THE EFFECTS OF GONADOTROPINS IN INDUCING MULTIPLE OVULATIONS IN THE BEEF COW

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ABSTRACT

THE EFFECTS OF GONADOTROPINS IN INDUCING MULTIPLE OVULATIONS IN THE BEEF COW

By

David Edwin Wildt

Ovulation induction in the beef cow was studied to determine the effects of various FSH-P and HCG levels in achieving a high incidence of double and triple ovulations. Experimental design allowed for examination of the effects of repetitive gonadotropin administration on behavioral and ovarian activity.

The study was conducted in three phases. Ovarian activity was monitored both before and after ovulation by rectal palpation.

Preliminary studies in Phase I included three gonadotropin regimes administered in consecutive cycles. Animal numbers and treatments were as follows: I) 7, 25 mg FSH-P on day 16, 2,000 IU HCG on day of estrus; II) 6, 25 mg FSH-P on day 16, 1,000 IU HCG on day of estrus; III) 6, 12.5 mg FSH-P on day 16, 1,000 IU HCG on day of estrus. Treatment III, utilizing low levels of FSH-P and HCG, resulted in the highest incidence of double ovulations without overstimulating the ovary. No refractory effect due to the repetitive gonadotropin treatment was observed, but the

incidence of standing estrus was reduced.

Phase II involved the utilization of two progestins in an attempt to induce overt estrus and to study the effects of the repetitive gonadotropin treatment of Phase I on the synchronized estrus. Our regime of 17α-hydroxyprogesterone-17 acetate administration was ineffective in synchronizing estrus within 8 days after withdrawal. The progestin, 17α-hydroxy-6α-methylprogesterone acetate, proved to be an adequate synchronizing agent but was only partially effective in increasing the incidence of standing heat.

During the post synchronized estrual cycle developed from the 17α-hydroxy-6α-methylprogesterone acetate, gonadotropins were administered to three animals each in the following two treatments: I) 25 mg FSH-P on day 16, 1,000 IU HCG on day of estrus; II) 12.5 mg FSH-P on day 16, 1,000 IU HCG on day of estrus. The animals were mated, slaughtered 7 to 10 days later, and the reproductive tracts were recovered. Treatment I resulted in excessive ovarian stimulation in one of the treated animals. The low levels of gonadotropin utilized in Treatment II resulted in multiple ovulations with no overstimulation of the ovary. Mean corpus luteum size was reduced in the animals frequently treated with gonadotropins when values were compared to those cited in literature.

Low level gonadotropin treatment was studied on a larger scale in Phase III. Sixteen postpartum cows receiving 12.5 mg FSH-P on day 16 and 1,000 IU HCG on day of estrus

achieved a highly significant increase ($p<0.01$) in ovulation rate in comparison to 30 controls. Over 56 percent of the gonadotropin treated cows achieved double ovulations while 69 percent ovulated two or three ova. Fertility was not affected at a later estrus in gonadotropin treated animals.

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INTRODUCTION

The efficiency of commercial cattle production can be vastly augmented by increasing the incidence of multiple births in the beef cow. Numerous studies have demonstrated that the administration of exogenous gonadotropic hormones at various levels and stages in the estrual cycle of the cow will result in enhanced follicular development and, consequently, increased ovulation numbers. Although some success has followed these endeavors, the application of most ovulation induction regimes is not practical on a commercial basis at the present time.

Many problems are inherent in attempting to regulate reproductive functions and fetal numbers. Previous research has demonstrated the importance of limiting calf numbers to two or three per cow, emphasizing the necessity of developing techniques for limiting the ovulation rate to one which will result in a high incidence of twin or triplet births. In addition, there is a need to examine, characterize, and determine effective dosage levels and stages of administration of available follicle stimulating and luteinizing agents. Finally, since follicle stimulating source administration must be timed precisely during the follicular phase of the cycle, extensive study and

development of accurate estrus synchronization techniques, coupled with proper gonadotropin treatment, will aid in accomplishing the goal of total regulated reproduction. The objectives of these investigations were:

1) To develop a treatment for inducing a high incidence of double or triple ovulations;

2) To study the effects of various FSH dosages in combination with low levels of HCG;

3) To observe the refractoriness of cattle to repeated doses of FSH and HCG.

LITERATURE REVIEW

Incidence of Natural Twinning

The frequency of natural twin births is low in all types of cattle. The summarized records of nearly a million parturitions in dairy cattle demonstrated that only 1.88 percent of all births resulted in twins. The corresponding rate for beef breeds was even less, being only 0.44 percent (Lush, 1945).

Summaries of cattle twinning rates based on the number of calvings revealed marked variations between breeds (Meadows and Lush, 1957), herds within breeds (Meadows and Lush, 1957), and families within herds (Lush, 1925).

Although twinning ability is inherited to some extent, selection is ineffective in increasing the incidence of natural twinning due to the low heritability of the trait (Lush, 1925; Lush, 1945; Pfau et al., 1948; Erb et al., 1960). Mechling and Carter (1964) studied the incidence of twinning in an Angus beef herd in Virginia which had been selected entirely on the basis of twinning since the 1930s. A systematic selection program utilized twins, daughters of twins, dams of twins, and cows with histories of twinning. Sires consisted only of twin Angus bulls or

bulls with records of twin offspring. After almost 30 years of intensive selection, the incidence of natural twinning in the selected herd was only 1.71 percent in comparison to two non-selected Virginia herds which had twinning frequencies of 1.64 and 0.81 percent respectively.

Ovulation Induction and Superovulation

Ovulation induction of prepuberal and postpuberal cattle, coupled with future advancements in ovum transfer, has potential for practical application. The benefits have been reviewed by Foote and Onuma (1970) and include: (a) reduction of the generation interval; (b) early progeny testing of females; (c) utilization of outstanding females as ovum donors; (d) transfer of embryos over great distances when the embryos have specific genetic distinctions; (e) isolation of prenatal maternal effects; (f) examination of the requirements for peak prolificacy and normal maturation; (g) a greater understanding of the effects of stress on conception and gestation; and, (h) the regulation of progeny numbers per female. In addition, the time interval between parturition and occurrence of estrus and ovulation can be shortened with the administration of gonadotropins (Oxenreider, 1968). The following review considers the significant advancements and knowledge achieved in the field of ovulation induction as it concerns the bovine specie. Due to the similarity in technique, gonadotropin research will be reviewed in both the prepuberal and postpuberal animal. The discussion will also be concerned with estrus

synchronization coupled with exogenous gonadotropin administration since the advantage of successful synchronization increases the potential of total regulated reproduction in the beef cow.

Early Gonadotropin Research

Gonadotropins were first investigated by Zondek and Aschheim (1927) and Smith and Engle (1927). These groups, utilizing laboratory animals, demonstrated the relationship of the anterior pituitary to the development and regulation of the genital system. Subsequent experimentation established that extracts of the anterior pituitary could be utilized to induce follicular stimulation and ovulation in immature female rats and rabbits (Casida, 1934). It was Casida et al., (1943), who between 1934 and 1941, first induced ovulation in cows and calves by subcutaneous injections of unfractionated ovine and bovine pituitary extracts or by subcutaneous injections of follicle stimulating extracts succeeded by an intravenous inoculation of luteinizing extract. Although this study met with limited success, it initiated experimentation with a variety of exogenous hormonal regimes to induce ovulation in the bovine.

The Ovulatory Induced Response

The ovulatory induced response is basically concerned with the stimulation of the follicular tissue of the ovary by a source of follicle-stimulating hormone (FSH) that will

result in a higher total level of FSH in the system than would normally be contributed by endogenous sources. In the past, the most commonly utilized source of exogenous FSH gonadotropin has been pregnant mare's serum (PMS) discussed by Cole and Hart (1930) and by Zondek (1930). More recently FSH-P, a partially purified pituitary extract, has received wide acceptance as an FSH source.

To further aid the ovulatory process, a form of luteinizing hormone is administered either intravenously or intramuscularly. The conventional preparation employed has been human chorionic gonadotropin (HCG) first described by Aschheim and Zondek (1927).

Induced Ovulation of the Prepuberal Calf

Superovulation of the prepuberal calf holds several distinct advantages when coupled with the successful transfer of ova including reduction of the generation interval by permitting early progeny tests for females and the production of the greatest possible number of progeny from outstanding genetic superiors (Onuma et al., 1969; Foote and Onuma, 1970). Such experimentation also allows the researcher to utilize an animal with none of the cyclic divergence or luteal characteristics of his mature counterparts (Foote and Onuma, 1970).

High ovulation rates, 40 to 80 percent, in prepuberal heifers have been obtained from exogenous gonadotropin treatments (Hafez, 1969); however, individual responses have been quite variable (Avery et al., 1962; Howe et al.,

1962; Jainudeen et al., 1966; Onuma et al., 1970) and fertilization depressed (Black, et al., 1953; Marden, 1953; Howe et al., 1962; Jainudeen et al., 1966; Onuma et al., 1969; Lineweaver and Hafez, 1970). Nutritional and management factors do not affect the widespread variability observed in numbers of follicles obtained (Onuma and Foote, 1969; Onuma et al., 1970). There is a reduced fertilization rate due to a large extent to the difficulties experienced when attempting to deposit semen beyond the cervical entrance of treated calves (Onuma et al., 1969).

Several attempts have been made to enhance the ovarian response of superovulated calves by administering exogenous steroids. Onuma et al., (1969) demonstrated that the administration of 17B-estradiol benzoate had no effect on follicle numbers or ovulation rate. Seidel et al., (1971) reported that 17B-estradiol injected after PMS administration reduced ovarian response to PMS but increased the numbers of fertilized ova recovered in calves. Additional evidence has indicated suppressed follicular growth and decreased ovulation rate in calves receiving this specific estrogen (Howe et al., 1962; Onuma et al., 1970). Howe and Black (1963) found that the addition of PMS and HCG with or without progesterone or a combination of progesterone and estrogen had no effect on sperm transport in the treated calf.

Calves rectally inseminated achieved significantly higher fertilization rates than younger calves which were

inseminated by other methods (Avery and Graham, 1962). Cleaved ova and recovery rate were lowered significantly in the presence of corpora lutea. Higher fertility was achieved when liquid semen was utilized in comparison to frozen semen and three inseminations were completed in contrast to two inseminations (Onuma et al., 1970).

Hafez (1969) has speculated that an essential hormonal balance or component is missing in the reproductive system of these prepuberal animals treated with PMS and HCG.

The recovery rate of fertilized ova from calves induced to ovulate is low (Jainudeen et al., 1966; Lineweaver and Hafez, 1970; Seidel et al., 1971). This, in part, appears to be due to the inability of the oviductal fimbria to pick up all of the ova ovulated, possibly because of the large ovaries produced in relation to the small, normal sized fimbriae (Onuma et al., 1969; Seidel et al., 1971). Apparently, however, the infantile condition of the oviduct does not interfere with the passage of ova through the reproductive tract (Marden, 1953).

Seidel and Foote (1969) and Seidel et al., (1971) have studied the age-ovarian response relationship after gonadotropin treatment. Calves superovulated at birth, 1, and 2 months of age had an average of 0, 9.4, and 28.2 ovulations and 0, 77.7, and 100 percent ovarian response, respectively.

Neville and Williams (1973) have recently reported attempts to induce estrus, ovulation, and conception in heifers averaging 327 days of age. Heifers received progestin

treatment with dihydroxyprogesterone acetophenide (DHPA) or medroxyprogesterone acetate (MAP). On the second day of progestin feeding, each animal received estradiol valerate and near the end of progestin feeding either PMS or FSH-P. Induction of estrus ranged from 50 to 100 percent and conception from 0 to 50 percent. PMS was found to result in higher ovulation rates and more mature follicles than FSH-P in these prepuberal animals.

Various hormonal regimes to induce multiple ovulations in the calf have been attempted (Casida et al., 1943; Black et al., 1953; Marden, 1953; Jainudeen et al., 1966; Onuma et al., 1969; Seidel and Foote, 1969; Lineweaver and Hafez, 1970; Onuma et al., 1970; Seidel et al., 1971; Neville and Williams, 1973). A summation of the most successful of these is shown in Table 1.

Ovulation Induction in Postpuberal Females

To achieve multiple ovulations, most research has been directed toward detection of the appropriate levels and sources of gonadotropins and the proper stage of the cycle for treatment. Numerous hormonal preparations and injection schedules have been utilized (Casida et al., 1943; Hammond and Bhattachanya, 1944; Dowling, 1949; Umbaugh, 1949; Avery et al., 1962; Avery and Graham, 1962; Gordon et al., 1962; Arbeiter, 1963; Hafez et al., 1963; Hafez et al., 1963; Schilling and Holm, 1963; Denny, 1964; Hafez et al., 1964; Peli and Castelli, 1964; Scanlon et al., 1968; Lamond and Hill, 1969; Schwartz and Shelby, 1969; Roussel and Beatty,

TABLE 1

SUMMARY OF GONADOTROPIN TREATMENTS
IN PREPUBERAL BOVINE FEMALES

Number of Animals	Age (wks)	Treatment	Number of Ovulations	Percent Ova Recovered	Reference
9	9-11	Day 1-5: 10 mg FSH-P Day 6: 10-150 mg LH	3.8	8.0	Onuma et al., (1969)
5	9	Day 1: 2,000 IU PMS Day 7: 25-150 mg LH*	15.4	45.6	Onuma et al., (1969)
4	8	Day 1: 1,500 IU PMS Day 6: 50 mg LH	28.2	5.7	Seidel et al., (1971)
10	4-24	Day 1: 2,000 IU PMS Day 6: 5-10 mg LH or HCG	16.3	27.0 - 60.0	Jainudeen et al., (1966)
4	8-9	Day 1: 1,500-2,000 IU PMS Day 6: 1,500 IU HCG	8.0	30.0	Onuma et al., (1970)
83	6-14	Day 1: 2,000 IU PMS followed by 10,000 IU HCG 120 hrs. later	45.0	---	Lineweaver and Hafez (1970)

*Armour PLH, PLHE, or NIH-LH

1970; Turman et al., 1971; Foote and Radmall, 1972; Johnson et al., 1972; Scanlon, 1972). The more successful treatments are shown in Table 2.

In the past, PMS has been the most common follicle stimulating preparation utilized. Because of its large molecular size and slow metabolism (Folley and Malpress, 1944), PMS exerts its effect over a longer period of time than purified FSH extracts (Rowson, 1951; Brock and Rowson, 1952). Hammond (1955) postulated that a single PMS injection is potent for four to five days compared to anterior pituitary FSH which is quickly metabolized and consequently requires priming dosages every 12 hours.

Disadvantages are evident with PMS use. Gordon et al., (1962) observed a high incidence of "silent heats" (absence of overt signs of estrus) when PMS was administered at low doses or late in the cycle. No effect on cycle length or mating behavior was noted. The tendency for PMS to result in refractoriness in treated cows will be discussed in detail later.

Although PMS and HCG have been the most extensively used gonadotropins, other preparations have been used such as unfractionated sheep pituitary extracts (Casida et al., 1943; Umbaugh, 1949; Black et al., 1953), beef pituitary extracts (Casida et al., 1943), horse anterior pituitary extracts (Dowling, 1949), and partially purified luteinizing hormone (Avery et al., 1962; Jainudeen et al., 1966; Seidel and Foote, 1969; Seidel et al., 1971). Recently FSH-P, a

TABLE 2

SUMMARY OF GONADOTROPIN TREATMENTS
IN POSTPUBERAL BOVINE FEMALES

Number of Animals	Age	Treatment	Number[1,2,3]	Conception Rate	Reference
81	Sexually mature	Day 3,4,5, or 6: 1,500 IU PMS and 2,000 IU PMS on day 16,17, or 18. Day of estrus: 2,500 IU HCG	23[2]	64.2%	Turman et al., (1971)
79	Sexually mature	Day 16: 1,500 or 2,000 IU PMS	11[3]	65.0%	Hafez et al., (1964)
416	Sexually mature	Day 16 or 17: 1,200 IU PMS or, Day 16 or 17: 1,600 IU PMS	2.5[1](1-17)[4] 2.7[1](1-15)[4]	76.2%	Gordon et al., (1962)
18	Sexually mature	Day 5: 2.5 mg ovine FSH Day 16: 3.0 mg ovine FSH	1.39[1](1-6)[4]	44.0%	Schwartz and Shelby (1969)
32	Sexually mature	Days 1-4[5]: 20,10,10,10 mg FSH and 5 mg LH each day. Day 5: 100 mg LH	21[1](4-55)[4]	61.0%	Avery et al.,(1962) Avery and Graham(1962)
89	Sexually mature	Day 16: 3,000 IU PMS Day of estrus: 2,000 IU HCG	9.0[1](1-55)[4]	68.0%	Scanlon et al.,(1968)

1 Number of ovulations 2 Number of multiple births 3 Percent of animals carrying viable twin embryos

4 Numbers in parentheses are ranges

5 Refers to injection day and not cycle day

pituitary extract with much of the LH activity removed, has been used extensively as a follicle stimulating source (Bellows et al., 1969; Onuma et al., 1969; Laster, 1972; Rich and Johnson, 1972). Some preliminary research has been completed using gonadotropin releasing hormone (GnRH) (Mauer and Rippel, 1972) as a stimulant of follicular growth. Results utilizing this particular preparation are inconclusive and require additional study.

The presence of a functional corpus luteum has a modifying effect on the ovarian response to gonadotropins and subsequent fertilization in the bovine (Hammond and Bhattachanya, 1944; Dowling, 1949; Avery et al., 1962; Hafez et al., 1963; Lamond, 1964). When the corpus luteum is present, ovulation is significantly decreased or completely obstructed (Dowling, 1949; Hafez et al., 1963; Laster, 1972). Furthermore, when ovulation does occur the ova do not become fertilized (Rowson, 1951), probably as a result of the inhibition of capacitation (Dukelow, 1971). Enucleation of the corpus luteum has been found to initiate estrus in cows prior to treatment for superovulation (Avery et al., 1962). In addition, this expression results in a higher fertility rate (Lamond, 1964). The reduction in the interval between enucleation treatment and subsequent estrus was superior by almost two days over daily subcutaneous injections of progesterone (Avery et al., 1962).

Contrary to some of the benefits postulated above from the expression of the corpus luteum, Hafez et al., (1965)

found no advantage to enucleating this structure and speculated that this process may have a detrimental effect on conception.

Ovarian response is also influenced by previous or subsequent treatment with estrogen during the superovulatory process. The administration of 17B-estradiol intramuscularly prior to PMS injection tended to reduce the number of developed follicles while treatment after PMS injection significantly increased the number of developed follicles and fertilized ova recovered. The estrogen had no effect on ovulation rate (Hafez et al., 1963).

Gonadotropin treatment is most effective when administered during the follicular phase, but no benefit is obtained by initiating single gonadotropin treatments prior to day 16 of the cycle (Casida et al., 1943; Dowling, 1949; Hafez et al., 1963). Hafez (1969) indicated that treatment during the luteal phase led to a high incidence of cystic follicles.

Follicular growth rate from a diameter of 1 to 2 mm is continual and independent of cyclic hormonal stimulation during the luteal state of the estrual period (Choudary et al., 1968; Marion et al., 1968). However, several studies have been completed in which gonadotropin injections have been given both in the luteal and follicular phase of the cycle. Schilling and Holm (1963) hypothesized that ripe follicles begin development during or just after the last heat period with only one continuing to maturation while the

others become atretic. They attempted to prevent this normal occurrence of atresia by injecting 1,000 to 1,500 IU PMS on day 5, e.g., during the luteal period. Enucleation of the corpora lutea and an additional injection of PMS between days 16 and 18 were followed by HCG administration on the day of estrus. This procedure resulted in 73 percent of the cows producing the desired number of two or three ovulations. Laster et al., (1971) confirmed this study when using a comparable treatment following post-estrus synchronization. Of the 57 animals treated, 40 percent had 2 corpora lutea and 33.3 percent single corpora lutea. Conception rate was only 36 percent. Schwartz and Shelby (1969), using similar regimes, obtained excessive stimulation (average 6.56 ovulations) and consequently low fertilization. When 2.5 mg of ovine FSH was administered on day 5 and 3 mg ovine FSH on day 16, a 1.39 ovulation rate and 44 percent conception rate were obtained.

Concerning the optimum number of injections of LH for highest induced ovulatory efficiency, Avery et al., (1962) found that a single injection of luteinizing hormone was superior to two consecutive daily doses of the same preparation.

It seems that a definite linear dose-response relationship exists for PMS (Gordon et al., 1962; Denny, 1964), although Hammond and Bhattachanya (1944) found no obvious correlation when doses from 800 to 1,500 IU PMS were utilized after enucleation of the corpora lutea. Bellows

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et al., (1969) indicated a dose-response for porcine FSH and also noted that dosages in excess of 12.5 mg FSH-P resulted in overstimulation of the ovary. Lineweaver and Hafez (1970) found a relationship between the amount of HCG administered and the ovarian response. As HCG levels increased from 2,000 to 10,000 IU, the number of large follicles developing decreased but the ovulation rate of these follicles increased significantly. Ovarian size increased as both the dose of HCG and time interval between PMS and HCG injection increased.

For the practical application of superovulation to cattle, a sequence of gonadotropin injections or mechanical measures is required that will result in a consistent percentage of two or three ovulations (Schilling and Holm, 1963; Laster et al., 1971). The results for achieving this goal have been variable. Evidence indicates that not only the level of treatment but other factors are involved in attaining only double ovulations. Laster et al., (1971) indicated that HCG injected on day 3 after PMS treatment (compared to the day of estrus) induced more double ovulations. Gordon et al., (1962) demonstrated that the survival rate of twin ova was significantly higher when one ovum was ovulated by each ovary in comparison to a double ovulation from a single ovary. Cows which ovulated two ova experienced a high rate of embryonic mortality during the first six weeks of gestation, and cows which carried three fetuses at six weeks usually aborted all of them prior to parturition.

No correlation between body weight and ovarian response to gonadotropin treatment exists (Gordon et al., 1962; Bellows et al., 1969). Gordon et al., (1962) found that the season of the year had no effect on inducing multiple ovulations. However, others have reported that variable seasonal factors such as temperature and humidity are definite determining factors in inducing ovulation (Stott and Williams, 1962; Hafez, 1969). The superovulatory response is also affected by the time interval from the injection of the gonadotropin to the subsequent estrus (Hammond, 1949; Scanlon et al., 1968). Hammond (1949) achieved the best results when estrus occurred three, four, or five days after the gonadotropin injection. Scanlon et al., (1968) reported a substantially higher ovulation rate as the interval between PMS and estrus increased from two to five days. Although limited evidence is available, it has also been reported that the induced ovulatory response is also affected by breed, genetic constitution, previous levels of hormonal injections, the postpartum interval, and the plane of nutrition (Hafez, 1969).

Refractoriness to Gonadotropins

Refractoriness to repeated injections of various gonadotropins in the bovine has occurred (Willet et al., 1952a; Willet et al., 1953; Hafez et al., 1964; Hafez et al., 1965; Jainudeen et al., 1966; Laster et al., 1971).

This condition is not common to all mammalian species. Fertility and litter size were typical when rabbits, after

weaning their previous litter, were again treated with purified luteinizing hormone and inseminated (Hafez et al., 1964).

Willet et al., (1952a) experienced a reduced number of corpora lutea with successive treatments of sheep and swine pituitary FSH or equine gonadotropin plus HCG. Hafez et al., (1964) reported reduced superovulatory response to 2,000 IU PMS injected during the second estrual cycle following first cycle treatment with 1,500 IU. Reports using similar treatments found complete refractoriness when the injections were repeated at 18 to 40 day intervals (Jainudeen et al., 1966), while no refractoriness was found 5 to 7 months after original treatment of PMS (Jainudeen et al., 1966). Gordon et al., (1962) reported that PMS treatment did not affect the superovulatory response if only one treatment was utilized per year. Willet et al., (1953), observing refractory cows, concluded that long periods of rest without gonadotropin treatment failed to reduce or eliminate the condition.

Concerning refractoriness due to LH preparations, Slyter et al., (1965) reported that follicle numbers were not significantly affected by daily 500 IU injections of HCG for 3 to 17 days. However, corpora lutea weight did decrease significantly during the longer treatments.

Refractoriness appears to be caused by the development of antibodies to the gonadotropins (Willet et al., 1953; Jainudeen et al., 1966). It is reported that the levels of

antigonadotropic activity obtained from PMS increases with successive treatments after the second injection and achieves maximal values 16 days after the fourth injection (Jainudeen et al., 1966). Other postulated factors attributed to the cause of refractoriness include the exhaustion of primary follicles in the ovary and varying degrees of damage to the ovaries as a result of treatment or surgery.

Excessive stimulation of the ovary can also have other effects. Hafez et al., (1963) noted that levels of 5,000 IU PMS caused a substantial number of hemorrhagic follicles. Hafez (1969) indicated that overstimulation of the ovary caused a reduced rate of ovum recovery by:

(a) the endogenous estrogen effect on ovum transport rate;
(b) the trapping by the follicles of some ova; and,
(c) undersize fimbriae to surround the ovarian surface.

Estrus Synchronization Prior to Gonadotropin Treatment

A combination of estrous cycle control and gonadotropin treatment is an important prerequisite for making the induction of multiple births more suitable for practical application.

One of the earliest studies in this field, completed by Nellor and Cole (1956), involved administration of various levels of crystalline progesterone followed by an injection of equine gonadotropin. This particular regime resulted in a 17 percent conception rate. In a later study, it was noted that cows which underwent progesterone synchronization

produced an average of 7.9 ovulations more than were produced by individuals superovulated but not synchronized (Avery et al., 1962). Osland and Ellington (1971) were successful by injecting two separate progesterone treatments of 750 mg each and 1,500 IU PMS on day 29. Estrus was demonstrated within 9 days in 86 to 91 percent of the test animals. The conception rate was 65 percent and three sets of twins and one set of triplets resulted from the treatment.

Several trials have been completed in which various levels and injection sequences of norethandrolone and FSH-P were utilized to induce multiple ovulations (Vincent and Mills, 1972). Of the FSH-P treated cows, 49 percent were estimated to have multiple ovulations (with an average of 1.9 corpora lutea per cow). Of the cows calving from first breeding, 24 percent achieved multiple births for a 126 percent calving rate. Vincent and Mills (1969), using norethandrolone to synchronize cycles and similar regimes of FSH-P, reported 55 percent of the cows attaining multiple ovulations with only 7 percent having more than 3 corpora lutea.

Lamond and Hill (1969) presented data that suggested that melengestrol acetate (MGA) and PMS could be used together to produce concurrent synchronization of the estrual cycle followed by subsequent multiple ovulation and satisfactory fertility. MGA, fed at the rate of 0.5 mg per animal daily for 18 days with PMS administered on day 14,

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

Bellows et al., (1969) fed medroxyprogesterone acetate (MAP) for 11 days with estradiol valerate administered on day 2 followed by various levels of FSH-P injected twice daily on days 8 through 12. This treatment resulted in the most predictable ovarian response. A total of 6.25 mg FSH-P resulted in the most desirable number of ovulations (2.1) and a fertilization rate of 93.8 percent.

Graves and Dziuk (1968) have studied the induction of ovulation in cattle with HCG after MAP treatment. An interval of 60 hours between withdrawal of MAP to the HCG injection followed 24 hours later by insemination gave optimum results: a 79 percent ovulation and 55 percent conception rate were achieved.

In comparing the effectiveness of MAP versus MGA, Reynolds et al., (1969) superovulated cows with FSH-P on days 8 through 12. In addition, 5 mg estradiol valerate was injected on day 2. The percentage of cows showing heat within 5 days after withdrawal and the conception rate at first and second cumulative services were: MAP 81.8, 55.6, and 100; MGA 78.6, 45.4, and 65.4. Subsequent to first service, the MAP treated cows carrying twins was 33 percent to 20 percent for the MGA cows.

Laster et al., (1971) successfully synchronized estrus with chloroacetoxy progesterone (CAP) and then used PMS and HCG in the subsequent cycle. Administration of 1,500 IU PMS

on day 5 and 2,000 IU PMS on day 17, after CAP-induced estrus, and with 2,500 or 4,000 IU HCG on day 3 following the second PMS injection gave the most repeatable ovulation rates. This treatment in 57 cows and heifers resulted in almost 53 percent of the animals achieving 2 or 3 ovulations. However, conception rate was only 36 percent to post treatment insemination.

A summary of successful synchronization and gonadotropin treatment is listed in Table 3.

TABLE 3

SUMMARY OF SYNCHRONIZATION AND GONADOTROPIN TREATMENTS IN BOVINE TRIALS

Number of Animals	Treatment	Number of Ovulations	Fertilization Rate	Reference
8	Days 1-11: Fed 180 mg MAP daily Day 2: 5 mg estradiol valerate Days 8-12:FSH-P(2x)[1]-total dosage 6.25 mg	2.1 (1-4)[2]	93.8%	Bellows et al., (1969)
6	Days 1-18: Fed .5 mg MGA daily Day 14: 3,500 IU PMS	6.7	95.0%	Lamond and Hill (1969)
12	Days 1-4: 5 mg norethandrolone Days 1-5: 9.4 mg FSH(2x)[1]	2.7	58.0%	Vincent and Mills (1972)
14	Days 1-16: Fed 180 mg MAP daily 60 hrs. after withdrawal: 500 IU HCG, 24 hrs. later: insemination	79[3]	55.0%	Graves and Dzuik (1968)
15	Days 1-11: Fed 180 mg MAP daily Day 2: 5 mg estradiol valerate Days 8-12: FSH-P(2x)[1]-total dosage 6.25 mg	1.27[4]	91.0%	Reynolds et al., (1969)
57	Cycle prior to treatment: Days 1-16: Fed 10 mg CAP daily. Days 17 and 18: Fed 5 mg CAP daily. Day 5 of next cycle: 1,500 IU PMS and 2,000 IU PMS on day 17. Day 20: 2,500 or 4,000 IU HCG	3.5	36.0%	Laster et al., (1971)

1 Twice daily 2 Range 3 Number of cows ovulated(%) 4 Number of viable embryos

GENERAL MATERIALS AND METHODS

This study was conducted in three consecutive stages from February to November 1972 at the Endocrine Research Unit facilities at Michigan State University. Although some methods and procedures were unique in each stage of the study, a number of factors were common to all phases and will be discussed in this section.

The experimental animals included a total of 6 heifers and 39 cows. The heifers and a single cow were maintained on a standard roughage diet consisting of approximately 35 pounds mixed hay daily from February through May. From June through August, this group was maintained on grass pasture. All cows in the Phase III trial were sustained on grass pasture and supplemented with sorghum silage daily.

The follicle stimulating source, FSH-P, was purchased from Armour-Baldwin Laboratories, Omaha, Nebraska. The luteinizing agent, HCG (A.P.L.), was donated by Ayerst Laboratories, New York, New York. Progestins were furnished by Upjohn Company, Kalamazoo, Michigan. The gonadotropins were lyophilized, standardized preparations. The biological activity of the HCG was 782 IU per milligram.

The FSH-P and HCG were reconstituted with 10 ml of physiological, isotonic 0.15 M sodium chloride prior to

injection. All gonadotropic injections were intramuscular.

Estrual behavior in all trials was determined by visual observations made twice daily, at approximately 8 a.m. and 5 p.m. During more critical periods (when the animals were scheduled to return to estrus or after progesterone withdrawal) visual observations were made at least three times daily.

Ovulation rate was detected in all animals by rectal palpation. Palpation schedules during the estrual cycle were unique to each stage of the study and will be discussed later. Ovarian activity was monitored closely both before and after ovulation. Rectal palpation, as described by Zemjanis (1962), detected both follicular and corpora lutea development and activity. Development of the palpation technique was undertaken in February 1972 and was facilitated by intense utilization of 15 heifers and 1 cow that had failed to conceive during a previous 90 day breeding season. Of these animals, seven were reproductively normal while nine contained abnormal characteristics such as small or cystic follicles, adhesions, or freemartinism. Extensive palpation of these animals provided a broad perspective of ovarian morphology and activity in both the normal and abnormal state.

Disposable shoulder length palpation gloves (Haver-Lockhart Laboratories, Shawnee, Kansas) and Roccal-D (Winthrop Laboratories, New York, New York), a disinfectant detergent compound, were used at each palpation.

The Student's t test was used for statistical analysis of ovulation data. The Chi Square procedure was employed for analysis of fertility results.

PHASE I

OVULATION INDUCTION WITH VARIOUS GONADOTROPIN LEVELS IN A REPETITIVE EXPERIMENTAL DESIGN

Materials and Methods

This trial was conducted from February 1972 through May 1972 and utilized six nulliparous heifers of Angus, Hereford-Angus, and Holstein-Angus descent and one pluriparous Angus cow. All animals had failed to conceive in a previous breeding season but were cycling normally and were anatomically and reproductively sound. None of the animals had received gonadotropin or other hormone treatment previously. All animals were observed cycling at least twice prior to the start of hormone administration. Ovarian activity was monitored by rectal palpation on day 16 of the estrual cycle, day of estrus, and three consecutive days post-estrus.

The gonadotropin injections were timed from a non-synchronized estrus. FSH-P and HCG were administered in a repetitive experimental design with both gonadotropins administered to the same experimental group for three consecutive estrual cycles.

The first treatment in Phase I was: (a) 25 mg FSH-P on day 16 of the estrous cycle; and, (b) 2,000 IU of HCG on day of scheduled or exhibited estrus.

Treatment 2, administered in the next cycle to the same experimental group, consisted of: (a) 25 mg FSH-P on day 16; and, (b) 1,000 IU HCG on the day of estrus.

Treatment 3, administered in the third cycle, was: (a) 12.5 mg FSH-P on day 16; and, (b) 1,000 IU HCG on day of estrus.

Results and Discussion

Phase I was designed to determine the effects of various gonadotropin levels on ovulation numbers. In addition, the effect of repetitive gonadotropin treatment on ovarian activity was studied.

Ovulation rates for each animal in each trial are presented in Table 4. All animals responded to gonadotropins administered in Trial 1. Heifer number 67 was excessively stimulated, producing five ovulations. This animal demonstrated an 11 and a 7 day cycle subsequent to the gonadotropin treated cycle. In addition to decreased cycle lengths, intense standing estrus and lengthened estrus periods for as long as six days were observed. Ovarian activity was quite variable, ranging from small cystic protrusions on the ovarian perimeter to an absence of any palpable structures on the ovarian surface. The raised tailhead, lowered loin characteristic was also observed. This condition persisted for approximately six weeks after the initial gonadotropin treatment after which time the animal's ovarian function became normal. The absence of overt signs of estrus became evident at this time. Due to difficulty experienced with this animal, heifer 67 was excluded from the subsequent two trials in Phase I.

Gonadotropin treatment in Trial 2, administered to the

TABLE 4

OVULATION NUMBERS ACHIEVED FROM
THREE GONADOTROPIN TREATMENTS

Animal Number	Trials		
	1. Day 16: 25 mg FSH-P Day of estrus: 2,000 IU HCG	2. Day 16: 25 mg FSH-P Day of estrus: 1,000 IU HCG	3. Day 16: 12.5 mg FSH-P Day of estrus: 1,000 IU HCG
220	3	4	1
032	2	1	1
503	2	2	0
$2F_4G$	3	1	2
32	2	0	2
91	1	0	2
67	5	-	-
Average	2.57	1.33	1.33

same experimental group, resulted in four of six animals ovulating with one of six producing double ovulations. In comparing Trials 1 and 2, it was apparent that either:
(1) antigonadotropic activity reduced ovulation; or,
(2) the reduced level of HCG was preventing ovulation of follicles that could be ovulated with higher levels of HCG.

This may be further examined in the results of Trial 3 in the subsequent cycle. Only one of six animals failed to ovulate while three of six produced the desired double ovulations, two of these heifers being those that had failed to ovulate in Trial 2.

Although average ovulation rate was reduced after treatment in Trial 1, it was apparent that low levels of gonadotropin, and not antigonadotropic activity, was responsible for reduced ovulation.

Because another objective of this study was to develop a treatment regime that would result in a high incidence of double or triple ovulations, the numbers of ovulations were tabulated and are presented in Table 5. Ovulation rate in each trial is expressed as the number of treated cows with each number of corpora lutea. Based on data from the total of 19 treated heifers, 9 (47%) had 2 or 3 corpora lutea.

In comparing individual treatments, Trial 1 resulted in the highest incidence of double or triple ovulations. It was in this group that severe behavioral and reproductive abnormalities were observed in one of the treated heifers.

Trial 2, which also utilized a high dosage level of FSH

TABLE 5

OVULATION RATES OF COWS ADMINISTERED WITH THREE GONADOTROPIN TREATMENTS

Treatment	No. of Treated Animals	No. of Corpora Lutea Per Cow					
		0	1	2	3	4	5
Trial 1							
Day 16: 25 mg FSH-P Day of estrus: 2,000 IU HCG	7	0	1	3	2	0	1
Trial 2							
Day 16: 25 mg FSH-P Day of estrus: 1,000 IU HCG	6	2	2	1	0	1	0
Trial 3							
Day 16: 12.5 mg FSH-P Day of estrus: 1,000 IU HCG	6	1	2	3	0	0	0

but reduced LH, gave variable results. The incidence of double or triple ovulations was only one in six while two of six animals were found with no corpora lutea on their ovaries following treatment.

Treatment in Trial 3 resulted in 50 percent of the heifers having double ovulations with no excessive ovarian stimulation.

Table 6 depicts the number of follicles greater than 12 mm in diameter on the day of estrus and subsequent number of ovulations in each of the treatment groups. With the high level of 25 mg FSH and 2,000 IU HCG, a large proportion of the follicles developed on day of estrus ovulated. This resulted in an abnormally high incidence of ovulations in one of the treated heifers. It seemed apparent in the subsequent two treatments that even though considerable numbers of follicles had developed from the 25 and 12.5 mg FSH treatment, the proportion of these follicles which ovulated was limited by the reduced dosage level of HCG utilized. This is further illustrated in Table 7. The 2,000 IU dose of HCG resulted in 86 percent ovulation of the follicles developed on the day of estrus. This is higher than the ovulatory rate (73%) observed in Trials 2 and 3 utilizing 1,000 IU HCG.

An apparent variable number of follicles on the day of estrus was observed in Trial 2 which consisted of 25 mg FSH in comparison with the even distribution of ovulatory follicles detected in Trial 3 which utilized only 12.5 mg

TABLE 6

OVULATORY FOLLICLE NUMBERS ON DAY OF ESTRUS AND OVULATION RATE IN RELATION TO THREE GONADOTROPIN TREATMENTS

Animal Numbers:	Follicle numbers on day of estrus Treatments: I.	II.	III.	Ovulation numbers I.	II.	III.
220	4	5	2	3	4	1
032	3	1	2	2	1	1
503	2	2	0	2	2	0
$2F_4G$	3	1	2	3	1	2
32	3	2	3	2	0	2
91	1	0	2	1	0	2
67	5	-	-	5	-	-

TABLE 7

PERCENTAGE OF FOLLICLES GREATER THAN 12 mm ON DAY OF ESTRUS THAT SUBSEQUENTLY OVULATED

	Total no. follicles in treated animals	Total no. ovulations in treated animals	Percentage
Trial 1 25 mg FSH plus 2,000 IU HCG	21	18	86
Trial 2 25 mg FSH plus 1,000 IU HCG	11	8	73
Trial 3 12.5 mg FSH plus 1,000 IU HCG	11	8	73

FSH. It could be postulated that this irregularity was caused by some minor antigenic activity alteration in follicle numbers due to the high levels of FSH used in Trial 1. Even so, the elevated dosage of FSH in Trial 2 had no effect on the distribution of ovulatory follicles observed in Trial 3.

Although ovulation numbers were not affected by any refractory response, standing estrus became less evident. The observance of standing heat was less apparent in the cycle following Trial 1 and completely absent after Trial 2 forcing estrus detection by the presence of vaginal discharge and rectal palpation. It may be speculated that repetitive gonadotropin administration can upset the delicate hormonal balance in the cycling heifer and reduce the incidence of behavioral estrus. Exogenous gonadotropic sources, specifically LH sources such as HCG, may have an inhibitory effect on estrogen release from the mature follicles thus reducing the intensity of standing estrus.

The results of Phase I indicate that multiple ovulations can be achieved with restricted levels of gonadotropins.

The data agree with earlier results (Bellows et al., 1969) which indicate that dosage levels in excess of 12.5 mg FSH-P result in overstimulation of the ovary. Refractoriness observed when PMS was repeatedly used (Willet et al., 1952a; Hafez et al., 1964; Jainudeen et al., 1966) was not observed with repetitive FSH-P administration.

Finally, these data agree with Slyter et al., (1965) who reported no refractory effects due to frequent injections of HCG.

PHASE II

ESTRUS SYNCHRONIZATION, OVULATION INDUCTION, AND SLAUGHTER DATA COLLECTION

Materials and Methods

These trials were conducted from June through August 1972. The experimental animals consisted of six heifers and one cow utilized in Phase I. The animals were maintained on grass pasture throughout the synchronization procedures and supplemented with mixed hay when necessary. During ovulation induction and breeding, heifers were confined to the feedlot and maintained on mixed hay.

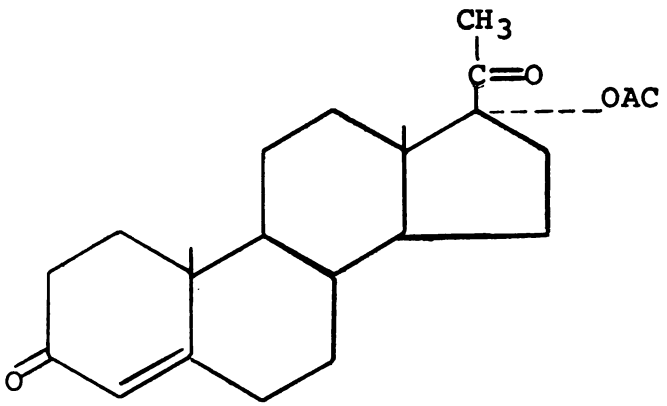
Two progestins were used as synchronizing agents. The structure of each is shown in Figures 1 and 2.

Prodox (17α-hydroxyprogesterone-17 acetate) was administered to individual heifers at the rate of 50 mg per head daily for seven consecutive days. A stock solution of the progestin was prepared by dissolving 2.8 gm Prodox in 140 ml of corn oil. Each dose consisted of a 2.5 ml injection in the rump region.

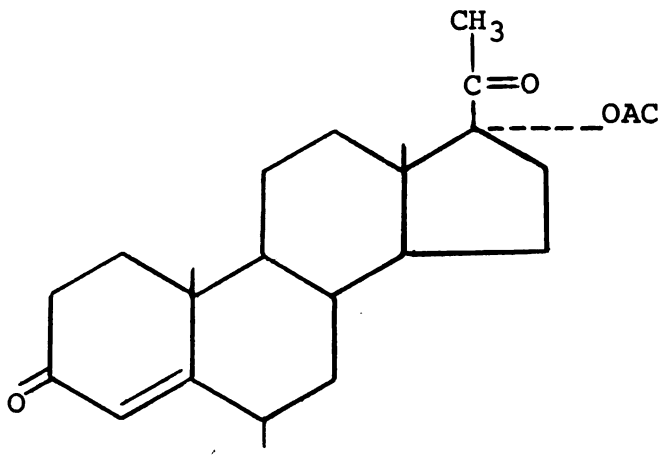
All animals were observed twice daily during progestin administration and 3 times daily for 14 days following hormone withdrawal.

Four weeks after the final Prodox injection, Provera (17α-hydroxy-6α-methylprogesterone acetate) was administered at the rate of 5 mg per head daily for 14 days. A standard solution of Provera was prepared by dissolving 525 mg of the progestin in 262.5 ml corn oil. The injection

Synchronizing Agents



Prodox
Figure 1. 17α-hydroxyprogesterone-17 acetate



Provera
Figure 2. 17α-hydroxy-6α-methylprogesterone acetate

dosage was 2.5 ml and was given in the same area as described for Prodox. Estrus observations were similar to those made during Prodox administration.

The post synchronized estrual cycle developed from the Provera was utilized in the final gonadotropin treatment which occurred 158 days after the initial gonadotropin treatment in Phase I.

The final treatment consisted of either 25 or 12.5 mg FSH-P (i.m.) on day 16 of the cycle. On day 18 of the cycle and each day thereafter, heifers were checked for standing estrus by being exposed to a proven Charolais bull and observed for one hour. On the day of scheduled or exhibited estrus, all heifers received 1,000 IU HCG (i.m.) and were allowed to mate naturally. On the day of HCG administration, each heifer was palpated. Because the animals were to be killed for tract recovery, palpation time was kept minimal. However, each ovary was palpated to determine the presence of mature follicles.

All heifers were killed at a local packing company 7 to 10 days after scheduled ovulation. The reproductive tracts were recovered, tagged, placed in damp towels, and transported to the Endocrine Research Unit within one hour of killing. The ovaries were removed and described relative to the shape, number and size of follicles, and number and size of existing corpora lutea. The length and width of each ovary was measured.

Oviducts were removed and flushed with 5 ml of 0.15 M

NaCl (in a 5 ml syringe, 24 gauge needle) into an 85 mm watchglass. The needle was inserted through the infundibular portion of the oviduct. Immediate examination of the flushings was made under a dissecting microscope.

Uterine horns were flushed with 25 ml of 0.15 M NaCl into individual 95 x 15 mm Petri dishes. Recovery procedures utilized a 50 ml syringe and 14 gauge needle directed from the tubouterine junction toward the oviduct. Flushings were immediately examined with a dissecting microscope for the presence of ova.

Recovered ova were removed from the medium by micropipette and transferred to a 75 x 25 mm glass slide. The slide was mounted on a Wild Heerbrugg inverted microscope with camera attachment. The presence of an ovum was documented with 8.5 x 10.5 cm black and white film.

Results and Discussion

Phase II of the study was designed to study the effect of various gonadotropin levels on ovulation rate following earlier progesterone administration. In addition, development of a successful ovum recovery technique for large animals was desired.

Estrus synchronization procedures were carried out in an effort to achieve three objectives: (1) to determine if progestin administration followed by withdrawal could induce a more observable estrus; (2) to identify any effects of previously administered exogenous gonadotropins on the effectiveness of the synchronized estrus; (3) to determine

if progestin sources facilitate gonadotropin treatment.

The results of the two progestin treatments are summarized in Table 8. The effectiveness of Prodox was poor, with only one of seven animals demonstrating any evident signs of standing estrus within eight days after termination of treatment. Copious vaginal discharge similar to that observed during estrus and normal cyclic ovarian activity was observed in one of the heifers during Prodox administration. This particular progestin is experimental and positive results have not been reported in literature.

It is evident that the ineffectiveness of Prodox was due to either inadequate daily levels of progestin or the period of administration. Studies have shown that reducing the levels of dihydroxyprogesterone acetophenide (DHPA) from 400 mg daily to 75 to 100 mg has rendered the progestin inadequate in synchronizing estrus (Wiltbank et al., 1967; Tripathi and Howel, 1969). Results with melengestrol acetate (MGA) show reduced effectiveness when the compound is administered for only 10 days in comparison with 14 day administration (DeBois and Bierschwal, 1970; Britt and Ulberg, 1970).

Provera resulted in four of seven heifers demonstrating evidence of estrus within seven days while all animals demonstrated indications of estrus within ten days of progestin withdrawal. Standing estrus was observed in only three of seven heifers. Estrus was confirmed in all animals by the

TABLE 8

TWO SYNCHRONIZATION TREATMENTS AND
THE EFFECTIVENESS OF EACH

Progestin	Animal Numbers	Treatment	Effectiveness
17α-Hydroxyprogesterone-17 acetate (Prodox)	7	50 mg daily (i.m.) for 7 days	1/7 in estrus within 8 days
17α-Hydroxy-6α-Methyl-Progesterone acetate (Provera)	7	5 mg daily (i.m.) for 14 days	4/7 in estrus within 7 days 7/7 in estrus within 10 days

presence of a clear vaginal discharge on the day of estrus and the observance of metestrus bleeding within three days after estrus. Normal ovarian activity was noted at this synchronized estrus.

It is suggested from these data, and in conjunction with the treatments and results obtained in Phase I, that repetitive gonadotropin administration of FSH-P and HCG, while having little effect on ovulation, may reduce the incidence of standing estrus. In addition, estrus synchronization with progestins is only partially effective in counteracting this effect and inducing a typical, prominent estrus.

Gonadotropin treatment levels and ovulation rates in the post synchronized estrual cycle are shown in Table 9. One ovary of one of the heifers was lost in the killing procedure and was excluded from the trial.

Observance of recent corpora lutea on the recovered ovaries demonstrated that animals receiving 25 mg FSH-P ovulated 2, 2, and 5 ova respectively while heifers receiving 12.5 mg ovulated 0, 3, and 2 ova. Increased levels of FSH, although producing a high incidence of double ovulations, again resulted in overstimulation of the ovaries of one of the experimental animals.

Ovarian size and weights are recorded in Table 10. No observable ovarian activity was noted upon examination of the ovaries of heifer number 91. These ovaries were reduced in size and weight in comparison to the majority of those

TABLE 9

OVULATION ACHIEVED IN PRESYNCHRONIZED GONADOTROPIN TREATED HEIFERS

Animal Number	Treatment on Day 16	Treatment on Day of Estrus	Ovulation Numbers
67	25 mg FSH-P	1,000 IU HCG	2
$2F_4G$	25 mg FSH-P	1,000 IU HCG	2
032	25 mg FSH-P	1,000 IU HCG	5
91	12.5 mg FSH-P	1,000 IU HCG	0
32	12.5 mg FSH-P	1,000 IU HCG	3
220	12.5 mg FSH-P	1,000 IU HCG	2

TABLE 10

OVARIAN SIZE AND WEIGHTS OF GONADOTROPIN TREATED HEIFERS

Animal Number	Left Ovary		Right Ovary	
	Size (cm)	Weight (gm)	Size (cm)	Weight (gm)
Treatment 1				
67	3.5 x 2.0	4.29	3.5 x 3.5	10.55
$2F_4G$	3.0 x 2.5	4.82	3.0 x 3.0	7.45
032	4.0 x 3.0	8.50	3.5 x 3.0	11.10
Treatment 2				
91	3.5 x 3.0	4.35	2.5 x 2.0	8.80
32	3.5 x 2.5	10.00	3.5 x 3.5	12.22
220	3.5 x 2.0	7.50	4.5 x 3.5	18.70
Average	3.6 x 2.6	6.58	3.4 x 3.1	11.47

recovered from other treated animals.

There does not seem to be a relationship between amounts of FSH-P administered and subsequent ovarian size and weight. Right ovaries were heavier as reported by Asdell (1955), but the measured dimensions of both the right and left ovary suggest no differences in total size.

Ovarian weights for both ovaries are reduced when compared to results from earlier studies. Asdell (1955) reported individual ovaries weigh 15 to 20 gm while McDonald (1969) reported a range of 10 to 20 gm. Although control animals were not available, it is possible that the frequency of gonadotropin administration in our study reduced ovarian mass.

The number and dimension of corpora lutea on left and right ovaries of gonadotropin treated heifers are reported in Table 11. There was a definite increase in the percentage of corpora lutea on the right ovary in comparison to the left. This confirmed earlier reports citing 60 to 65 percent of ovulations from the right ovary (Asdell, 1955; Salisbury and Vandemark, 1961; McDonald, 1969).

There was no significant difference in mean corpus luteum size between ovaries. Corpora lutea ranged from 0.4 to 1.5 cm with a mean size of 0.9 cm based on 14 ovulations. Earlier studies have reported a mean mature corpus luteum size of 2.0 to 2.5 cm on day 10 of the estrual cycle (Asdell, 1955; McDonald, 1969). The reduced corpus luteum size may have been due to immaturity of the corpus, the younger age

TABLE 11

NUMBER AND SIZE OF CORPORA LUTEA ON LEFT AND RIGHT OVARIES OF GONADOTROPIN TREATED HEIFERS

Animal Number	Left Ovary		Right Ovary	
	No. of CL	Mean Size (cm)	No. of CL	Mean Size (cm)
67	0	-	2	1.25
$2F_4G$	1	.40	1	.50
032	2	1.25	3	.77
91	0	-	0	-
32	2	1.00	1	1.00
220	0	-	2	.88
Percent of total CL	35.7		64.3	
Mean CL size	0.98		0.89	
Total Mean CL size	.93			

of the cattle, and/or the gonadotropin treatment.

Follicle numbers of various sizes on each ovary are shown in Table 12. The number of follicles was variable with no evidence of a correlation between administered FSH levels and total number of follicles. This was equally true for follicles one cm or more in diameter. Laster (1972) demonstrated a significant increase in numbers of follicles greater than one cm with increased FSH-P. This dose-response was not significant with follicles 0.5 cm or greater.

Reduced follicle numbers were observed in animal 91 which failed to ovulate following treatment. Even so, heifer 032 with few follicles had several fresh corpora lutea on the ovarian perimeter.

The follicle destined to ovulate in the cycling cow is 1.0 to 1.2 cm in diameter (Rajakoski, 1960; Marion et al., 1968). A total of 15 ovulatory follicles within this range were observed in 4 of the 6 heifers on days 7 to 10 of the cycle. Follicular development during the subsequent cycle would not be so advanced as to produce follicles of such dimensions. It can thus be postulated that these follicles were unovulated remnants of the gonadotropin treated cycle. This supports earlier discussion which speculated that reduced ovulation rate and increased incidence of double and triple ovulations were due to the decreased amounts of administered LH.

Ova recovery rate was less than 10 percent. One of 14

TABLE 12

NUMBER OF FOLLICLES OF VARIOUS SIZES
ON THE LEFT AND RIGHT OVARIES

Animal Number	Ovary	Follicle Size in cm 1.5	1.0	0.75	0.50	<0.50	Total
67	Right	-	-	-	4	6	10
	Left	-	-	-	5	12	17
	Total	-	-	-	9	18	27
$2F_4G$	Right	2	2	-	2	4	10
	Left	-	-	1	2	14	17
	Total	2	2	1	4	18	27
032	Right	1	-	1	3	2	7
	Left	2	-	-	1	-	3
	Total	3	-	1	4	2	10
91	Right	-	-	1	0	1	2
	Left	-	-	-	3	0	3
	Total	-	-	1	3	1	5
32	Right	2	-	-	4	19	25
	Left	-	1	-	-	22	23
	Total	2	1	-	4	41	48
220	Right	-	3	-	4	3	10
	Left	-	2	-	1	-	3
	Total	-	5	-	5	3	13

ova was recovered from the right oviduct of animal 220 and is shown in Figure 3. The ovum resembled fertilized ova at a similar age and stage of development recovered from the horse (Oguri and Tsutsumi, 1972).

Recoveries of ova from gonadotropin treated cows range from 25 to 75 percent (Dowling, 1949; Avery and Graham, 1962; Hafez et al., 1963; Rowson and Moor, 1966). Optimum time for ovum recovery is two to six days after estrus or LH administration (Foote and Onuma, 1970). Because mature corpora lutea were to be observed in this study, slaughter and ovum recovery occurred 7 to 10 days after LH administration. Consequently, most of the ovulated ova had entered the uterus and were difficult to recover.

This phase of the study supported earlier results demonstrating that low levels of gonadotropins can produce restricted ovulation. The effectiveness of such treatment was not influenced by earlier repeated gonadotropin administration. Results indicated an inverse relationship between frequency of gonadotropin administration and corpora lutea size.

Estrus synchronization was not totally effective in increasing the incidence of overt estrus that is lost following frequent gonadotropin use.

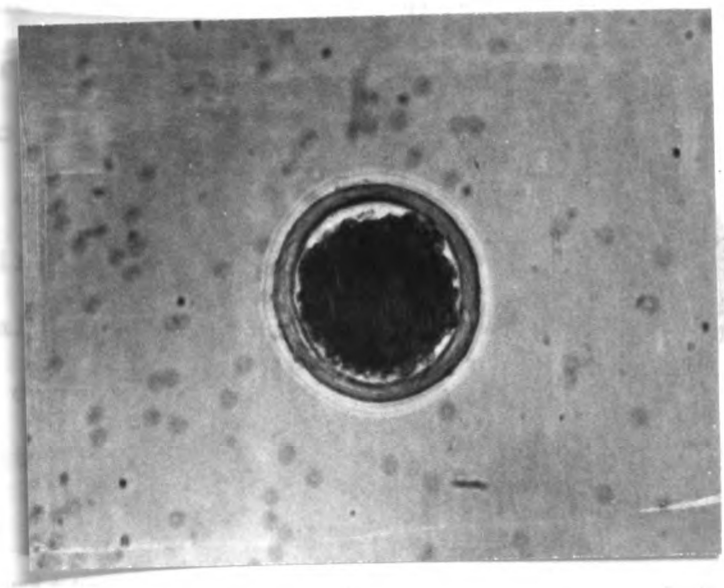


Figure 3. Ovum recovered from right oviduct (Magnified 200x)

PHASE III

OVULATION INDUCTION WITH LOW DOSE GONADOTROPINS FROM A NON-SYNCHRONIZED ESTRUS

Materials and Methods

Phase III, conducted from September through October 1972, used 38 pluriparous cows experiencing their first or second estrus after parturition. None of the animals had previously received gonadotropin or other hormone treatment. All cows were checked for estrus two or three times daily. Estrus was confirmed in all animals by standing heat, the mounting of other animals, and/or the presence of clear vaginal discharge.

Two groups of control cows were utilized. Control Group I consisted of a random group of 15 cows demonstrating their first postpartum estrus. Ovarian data were collected by rectal palpation on day of observed heat and on the seventh day after estrus.

Control Group II consisted of 15 cows selected randomly that had experienced at least one estrus prior to being admitted to the experiment. Three cows used in Control Group I were also entered in Control Group II. These animals were palpated three to five days prior to scheduled estrus, on the day of the second observed estrus, and on the seventh day following estrus.

Sixteen of the postpartum cows were assigned to a treated group and received 12.5 mg FSH-P on day 16 and an

ovulatory dose of 1,000 IU HCG on the day of estrus. Five cows in this group were previously included in Control Group I. All treated cows were rectally palpated on day 16 of the cycle, on the day of estrus, and on days 3 and 7 following estrus.

Thirty of the cows used in Phase III were utilized in a subsequent study by another investigator. Estrus was synchronized in one to three cycles following the use of the cows in the treated or control groups of Phase III. Animals were artificially inseminated and then exposed under pasture conditions to 5 bulls for approximately 90 days.

Results and Discussion

Ovulation results of control and gonadotropin treated postpartum cows are presented in Table 13. Nine of 15 (60%) of the cows in Control Group I ovulated at the first postpartum estrus. Mean ovulation rate for all cows was 0.6 while the average for those cows ovulating was 1.0.

In Control Group II, 10 of 15 (66.6%) ovulated with 9 of 15 producing one ovulation. One cow produced a double ovulation. The mean ovulation rate was 0.73 and the mean of those cows ovulating 1.10. Ovulation rate between the first and second estrus control groups was not significant.

Of the 16 gonadotropin treated cows, 9 (56.3%) had double ovulations. Eleven of 16 (68.7%) had 2 or 3 corpora lutea on their ovaries on day 7. One of the 16 treated animals failed to ovulate and no excessive ovarian stimulation was observed. Mean ovulation rate for all cows was

TABLE 13

OVULATION DATA ON CONTROL AND GONADOTROPIN TREATED POSTPARTUM COWS

First estrus controls-I		Second estrus controls-II		12.5 mg FSH-P-1,000 IU HCG	
Animal Number	Ovulation Number	Animal Number	Ovulation Number	Animal Number	Ovulation Number
85	1	77	1	19	2
5	1	80	2	10	2
16	1	52	1	47	1
30	0	68	0	20	1
27	1	57	0	26	2
17	1	14	0	73	3
12	0	75	1	11	0
77	0	101	1	31	2
81	1	15	0	62	1
65	0	109	1	45	2
68	1	44	0	30	1
57	0	7	1	9	2
76	1	107	1	1	3
9	1	93	1	27	2
1	0	78	1	65	2
				81	2
Cows ovulating=9/15 (60%)		Cows ovulating=10/15 (66.6%)		Cows ovulating=15/16(93.7%)	
Mean ovulation rate .60		Mean ovulation rate .73		Mean ovulation rate 1.75	
Mean of those cows ovulating 1.0		Mean of those cows ovulating 1.1		Mean of those cows ovulating 1.87	

1.75 and the mean of cows ovulating was 1.87.

Fertility was not measured at the gonadotropin treated estrus. Fertility was measured in 30 of the cows one to three cycles following palpation of control or gonadotropin treated animals. First service conception rates for treated and control groups are presented in Table 14. Of the 16 control cows in which fertility was measured, 5 (31.2%) failed to conceive at first service. Fertility was measured in 14 of the gonadotropin treated cows. Three of 14 (21.6%) failed to conceive at first breeding. Statistical analysis demonstrated no significant difference between fertility in the treated group and fertility achieved in the combined control groups.

The results of Table 14 also indicate that the time interval from gonadotropin administration to breeding was not related to the ability of the cow to conceive at first service.

Phase III demonstrated the effectiveness of low level gonadotropin in inducing a high incidence of double and triple ovulations on a large scale in postpartum cows. Earlier gonadotropin treatments were found to have no effect on conception rate, even as early as the cycle following gonadotropin administration.

TABLE 14

FERTILITY IN CONTROL AND EARLIER GONADOTROPIN TREATED COWS AS MEASURED BY FAILURE TO RETURN TO ESTRUS AFTER ARTIFICIAL INSEMINATION

Control Groups		Treated Group		
Animal Number	Failure to return to estrus	Animal Number	Time Interval from treatment to breeding (days)	Failure to return to estrus
5	+	81	19	+
16	+	27	20	+
17	+	30	21	+
27	+	1	22	+
44	+	9	22	+
57	+	20	26	+
68	+	73	26	-
77	+	26	28	+
78	+	10	29	+
80	+	31	29	+
107	+	19	35	-
12	-	47	37	-
76	-	45	38	+
93	-	62	38	+
101	-			
109	-			

SUMMARY AND CONCLUSIONS

A practical, reliable gonadotropin regime was developed to induce a high incidence of double and triple ovulations in the beef cow. In addition, the study was designed to determine the effects of repetitive gonadotropin administration on ovulation. Estrus synchronization was utilized in portions of the study to determine the effects of progestins in combination with gonadotropins. The following conclusions resulted from the data obtained:

1. low level administered gonadotropins, specifically 12.5 mg FSH-P on day 16 and 1,000 IU HCG on day of estrus, resulted in 68.7 percent of the treated cows achieving double or triple ovulations;
2. fertility of cows, mated as early as the first cycle following low dose gonadotropin treatment, was not affected;
3. FSH-P levels greater than 12.5 mg resulted in overstimulation of the ovary;
4. although the number of ovulations was not affected by repeated gonadotropin administration, the incidence of standing heat was reduced;
5. previous repetitive gonadotropin treatment was not a factor in determining the effectiveness of

exogenous progestins;

6. synchronizing agents were only partially effective in inducing overt signs of estrus lost following frequent gonadotropin administration;
7. mean corpus luteum size in animals frequently receiving gonadotropins was reduced when compared to values cited in literature;
8. the right ovary was consistently heavier and produced more ovulations than the left ovary.

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LITERATURE CITED

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APPENDIX A

LAPAROSCOPY OF THE COW

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LAPAROSCOPY OF THE COW

The laparoscopy technique was attempted in the cow to assist in confirmation and documentation of ovarian activity detected by rectal palpation. Laparoscopy has been successfully used in many species including human and nonhuman primates, rabbits, rodents, and, more recently, the pig. Five attempts were made to laparoscope individual cows at the Endocrine Research Unit facilities in February 1972.

The laparoscope apparatus included a 135 degree pediatric laparoscope, 5 mm in diameter, a trocar-cannula, a fiber optic cable, and a Model 4000 projector light source (Richard Wolf, GMBH, 7134 Knittlingen, West Germany).

Cows were placed in a squeeze chute and lightly tranquilized (i.m.) with Sparine (Promazine Hydrochloride, Wyeth Laboratories, Inc.) at 1.2 mg/kg of body weight. The site of the laparoscope insertion was between the last rib and the external angle of the ilium. The hair in the area of incision was clipped and the area was washed with Zephiran (benzalkonium chloride). Xylocaine (2%) (Lidocaine Hydrochloride, Jensen-Salsbery Laboratories), a local anesthetic, was injected into the skin and underlying tissues

in the immediate incision area. A 2 cm incision was made, the trocar-cannula inserted, followed by insertion of the laparoscope through the cannula. Observations were facilitated by admitting 5 percent CO_2/air into the abdominal cavity.

Unsuccessful attempts indicated that the inadequate length of the laparoscope was preventing observation of internal structures. This was confirmed in another attempt in which rectal palpation and laparoscopy were employed simultaneously. The ovary was grasped and moved to the lateral abdominal wall in an effort to position it in proximity to the inserted laparoscope. Observation of the ovary was unsuccessful and the end of the laparoscope was not detected protruding through the inner abdominal wall.

Although limited success was achieved in this study, the potential of laparoscopy may be beneficial in the study of ovarian activity in the smaller or younger bovine animal.

APPENDIX B

PUBLICATIONS BY THE AUTHOR

APPENDIX B

PUBLICATIONS BY THE AUTHOR

Controlled Ovulation in Beef Using FSH and HCG. D. E. Wildt and W. Richard Dukelow. J. Anim. Sci. 35:1125.

Control of Ovulation in Squirrel Monkeys. R. M. Harrison, D. E. Wildt, and W. R. Dukelow. Fed. Amer. Soc. Exp. Biol. Proceedings, Vol. 32; March 1973.

APPENDIX C

ABSTRACTS

CONTROLLED OVULATION IN BEEF USING FSH AND HCG[1]

D. E. Wildt and W. Richard Dukelow[2]

The objective of this study was to determine the effects of porcine-FSH and low dosages of HCG in inducing multiple ovulation in mature Angus, Angus-Hereford, and Angus-Holstein heifers. All animals were observed for two estrous cycles before treatments were begun. The number of animals and the treatments employed were as follows: (I) 7, 25 mg FSH-P, 2,000 IU HCG; (II) 6, 25 mg FSH-P, 1,000 IU HCG; (III) 6, 12.5 mg FSH-P, 1,000 IU HCG. The heifers were rectally palpated on day 16 of the cycle and injected with a single dose of FSH-P (i.m.) on that day. The animals were checked for estrus twice daily. They were rectally palpated and injected (i.m.) with HCG on the day of scheduled estrus (or earlier if signs of estrus were evident prior to the 21st day). The heifers were then palpated daily for 3 days. The number of ovulations, the number of animals with twin ovulations, and the number of

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[1]Presented at the Midwestern Section-American Society of Animal Science Meetings, Chicago, Ill., Nov. 24-25, 1972.

[2]Endocrine Research Unit (Ctr. Lab. Animal Res., Depts. An. Hus. & Physiol.).

animals excessively stimulated (>3 ovulations) for each group were as follows: (I) 2.6, 3/7, 1/7; (II) 1.3, 1/6, 1/6; (III) 1.3, 3/6, 0/6. The above data demonstrate that controlled multiple ovulations may be produced with restricted levels of FSH-P and HCG and that the desirable twin ovulation objective may be attained without excessive ovarian stimulation. (This work supported by NIH Research Career Development Award No. 1-K4-HD35, 306-01 and the Michigan Agricultural Experiment Station.)

CONTROL OF OVULATION IN SQUIRREL MONKEYS[1]

R. M. Harrison, D. E. Wildt,
and W. Richard Dukelow[2]

Ovulation induced in squirrel monkeys with progesterone-FSH-HCG can be inhibited by megestrol acetate (MA) injections. We studied MA administration by silastic implants, disposition *in vivo*, and rate of release. The effectiveness of MA implants depends on induction pretreatment and time *in situ*. Monkeys receiving progesterone pretreatment ovulated at a lower rate (2/8; 25%) than those not receiving progesterone (12/20; 60%). Monkeys with implants 11 days prior to FSH ovulated at a rate of 62% (10/16) compared to 33% (4/12) for those with implants 29 days. Using MA-H^3 implants, concentration of the progestin was highest in ovaries and oviducts, less in adrenals, uterus and vagina, and not found in the pituitary. *In vivo* release rate was determined with 40 implants in 20 monkeys. To determine *in vitro* release rate, each implant was incubated in 10 ml of TC-199 at 37°C for 5 weeks. One ml

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[1]Presented at the Fed. of Amer. Soc. for Exp. Biol. Meeting, Atlantic City, N.J., April 16-20, 1973.

[2]Endocrine Research Unit (Ctr. Lab. Animal Res., Depts. An. Hus. & Physiol.).

aliquots were removed and replaced daily and implants were transferred each week to fresh medium. The *in vivo* release rate was 56 ± 12 mg/day, whereas the *in vitro* rate was 13 ± 5 mg/day. *In vitro* release was influenced by build-up in the medium within 48 hours, and the rate was not linear over the five week period.

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