were conducted with prepuberal heifers to determine the influence 6-methyl-17-acetoxyprogesterone (MAP), pregnant mare serum (PMS), and human chorionic gonadotropin (HCG) might exert on stimulating the occurrence and development of a cyclic pattern of estrus. Two trials to study the degree of synchronization of estrus and subsequent fertility after synchronization of post-puberal heifers were conducted. The first such trial involved individual feeding with fertility after mating at the induced estrus compared to mating at the second estrus after an 18-day MAP treatment period. The second synchronization trial involved 84 weaner heifers, half of which were fed 180 mg. MAP per head per day. Synchronization of observed estrus and fertility at the second heat were studied. Two trials using post-partum cows were conducted. In the first, only MAP was administered in an attempt to shorten the period of reproductive quiescence following calving. In another trial the effect of estradiol-17β (ECP) treatment on the interval from parturition to uterine involution and first estrus were studied. In addition, the influence of MAP alone or in combination with ECP on the degree of synchronization of estrus and subsequent fertility was studied.

In Trial I a single intramuscular injection of 1000 IU PMS did stimulate estrual activity in 13 of 15 prepuberal heifers whose average age was 225 days. The injection of PMS alone did stimulate ovulation in four of nine heifers. This would indicate that PMS was sufficient treatment to produce both follicular development and ovulation. When these PMS treatments were followed by an intravenous injection of 1000 IU of HCG ovulation occurred in six of nine heifers palpated. Injections of HCG caused luteinization of some follicles in all animals without a resultant ovulation. This could be the result of the intravenous injection of a large dose of luteinizing hormone. The use of MAP either before or after PMS and HCG had no apparent influence on stimulating cyclic activity. The feeding of 180 mg. MAP per head per day stimulated only two of five heifers to exhibit estrus after cessation of MAP feeding with conception occurring in both. The use of PMS and HCG without MAP resulted in all heifers of a younger group (five of five) exhibiting an observed estrus. Three heifers of this group were later observed in estrus but not at a normal interval. Only the MAP plus PMS and HCG treatment group had one heifer which exhibited an uninduced estrus at what was considered a normal interval. Seven of 20 heifers subjected to these various treatments became pregnant to the service of a fertile teaser bull. The use of MAP alone or in combination with PMS and HCG was ineffective in stimulating cyclic estrual behavior. Apparently these treatments were not capable of triggering the onset of puberty.

In Trial II the effect of a treatment of 1000 IU PMS followed by 180 mg. MAP per head per day was compared to 1000 IU PMS alone on estrual activity in 300-day-old prepuberal heifers. Following the PMS treatment one of five heifers came into estrus before the MAP treatment was begun. After cessation of MAP treatment three of four heifers exhibited estrus with one exhibiting another heat period 19 days later. In the group administered PMS alone two of four heifers were observed in estrus. The failure to respond to PMS was not expected in either group. Six of the heifers in these groups had received a prior 1000 IU PMS injection. Apparently the ovaries had become refractory to PMS, although only one previous dose would not be expected to accomplish this. Whereas, treatment with MAP after PMS was not successful in Trial I, three of four heifers exhibited an induced estrus in Trial II after MAP feeding was terminated. Possibly the increase in age (average of 3 months) of these heifers between the first and this second treatment was a factor in expression of an induced estrus. Another possibility is the need for the presence of a corpus luteum before further estrusl activity usually occurs.

In Trial III administration of 180 mg. MAP per head per day for 18 days to 17 prepuberal heifers whose average age was 375 days stimulated the exhibition of estrus in seven heifers (41.2 percent) with six and four observed in a second and third post-treatment estrus, respectively. The administration of 1000 IU PMS to eight heifers of this group pretreated with MAP which had shown no prior sexual behavior resulted in seven exhibiting heat. None, however, returned to estrus soon after this initial heat period. These heifers were more responsive to MAP than any group treated previously. Since they were approximately 11 months old they could possibly be nearing an age when the puberal estrus normally occurs. This factor was probably of primary importance in determining degree of response.

In Trial IV the use of 180 mg. MAP per head per day for 18 days in a group of 38 lactating post-partum two-year-old beef cows given for 18 days beginning on day 25 post-calving significantly (P .05) increased the interval from calving to first estrus in the 19 treated cows by 16.9 days (74.9 vs 58.0 days). Four heifers (21 percent) were observed in estrus within three to six days after termination of MAP feeding. Percent conception at first post-partum estrus was not statistically different nor did level of nutrition significantly affect reproductive activity after parturition. The standard deviations for calving interval and days from calving to conception were large indicating considerable variation was associated with these measures of post-partum reproductive activity. Incorporation of MAP into the diet of noncycling cows did not produce the desired response of reducing post-partum interval but instead lengthened it significantly by approximately 17 days.

In Trial V 180 mg. MAP per head per day for 18 days was used to synchronize 60 heifers. Artificial insemination was carried out at the first induced estrus in 30 heifers and at the second post-treatment estrus in the other 30 heifers. Each group of 30 heifers was further divided into two groups of 15. All heifers had been wintered at a moderate energy intake (0.5 lb.) gain per day. Then two months before start of breeding two groups of 15 each were raised to a high level of energy (1 pound gain per day) before breeding, while the other group was maintained at the moderate energy level. Varying the energy intake in these heifers for 60 days preceding breeding was not found to have a statistically significant influence on the time of occurrence of estrus nor conception rate. With regard to synchronization, 55 heifers (91.7 percent) were observed exhibiting an induced estrus within six days after treatment ended, with 60 percent doing so on day two following the termination of treatment. Of the heifers that were allowed to recycle before breeding or did not conceive to first mating, 75 percent were in estrus on day 20, 21, 22,and 23. The second heat periods were observed over a wider range of days, but were still obviously synchronized. Eight of 22 heifers (36.4 percent) conceived to first service when artificially inseminated at the induced estrus. In the group artificially inseminated at the second post-treatment estrus first service conception was again extremely low, with eight of 24 conceiving (33.3 percent). There was very little difference between level of nutrition and its influence on first service conception rate in the group bred at first estrus (33.3 and 40.0 percent). A large difference was noted between the high and moderate levels in the groups bred at second estrus. The high level group had 53.8 percent (7 of 13) conceive to first service compared to only 9.1 percent conception rate in the moderate level group (1 of 11 heifers). This low first service conception rate was also reflected in services required per conception where values of 1.5 and 1.9 were noted for the high and moderate energy groups, respectively. The number of services per conception for the group bred at first estrus was 1.7 and 1.5 for the high and moderate energy groups, respectively. The first service conception rates in this trial were very low when insemination occurred at either the first or second post-treatment estrus. The low conception rate at the induced estrus was expected but was not anticipated when breeding was begun at the second post-treatment estrus. Possible explanations for the low first service conception rate observed were the presence of low level cervical infection or low fertilizing capacity of the semen used, which was not believed to be true.

The degree of synchronization in this trial was very good, but conception rates after synchronization were low. Varying energy intake before the breeding season was not noted to have any significant influence on the occurrence of estrus or conception data in the groups bred at induced estrus. The difference between conception rates of the high energy and moderate energy group bred at the second post-treatment estrus was quite large but cannot be explained other than it was probably a difference due to chance.

The purpose of Trial IV was to study the effects of group feeding 180 mg. MAP per head per day for 18 days on the first service conception rate of yearling Hereford and Angus heifers bred naturally at the second post-treatment estrus. Of 41 heifers receiving the MAP treatment, 26.8 percent (11) were observed in estrus during the feeding period, varying in occurrence from the onset of treatment to four days before cessation of treatment. This percentage was higher than the 15 percent observed in estrus during treatment in the previous individually fed MAP trial. The failure to obtain the two lb. daily allotment of feed in the group fed lot could be the cause of increased estrual activity between the two trials. The degree of synchronization was good with 80.5 percent of the heifers observed in estrus in a two- to six-day period. When allowed to recycle and then placed in breeding pastures for natural mating at the second post-treatment estrual period, 75.9 percent of the 38 treated heifers that had recorded matings did so in the first eight days of the breeding season. The use of MAP significantly ($P < .005$) reduced the variation in time of occurrence of estrus after the breeding season began. Rectal palpations 90 days after termination of the breeding season revealed the conception rate at first service was 63.4 percent (26 of 41) compared to 52.4 percent (22 of 42) for the control lot. The difference was not significant but did indicate that MAP might enhance conception rate when breeding is carried out at the second post-treatment estrus.

In Trial VII, 5 mg. ECP was administered to 11 three-year-old and 17 two-year-old cows 8-15 days after calving in an attempt to shorten the interval from parturition to involution of the uterus and occurrence of first estrus. The ECP injection decreased significantly ($P < .01$) the mean interval from parturition to uterine involution in pluriparous cows, but not in the primiparous cows. The interval in the treated cows was five days shorter than it was in the nontreated cows (39.6 vs 44.4 days). A significant ($P < .10$) interaction between age and treatment was noted. As age increase treatment had a greater influence on the uterus and facilitated its return to normal size and tonus. These results suggest that estrogen hastens uterine involution in cows that have produced more than one calf. In contrast somewhat to the preceding results was the observation that the mean interval from calving to occurrence of first estrus was significantly ($P < .05$) reduced in the two-year-old treated cows, but not in the three-year-old cows.

The 25 two-year-old cows that received 5 mg. ECP intramuscularly had an average interval from parturition to first estrus of 36.3 days compared to an interval of 45.7 days for the untreated group. The mean intervals for the three-year-old treated and untreated group, respectively, were 29.8 and 44.5 days. Only five of the 14 treated cows were observed in estrus before an oral progestogen was given. In the untreated group 11 of 16 cows exhibited heat. The average interval from parturition to first estrus was biased downward in all four groups because all cows did not have an opportunity to return to estrual activity before a progestogen treatment to synchronize estrus was initiated.

An attempt was made to synchronize the occurrence of estrus in post-partum cows using 180 mg. MAP per head per day for 18 days, alone or in combination with a single 1 mg. injection of ECP on the second day of the MAP feeding period. Considering the prior ECP or control treatment 8-15 days post-calving, this resulted in the formation of six subtreatment groups. If post-calving treatment prior to the MAP treatment was not considered, the percent of cows in estrus during the period 1-6 days after the last MAP feeding in the control, MAP and MAP+ECP groups, respectively, was 44.8, 46.7, and 44.8 percent. Treatment 8-15 days post-calving was noted to have major influence on the occurrence of estrus after cessation of MAP feeding. In the groups receiving no ECP, there was 55.6 percent of the control group, 58.8 percent of the MAP group, and 43.8 percent of the MAP+ECP group observed in estrus within 1-6 days after the last feeding. In comparison there were 27.3, 30.8, and 46.2 percent of the cows in estrus in the control, MAP, and MAP+ECP groups, respectively, that had received a single 5 mg. ECP injection within 8-15 days after calving. Of the 51 cows not pretreated with ECP, 27 (52.9 percent) were observed in estrus within six days after MAP feeding was stopped; whereas, 13 of 37 cows (35.1 percent) which had received the 5 mg. ECP pretreatment were observed in heat in a similar time. Age of cows was also noted to have an effect on the occurrence of an induced estrus after the termination of the MAP feeding period. Only 13 of 36 treated two-year-old cows (36.1 percent) exhibited an induced estrus while 14 of 23 treated three-year-old cows (60.0 percent) exhibited an induced estrus within 1-6 days following the termination of the feeding period. If ECP had an inhibitory influence on the occurrence of a post-MAP treatment induced estrus, the three-year-old cows were able to overcome it somewhat.

The conception results obtained after attempting to synchronize the occurrence of estrus with MAP or MAP+ECP were noted also to be influenced by the pretreatment with ECP. The two-year-old cows which received the 5 mg. ECP injection 8-15 days post-calving had a 69.6 percent first service conception rate (16 of 23) and all cows conceived during the 90-day breeding season. The cows which had been pretreated as controls had only a 46.7 percent first service conception rate (14 of 30) which was significantly ($P < .05$) less than for the ECP pretreatment group. Also only 76.7 percent (23 of 30) of the control pretreated groups conceived during the breeding season. Those cows treated with 180 mg. MAP alone had a higher first service conception rate than those treated as controls or with 180 mg. MAP alone had a higher first service conception rate than those treated as controls or with 180 mg. MAP plus 1 mg. ECP (66.7 vs 55.6 and 47.1 percent, respectively). The first service conception rates of the subgroups are as follows: control-control, 36.4 percent; ECP-control, 85.7 percent; control-MAP, 80.0 percent; ECP-MAP, 50.0 percent; control-MAP-ECP, 22.2 percent; and, the ECP-MAP+ECP was 75.0 percent. Only the differences associated with the ECP-control, control-MAP, and ECP-MAP+ECP were significantly increased over the control-control group to which all were compared.

The pretreatment with 5 mg. ECP apparently had more influence on later conception rate than any other factor. Conception was much higher in all ECP treated groups than the control-control group with the exception of the control-MAP group. All of the cows pretreated with ECP conceived during the breeding season; whereas, only 76.7 percent of the control pretreated group did so. Apparently the ECP had some stimulatory effect on the reproductive tract resulting in the production of more viable ova or a more suitable uterine environment for implantation to occur.

The association of level of milk production with response to the various hormones was also studied. The data obtained indicated that level of milk production had very little influence on reproductive activity during the post-partum interval. Cows producing over nine lbs. milk per day at two years of age had an interval of 36.8 and 49.8 days, respectively, to uterine involution and first estrus; whereas, those producing less than nine lbs. per day had intervals of 39.3 and 48.5 days, respectively, to involution and first estrus. These same intervals in the three-year-old cows were slightly longer. In the high-producing cows they were 44.0 and 51.7 days compared to 41.6 and 52.9 days, respectively, in the low-producing cows. The results pertaining to the occurrence of the induced estrus within 1-6 days after treatment were comparable as were the conception results between level of milk production groups. The first service conception was 56.7 for the low-producing group and 56.5 for the high-producing group. Total conception results were also approximately equal. Level of milk production was concluded not to have a significant association with the post-partum reproductive performance of the cows in this study.

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APPENDIX

TABLE XXIII

IDENTIFICATION OF ANIMALS USED IN THE PREPUBERAL STUDY

Brand	Ear Mark	Birth Date[a]	Beginning Treatment Weight	Age in Days at 1st Trt.	Breeding	First Treatment	Subsequent Trt.
362	T10	1-14	400	367	Angus	P[b]	
1	428	3-7	420	314	Crossbred	P	
2	420	2-24	495	326	Crossbred	P	
3	408	2-14	555	336	Crossbred	P	
4	427	3-6	405	315	Crossbred	P	PMS
5	422	2-26	510	324	Crossbred	P	
6	406	2-12	525	338	Crossbred	P	PMS
7	426	3-3	495	318	Crossbred	P	
8	424	3-1	485	320	Crossbred	P	
9	411	2-17	550	333	Crossbred	P	
10	432	3-10	525	311	Crossbred	P	PMS
11	423	2-27	470	323	Crossbred	P	PMS
12	436	3-19	480	302	Crossbred	P	
13	429	3-8	405	313	Crossbred	P	
14	402	2-10	495	340	Crossbred	P	PMS
17	410	2-16	460	334	Crossbred	P	PMS
18	419	2-23	445	327	Crossbred	P	PMS
20	T42	10-9	540	236	Crossbred	PMS-HCG-P	
46	T43	10-19	360	234	Crossbred	P-PMS-HCG	PMS
28	T46	10-29	355	237	Crossbred	PMS-HCG	
33	T47	10-30	460	215	Hereford	PMS-HCG-P	PMS-P
39	T92	11-6	430	216	Crossbred	P-PMS-HCG	PMS-P
21	T96	11-10	480	212	Crossbred	P	PMS
23	T97	11-13	395	209	Crossbred	P	PMS
29	7171	10-11	405	242	Angus	P-PMS-	
22	7335	10-15	360	238	Angus	HCG	PMS
41	0335	10-19	375	226	Angus	PMS-HCG-P	PMS-P
26	9334	11-12	340	223	Angus	PMS-HCG	
47	9335	11-30	320	206	Angus	PMS-HCG	
44	9336	12-1	320	205	Angus	PMS-HCG	
34	2477	11-12	330	208	Angus	PMS-HCG	
37	2478	10-29	395	224	Angus	P	PMS-P
27	2480	10-17	370	236	Angus	P-PMS-HCG	PMS-P
38	2496	10-15	370	238	Angus	P	PMS-P
40	1475	10-23	385	222	Angus	PMS-HCG-P	PMS

TABLE XXIII (Continued)

Brand	Ear Mark	Birth Date[a]	Beginning Treatment Weight	Age in Days at 1st Trt.	Breeding	First Treatment	Subsequent Trt.
42	1494	10-11	400	234	Angus	PMS-HCG-P	PMS
36	1486	10-21	440	233	Angus	P-PMS-HCG	PMS-P

[a]Heifers 362 and 1-18 were born in the spring, 1964. Heifers with brand numbers higher than 18 were born in the fall, 1963.

[b]P in this instance refers to the MAP treatment.

VITA

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Doctor of Philosophy

Thesis: A STUDY OF THE EFFECTS OF VARIOUS HORMONE TREATMENTS ON THE REPRODUCTIVE ACTIVITY OF PREPUBERAL AND POST-PUBERAL BEEF HEIFERS AND LACTATING BEEF COWS

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