EFFICACY OF AN EQUINE PITUITARY EXTRACT TO SUPEROVULATE COWS

By

Myron Lindle Danner

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

1981

G-115742

ABSTRACT

EFFICACY OF AN EQUINE PITUITARY EXTRACT
TO SUPEROVULATE COWS

By

Myron Lindle Danner

Three trials utilizing sixty-one animals were conducted to study the efficacy of a commercially available equine pituitary extract (trade name--Pitropin; Biological Specialties, Middletown, Wisconsin) for inducing multiple ovulations in beef cows. Variables included: dose of equine pituitary extract (EPE), addition of HCG, number of days for injections and number of injections per day. A comparison of EPE with pituitary FSH and LH of domestic animal origin (FSH-LH) was made. Estrous cycles of cows within each trial were synchronized with 2 injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) given 11 days apart. Gonadotropin injections began on day 12 of the cycle (0 = day of estrus) followed 72 hr later by $PGF_{2\alpha}$. Multiple inseminations were performed starting 12 hr after onset of estrus. Cows were slaughtered 7 days postestrus and reproductive tracts were removed for study.

EPE was a very potent stimulus for follicular growth and ovulation although variation between animals was high. The range in number of ovulations for EPE treated cows was 0 to 96. Addition of HCG to EPE treatments was contraindicated. To be effective, a total dose of 750 Fevold-Hisaw Rat Units of EPE administered over at least 3 consecutive days was necessary. Seventy-five percent of cows receiving this

treatment responded with ≥ 5 ovulations. Once daily injections of EPE were adequate. FSH-LH decreased the number of unovulated follicles ≥ 10 mm (0.8 vs 3.1) and increased the number of embryos recovered (11.2 vs 6.2) over EPE treated cows.

Blood samples were taken during the gonadotropin treatment period and the relationship between serum concentration of progesterone and estradiol 17β and the resulting ovarian response was determined. Cows having ≤ 3 ovulations were classified as a poor ovulatory response while those having ≥ 5 ovulations were classified as a good ovulatory response. High progesterone level during the period from initiation of gonadotropin injection to $PGF_{2\alpha}$ injection was associated with a good ovulatory response. No relationship between progesterone level following $PGF_{2\alpha}$ injection or estradiol 17β concentration during the gonadotropin treatment and the resulting ovulatory response was observed. A positive correlation between the progesterone level on day 7 postestrus and the number of corpora lutea present was observed.

ACKNOWLEDGMENTS

The author is grateful to the members of his Graduate Committee: Dr. W. D. Oxender, Veterinary Medicine; Dr. R. L. Fogwell, Dairy Science; Dr. W. R. Dukelow, Dr. D. R. Hawkins, Dr. H. D. Ritchie, and Dr. R. H. Nelson, Animal Husbandry. Their guidance in planning and conducting this investigation and critique of this dissertation is deeply appreciated.

Sincere appreciation is expressed to the Animal Husbandry Department, Dr. R. H. Nelson, Chairman, for providing facilities and research animals with which to work. The excellence of both facilities and cattle enhance the value of this dissertation.

Appreciation is extended to the Dairy Science Department, Dr. H. D. Hafs, Chairman, for the use of facilities and equipment to complete hormone assays.

Gratitude is expressed to Dr. R. D. Baker, American Embryos, Middleville, Michigan, for assistance in evaluation of embryos; to Dr. W. T. Magee and Dr. J. L. Gill for guidance with statistical analysis; to the graduate students of the Animal Husbandry and Dairy Science Departments for assistance in data collection; to the personnel of the Beef Cattle Research Center for care of experimental animals; and to Mrs. Grace Rutherford for her excellent typing of this manuscript.

Sincere thanks are expressed to the author's wife, Kathy, for her help and support throughout the graduate program. Special appreciation is also extended to the author's parents.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Early developments in embryo transfer techniques were stimulated by the increased financial gains through multiplying exotic breeds of cattle. Present interest in embryo transfer is stimulated by newly developed techniques which can be used for genetic improvement by progressive cattle breeders. Recent improvement of nonsurgical techniques for collection of embryos has decreased chances of causing sterility in genetically valuable donor cows. In combination with superovulation, nonsurgical collection techniques make it possible to obtain 5 to 25 embryos from a single collection. This procedure dramatically increases the number of offspring which can be obtained from females genetically superior for production of meat or milk. Furthermore, potential exists for the import and export of embryos rather than live animals reducing transportation costs and the risk of spreading diseases.

Artificial insemination (A.I.) has made leading sires available to all breeders at a reasonable cost. Given total use of superior sires available through A.I., considerable genetic progress can be realized over use of average natural service sires. However, only 50 percent of the genotype for any calf is from the sire. This leaves 50 percent of the genotype contributed by the dam and consequently, a heretofore untapped resource for genetic improvement of livestock. Embryo transfer techniques may increase the potential for genetic

improvement from superior females by decreasing the generation interval (ova can be collected from young females) and increasing the number of calves per female.

Gordon (1975), in a review of embryo transfer in cattle, concluded that superovulation must be regarded as a major problem blocking progress in expanding the use of embryo transfer. Extreme variation and lack of repeatability in superovulatory responses of donor females are just two problems cited by numerous investigators. Improved methods for superovulation in donor females are necessary to increase the use of embryo transfer.

The purpose of this dissertation research was to study superovulation in cows using equine pituitary gonadotropin extract (EPE). Specific objectives in this study were:

1. To determine the dosage and treatment schedule for administration of EPE to give optimal superovulation response in beef cows.
2. To determine the relative efficacy of EPE and pituitary extracts of domestic animal origin to induce multiple ovulations.
3. To determine relationships between serum hormone concentrations (progesterone and estradiol 17β) throughout the gonadotropin treatment period and the resulting ovarian response.

LITERATURE REVIEW

Superovulation in Cows

The ultimate objective of superovulation is to increase the number of normal fertile eggs or embryos per donor. The basic principle is to stimulate extensive follicular development through intramuscular or subcutaneous administration of a preparation with follicle-stimulating hormone (FSH) activity at levels in excess of normal endogenous levels. Preparations utilized with FSH activity include pregnant mares' serum gonadotropin (PMSG) and cattle, sheep, swine or horse pituitary extracts. In addition, a preparation is occasionally injected intravenously or intramuscularly to help assure ovulation. Such preparations include pituitary luteinizing hormone (LH) or human chorionic gonadotropin (HCG)

In a review of superovulation, Gordon (1975) summarized data of 436 sexually mature cattle in six studies. Cows had been treated with both pituitary extracts and PMSG. He found that the average number of eggs released was 18.1 ± 8.1. Of these, 9.8 ± 5.1 (54%) were recovered and 4.3 ± 0.9 (44% of eggs recovered) were considered to be fertilized.

In 1974, Graham reported the results from seven embryo transfer units. Of the donors treated, 73.5% responded to the gonadotropin treatment, yielding an average of 8.2 ova. Of the collected ova, 65.5% were fertilized, giving an average of 5.3 per

donor that were considered transferable. Of those, only 2.2 per donor resulted in pregnancies. The number of recipient pregnancies from one recovery attempt ranged from 0 to 32.

In a more recent review, Betteridge (1977) summarized data from 1,343 donors in 19 studies. The range in the average number of ovulations per flushed donor from all studies was from 3.5 to 18.5 with a mean of 10.2. The number of embryos and unfertilized ova recovered varied from 2.6 to 12.3 with a mean of 6.2 (61% of ovulations). The range in number of fertile embryos recovered was from 2.0 to 9.6 with a mean of 4.7 (76% of eggs recovered).

In studying superovulation, it becomes obvious that large variation exists in the number of eggs released, recovered, and fertilized. A number of factors which have been suggested as possible sources of variation will be discussed. These are:

- Nature of gonadotropin,
- Dose-response relationship,
- Batch of gonadotropin and method of administration,
- Time of estrous cycle to initiate gonadotropin treatment,
- Interval between gonadotropin treatment and estrus,
- Population of ovarian follicles,
- Endocrine environment during superovulation,
- Immunological response,
- Breed differences,
- Seasonal effects, and
- Nutritional effects.

Nature of Gonadotropin

In 1939, Fevold showed that among the mare, the sow, the ewe, and the cow, the pituitary of the cow is lowest in FSH, and the pituitary of the mare is nearly ten times richer in FSH than the pituitary of either the sow or the ewe. The relative FSH and LH levels in these species are shown in Figure 1. Among the four animals mentioned above, the ewe pituitary is the highest in LH, followed by that of the sow, the mare, and the cow, in that order.

Although critical data comparing pituitary extracts from different species are lacking, cattle, sheep, pig, and horse pituitary extracts have all been used with success to superovulate cattle (Gordon, 1975). Because of their short half-lives, pituitary extracts have to be administered daily or twice daily for periods of up to five days. Another commonly used gonadrotropin has been PMSG which has a longer half-life. With PMSG, the normal treatment has been to administer a single dose usually between 1500 and 3500 I.U.

Limited data comparing pituitary extracts to PMSG do exist. In sheep there is firm evidence that a crude horse pituitary extract of FSH (HAP) can be superior to PMSG in achieving effective high superovulation (Moore and Shelton, 1962, 1974; Shelton and Moore, 1967). However in cattle this has not been confirmed. Dowling (1949) observed an average ovulation rate of 12.0 for PMSG treated cows and 6.5 for HAP but recovered a higher number of fertilized eggs with HAP. Gordon (1975) reported an average ovulation rate of 19.3 with PMSG and 7.3 with HAP.

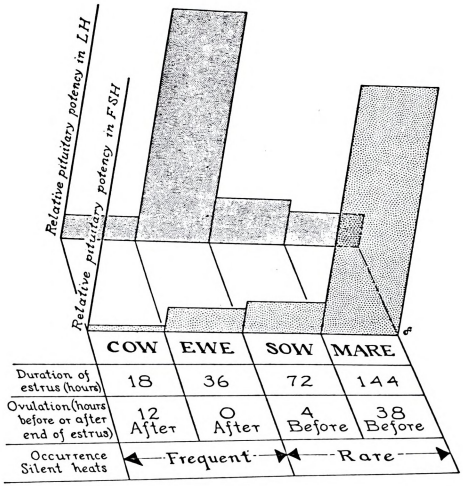


Figure 1. The relative FSH and LH potency of the pituitary in the cow, ewe, sow, and mare. *(Taken from Salisbury and VanDemark, 1961.)*

Comparing PMSG to HAP, Moore (1975a) found both to be equally effective in superovulating mature cows. In heifers there was little difference in ovulatory response due to PMSG or HAP, however HAP resulted in three to four times as many large unovulated follicles. Moore concluded that due to availability and need for fewer injections, PMSG provided a simpler treatment to obtain fertilized cow eggs than HAP.

Pituitary follicle stimulating hormone of domestic animal origin (FSH) has been more extensively studied than HAP. FSH is available commercially and marketed in 50 mg lots according to an Armour standard. It is quite common to use a treatment consisting of a 5:1 mixture of FSH and pituitary luteinizing hormone (LH) (Elsden et al., 1976). Elsden et al. (1978) compared FSH-LH to PMSG and observed more corpora lutea, ova and pregnancies in cows treated with FSH-LH than in cows treated with PMSG. Mean numbers of corpora lutea, ova and pregnancies were 6.2, 2.0 and 1.2 for PMSG and 11.4, 7.9 and 4.2 for FSH-LH, respectively.

Hasler (1978) observed no significant differences in numbers of corpora lutea, ova recovered, fertilized ova or pregnancy rate following transfer of fertilized ova after treatment of cows with FSH-LH or PMSG. However, in cows considered to be infertile, those treated with FSH-LH produced more corpora lutea and fertilized ova than cows treated with PMSG. Number of corpora lutea were 11.9 ± 9.6 vs 4.9 ± 2.4 while number of fertilized ova were 4.4 ± 8.0 vs 0.6 ± 0.9 for FSH-LH and PMSG, respectively. In an investigation comparing

efficacy of PMSG to FSH for production of twins, Laster (1973) indicated that FSH may have greater potential to induce high numbers of ovulations than PMSG.

Dose-Response Relationship

A definite dose-response relationship has been demonstrated for PMSG and pituitary extracts. Sreenan and Beehan (1976) injected three levels of PMSG and observed the mean ovulation response. PMSG dose levels (IU) were 1500, 2000 and 2500, which resulted in 7.8 ± 1.4, 12.1 ± 2.3 and 13.1 ± 3.9 ovulations, respectively. Percentage of ova recovered was 65, 56 and 51 for the three levels, respectively. There was a tendency for a lower proportion of ova recovered at the high level of PMSG.

Moore (1975a) gave PMSG or horse pituitary extract (HAP) at three dose levels. There was a significant linear effect of PMSG and HAP on the numbers of CL and numbers of follicles. Percentage of eggs recovered decreased as dose of gonadotropin increased. However, the fertilization rate was consistent across treatments.

Combined results of eight studies utilizing repeated injections of FSH are presented in Table 1 (Bellows et al., 1969). As dose level increased, ovulation rate increased, as did the range in number of ovulations per heifer. Ova recovery and fertilization rates were lower at the higher dose levels.

Gordon (1975) observed that the higher the PMSG level, the greater the variability in response. He reported that at PMSG dose levels of 1000 IU and even 2000 IU, some proportion of cattle may not

TABLE 1. EFFECTS OF DOSE LEVEL OF PORCINE FSH ON OVULATION, RECOVERY AND FERTILIZATION RATES

Total Dose[a] (mg eq)	Number Animals	Ovulations		Ova	
		Mean	Range	Recovered	Fertilized[b]
				%	%
3.1	8	1.1	1-2	88	93
6.2	18	2.2	1-6	98	95
10.0	81	2.6	0-8	--[c]	--[c]
12.5	13	6.5	1-14	79	75
25.0	11	14.0	1-32	48	79
50.0	4	17.8	13-25	58	52

[a]MAP fed 180 mg/day for 11 days, first MAP feeding = day 1; 5 mg estradiol injected day 2; gonadotropin injected 2X/day on days 8, 9, 10, 11 and 12.

[b]Includes all cleaved ova.

[c]Data not available.

be induced to superovulate. However at 3000 IU, practically all cattle can be expected to release additional eggs, but the variation may be enormous. Gordon found that response to 3000 IU PMSG varied from one to 112 ovulations. The work of Newcomb et al. (1979) supports a dose-response relationship.

Batch of Gonadotropin and Method of Administration

It has been suggested that the superovulatory effect of PMSG can vary with the particular batch employed. Stewart et al. (1976), using rat testis radioreceptor assays to measure FSH and LH activity,

showed the FSH:LH ratio to remain constant (about 1:5) in unextracted serum from six different mares throughout the period between days 40 and 80 of gestation. They also tested six batches of commercially available PMSG and found no significant difference in the FSH:LH ratios. Further, using the same assayed batches to superovulate cattle and sheep, they found no significant variation in mean ovulation rates between groups. They concluded that variation between animals in response to PMSG is unlikely to be due to differences in the FSH:LH ratio of the preparation used.

Similarly, using rat testis radioreceptor assays, Newcomb et al. (1979) found no significant differences in the FSH:LH ratio of three different batches of PMSG. When administered to heifers, no differences were found in ovarian response from the three batches. Gordon (1975) also showed little difference in ovarian response of cows from two different batches of PMSG.

In contrast, Humphrey et al. (1979) observed FSH activity was significantly higher in serum of pregnant mares at 60 and 90 days of gestation when compared to days 45 or 120. In Shorthorn cows, high FSH/LH ratio PMSG induced more cows to ovulate than low FSH/LH ratio PMSG (88% vs 50%). Addition of HCG to medium ratio PMSG reduced ovulatory success to 37%. They concluded that both high and low FSH/LH ratio PMSG could induce follicular activity; however preparations with high FSH/LH PMSG were more conducive to the induction of ovulation.

The way in which PMSG is administered to the cow on day 16 of the cycle can markedly affect the ovulatory response. When a dose of 2500 IU was given in low volume (2.5 ml) by intramuscular injection, the superovulatory effect was substantially greater than when the same dose was given in a 25 ml volume subcutaneously (Gordon, 1975).

Time of Estrous Cycle to Initiate Gonadotropin Treatment

Most early superovulation treatments were initiated on day 16 of the cow's cycle to coincide with the follicular phase. However, the development of $PGF_{2\alpha}$ and progestogens for controlling the time of ovulation has opened new possibilities of beginning superovulation earlier in the cycle. The ability to control the time of ovulation has numerous advantages in a commercial embryo transfer program.

Elsden et al. (1974) compared responses of cows treated with PMSG during the mid-luteal phase of the cycle followed by $PGF_{2\alpha}$ 48 hr later to response of cows given PMSG on day 16 of the cycle. All cows given PMSG followed by $PGF_{2\alpha}$ ovulated with the mean ovulation rate being 13.2 ± 1.9. Of cows treated with PMSG alone on day 16, 50% responded with 8.0 ± 1.5 ovulations, 20% did not ovulate but had cystic follicles and 30% did not respond. These results suggest that the use of PMSG together with $PGF_{2\alpha}$ was superior to PMSG alone in terms of the proportion of animals ovulating and the higher ovulation rates achieved in the animals which responded. In agreement are Seidel et al. (1975) and Nelson et al. (1976) who observed animals brought into estrus by $PGF_{2\alpha}$ treatment after PMSG had higher

superovulation rates than those treated with PMSG on day 16 of the natural cycle.

Ford and Stormshak (1978) investigated gonadotropin-induced follicular development and ovulation during the three-day period after the cow had ovulated spontaneously. Treatment of heifers with PMSG failed to stimulate follicular growth during metestrus, as determined by palpation.

Phillippo and Rowson (1975) compared the ovulatory response of cows whose treatment was begun during different days of the cycle. Cows were grouped into the following periods: days 3-7, 8-12, and 13-16. The percentage responding with three or more ovulations were 37.5, 77.6 and 55.5, respectively. Responses of cows treated prior to day 8 were considerably lower. Sreenan (1976) also demonstrated that treatment initiated during the mid-luteal phase (days 8-12) gave higher ovulation rates and yields of embryos than treatment begun earlier.

Newcomb et al. (1979) injected cows with PMSG from days 9-12 and observed no systematic effect of day on response. They concluded that when PMSG is administered during the mid-luteal phase, after day 8, there is no significant effect of day of treatment on response.

Interval Between Gonadotropin Treatment and Estrus

It has been reported that following the use of PMSG during the follicular phase of the cycle, a definite relationship exists between the mean percentage of follicles ovulating and the time

interval separating PMSG and estrus. Gengenbach et al. (1978), utilizing various combinations of $PGF_{2\alpha}$ and PMSG, grouped animals according to ovulation rates and observed significant differences in the interval to estrus. Heifers with the highest ovulation rates tended to have the longest interval to estrus. Two heifers which did not show estrus until 120 and 144 hrs after treatment had 16 and 19 ovulations, respectively.

Sreenan and Beehan (1976) also reported that the longer the interval, the higher the proportion of total ovarian response that is represented as ovulations. Animals with an interval from PMSG to estrus of 3 days had the lowest percentage of ovulations with 59%. Those with an interval of 4 days ovulated 80% while animals with an interval of 5-7 days ovulated 97% of stimulated follicles.

Using various combinations of PMSG and $PGF_{2\alpha}$, Henricks and Hill (1978) recorded the days from PMSG to estrus and the number of ovulations. The treatment group having the least days from PMSG to estrus, 2.7, also had the fewest ovulations, 2.3. Treatment groups averaging 4.2 and 5.3 days from PMSG to estrus produced 5.7 and 4.6 ovulations, respectively.

In contrast, Lopez-Barbella et al. (1979) found an increasing interval from PMSG to observed estrus coincided with a decrease in ovulation rate (72 hrs, 5.50 ± 1.29 CL vs 97 to 144 hrs, 0.67 ± 0.82 CL). Furthermore, Moore (1975a) observed the time elapsing between treatment with PMSG or HAP and the onset of estrus in 140 mature cows. No apparent effect upon ovarian response was seen. However, mean number

of corpora lutea was 2.9 while large unovulated follicles averaged 3.7. This is a very poor response and could suggest subfertility of experimental animals.

Using PMSG followed by HCG, Hafez et al. (1963) observed that the longer the interval from PMSG to HCG injection, the greater the percentage of follicles that ovulated. They concluded that at least 5 days should elapse between PMSG and HCG injections for a high ovulation percentage.

Betteridge (1977) reviewed work covering superovulation of prepuberal calves. The most successful treatment regimen consisted of inserting vaginal sponges impregnated with 60 mg fluorogestone acetate (FGA) at the time of PMSG treatment and leaving them in place for 4 days. The 4 days of FGA blocked ovulation until day 6. Beginning 41 hrs after sponge withdrawal, 75.3% of 93 calves averaged 13.9 ovulations each and they were grouped within a 20-hr ovulatory period. Without FGA the time span over which ovulations occurred was prolonged.

Population of Ovarian Follicles

During fetal life in cattle, the definitive stock of oocytes is constituted which will be used during the entire sexual life. At the end of fetal life, follicle growth cycles succeed one another, causing constant formation of graafian follicles which disappear by atresia. It is only following puberty that regular estrous cycles and ovulation commence. However, follicular growth and atresia continue to take place throughout the reproductive life cycle of the cow.

Sreenan and Beehan (1976) have suggested that changes in the ovarian population of follicles could affect the response to superovulation. Rajakoski (1960) characterized changes taking place in the ovarian follicular system during one cycle in sexually mature heifers. He observed that during the bovine sexual cycle, follicles ≥ 5 mm diameter go through two growth phases. The first of these occurs during the third and fourth days of the cycle and gives an increased number of medium-sized follicles and a single large follicle which undergoes atresia during the eleventh and twelfth days of the cycle. A second similar growth wave appears between the twelfth and fourteenth days of the cycle and leads to the development of a large follicle which attains maturity during the first and second days of the subsequent cycle and then ovulates. Rajakoski (1960), however, stated that there was tremendous variation due to individual differences.

In a similar study Cahill et al. (1979) studied ovarian follicular populations in two breeds of ewes which differed in their ovulation rates. Mean ovulation rate for Romanov ewes was 3.1 while Ile-de-France averaged 1.4 ovulations. The researchers observed half as many small follicles but 1.5 to 2.0 times more large follicles in the ovaries of the Romanov ewes compared to those of Ile-de-France ewes. They concluded that the higher ovulation rate in the Romanov ewe is due to the greater number of large follicles available to be stimulated for ovulation.

Follicles ≥ 5 mm diameter generally are held to be responsive to gonadotropin stimulation. Changes in the population of these follicles as demonstrated by Rajakoski (1960) could very likely explain some of the variation in individual response to superovulation.

Endocrine Environment During Superovulation

Betteridge (1977) has described knowledge of the endocrinology of superovulation as fragmentary and disputed. Understanding hormonal interrelations in cows with induced multiple ovulations may provide a key to obtaining greater precision in response.

Numerous researchers have shown circulating estrogen levels rise tremendously and at estrus may be three to four times higher in superovulated compared to untreated cattle (Lemon and Saumande, 1972; Henricks et al., 1973; Hallford, Turman, Wetteman and Pope, 1975). Booth et al. (1975) found a temporary decline in circulating estrogen levels followed by a secondary rise around days 5 and 6 post estrus when levels were eight times those found in normally cycling animals. They fell to normal levels by day 12. Spilman et al. (1973) also observed secondary peaks of estrogen in superovulated calves.

Estrogen concentrations have been positively correlated with the subsequent number of CL (Henricks et al., 1973; Henricks and Hill, 1978). However it is also possible, as observed by Booth et al. (1975), that the high estrogen levels can be due to large numbers of unovulated follicles > 15 mm diameter. Using prepuberal

calves, Spilman et al. (1973) found circulating estrogen levels are greatly elevated before ovulation. Levels were well correlated with the degree of follicular development but not with the number of ovulations. Gengenbach et al. (1978) reported no relationship between estradiol concentrations prior to estrus and the number of CL formed.

In rabbits it is known that estrogen can accelerate the transport of eggs in the oviduct (Harper and Change, 1971). It therefore seems likely that, in the superovulated cow, the high levels of estrogen which occur after ovulation, could modify the motility of the oviduct and uterus causing premature transport of eggs into the uterus or expulsion into the vagina. It is also possible that high levels of estrogen may bring about premature shedding of the zona pellucida, which would lead to a subsequent degeneration of the egg (Dickman, 1969).

A steep rise in the plasma progesterone of superovulated heifers after day 2 has been found by numerous workers (Booth et al., 1975; Spilman et al., 1973; Gengenbach et al., 1978; Henricks and Hill, 1978; Henricks et al., 1973). Levels as high as 60-100 ng/ml have been reported. Lopez-Barbella et al. (1979) observed a coefficient of correlation of 0.62 between number of CL and plasma progesterone level 13 days after estrus.

Gengenbach et al. (1978) conducted a study to determine if differences in plasma progesterone concentration at the time of PMSG administration affected the variability of ovulatory response to PMSG.

They reported that duration of increased plasma progesterone concentrations, particularly following the highest dose of PMSG, seemed to be more important in determining ovarian response than changes in progesterone prior to PMSG treatment. Five of eight heifers treated with 2000 IU of PMSG having low progesterone concentrations before the end of the 84 hr post-treatment period, averaged 1.6 corpora lutea and 4.6 large follicles and returned to estrus 51.4 hrs after treatment. The heifers with higher progesterone concentrations during this period averaged 12.3 corpora lutea and three large follicles and returned to estrus after 120 hrs.

Avery et al. (1962) pretreated cows with progesterone for 10 days prior to superovulating them with FSH. Cows receiving pretreatment with progesterone produced an average of 7.9 more ovulations than cows not receiving prior progesterone treatment.

Lopez-Barbella et al. (1979) grouped cows according to number of corpora lutea (CL): 0 to 1; 2 to 3; and greater than 3. LH, progesterone and estrogen changes with time were similar in all groups following PMSG treatment, although progesterone and estrogen concentrations were higher in cows with a larger number of CL. In a similar manner, Solti et al. (1978) studied the plasma progesterone level at the time of PMSG administration and the subsequent number of corpora lutea. No correlation was found.

Spillman et al. (1973) state that PMSG leads to release of endogenous LH within 24 to 48 hrs of injection. This was not the

experience of others (Henricks et al., 1973; Lemon and Saumande, 1974; Saumande and Pelletier, 1975; Hallford, Turman, Wetteman and Pope, 1975) who describe no LH peaks before the one coinciding with estrus. Hallford et al. (1979) failed to detect a relationship between plasma LH and reproductive criteria after PMSG treatment.

Betteridge (1977) in reviewing superovulation of prepuberal calves found no evidence that PMSG alone induced an immediate release of endogenous LH into the circulation. Instead, it resulted in a short-lived peak of 3 to 4 ng/ml for up to 8 hrs between 108 and 132 hrs after PMSG which is considered insufficient to bring about ovulation. The use of FGA improved results by leading to a much greater LH release for 8 to 16 hrs with peak values of 11 to 72 ng/ml 12 to 20 hrs after FGA withdrawal. This matches LH release in normally cycling adult cows and was sufficient to lead to multiple ovulation some 20 hrs later. There was also an FSH peak coincident with the major LH peak.

Immunological Response

Seidel et al. (1978) have discussed the possibility of immunological response to repeated injections of gonadotropins which may limit the number of times a donor may be superovulated. Both PMSG and FSH are proteins and, therefore, potential inducers of anaphylaxis. This antigenicity also implies that repeated injections may stimulate the production of antigonadotropins which may inhibit subsequent responses or perhaps even interfere with endogenous gonadotropins.

Jainudeen et al. (1966) studied the use of repeated injections of gonadotropins to superovulate cows. Multiple ovulations were obtained in cows after the first PMSG injection. A larger dose of PMSG injected 5 to 7 months later also produced a similar ovulatory response; however, the same dose of PMSG repeatedly injected at subsequent estrous cycles failed to stimulate the ovaries. Using immature intact female rats they assayed antigonadotropic activity in the blood serum of treated cows. They found the level of anti-gonadotropic activity was low prior to the second PMSG injection but increased with successive treatments and attained maximal values 16 days after the fourth PMSG injection. Antigonadotropins in PMSG-treated cows inhibited the follicular stimulating properties of PMSG, but had no adverse affect on follicular development and ovulation resulting from endogenously secreted gonadotropins. They concluded that repeated therapeutic doses of PMSG failed to induce multiple ovulations in the cow and the failure was due to the presence of antigonadotropins. These results are in accord with the findings of Willet et al. (1953) and Havez et al. (1964).

Similarly, Hallford et al. (1979) observed three of six cows previously treated with PMSG failed to ovulate and none ovulated more than one egg when PMSG was administered on day 17 or days 5 and 17 of the previous cycle.

Newcomb et al. (1979) also observed fewer ovulations but no reduction in the number of follicles after a second PMSG treatment to cows. The mean interval between the two treatments was 51 days.

Turman et al. (1978) conducted a study to determine if PMSG injections given one year may adversely affect the superovulatory response of cows to PMSG injections the following year. Treatment with PMSG the previous year reduced the superovulatory response of cows to PMSG. Cows that had never been previously treated had a significantly greater ovulation rate (5.3 vs 1.8), a wider range in ovulations (1-16 vs 0-5) with more cows ovulating four or more eggs (45% vs 9%) than did cows that had been previously treated.

Seidel et al. (1978) have not encountered any difficulties with immunological response but concede that data on this problem are scarce. They suggest that the possibility of endocrine pathology may be reduced if the donor cow is allowed to carry a pregnancy after several superovulation treatments.

Breed Differences

There is evidence of a breed response relationship, with beef breeds showing a greater sensitivity to PMSG than dairy breeds. Sreenan and Beehan (1976) have reported higher mean ovulating responses in Hereford and Angus beef cattle as compared with Freisians, all treated with a standard dose of PMSG (3000 IU) in the follicular phase of the cycle. Likewise, Mariana et al. (1970) reported a higher ovulation response in Charolais than in Freisian cattle following 1600 IU PMSG.

Further breed differences in responsiveness to PMSG are recorded by Shea et al. (1976), 178 Simmental, 79 Limousin, 54 Chianina and 93 Maine Anjou donors averaged 15.2, 13.6, 12.4

and 9.9 ovulations, respectively. The Maine-Anjou ovulation rate was significantly ($P \leq .05$) poorer than for other breeds.

Seasonal Effects

Gordon (1975) presented evidence that seasonal fluctuations in ovarian response to PMSG did exist. Using 3000 IU PMSG on day 16 of the cycle, he observed the highest average number of ovulations (17.9) and total follicle development (CL + large follicles, 26.8) in the period from February to April. Remaining periods of the year were similar to each other with responses of: November to January, 10.0 ovulations and 17.5 CL + large follicles; May to July, 9.5 ovulations and 15.0 CL + large follicles; and August to September, 8.9 ovulations and 12.2 CL + large follicles.

Subsequent data have not supported the existence of seasonal variations very well, however. In a study of 582 cows, data reported by Shea et al. (1976) showed similar responses in all quarters of the year. Sreenan and Beehan (unpublished, reported in Betteridge, 1977) also observed nearly equal ovulation responses during different seasons of the year.

Rajokoski (1960) observed the total number of follicles < 5 mm diameter varied for the seasons with the lowest mean total, 73.1 per heifer, during the autumn and a distinct increase during the winter and spring to 118.5 and 116.5 per heifer, respectively. However, there was no systematic seasonal variation in the number of follicles with a diameter ≥ 5 mm.

Nutritional Effects

Nutrition is known to have dramatic effects upon reproductive processes in cattle. Inadequate rations for growing heifers result in both reduced body weight and increased age at puberty (Wiltbank et al., 1969). Restricted diets can also increase the post-partum interval in mature cows (Dunn et al., 1969).

Ovulation rate in sheep is known to be affected by nutrition (Ensminger, 1970). It is widely held that flushing, the practice of feeding ewes more generously during the period of 2 to 8 weeks immediately prior to breeding, will result in a 15 to 20% increase in the lamb crop. Level of nutrition has also been demonstrated to affect the ovarian response to PMSG in ewes (Allen and Lamming, 1961).

Similarly in swine, gilts on a high energy ration have a greater number of ovulations than those on a low energy ration (Sorenson et al., 1961; Self et al., 1955; Zimmerman et al., 1960). However it has also been shown that a higher percentage of live embryos at 40 days following breeding was obtained on gilts fed the low energy ration (Sorenson et al., 1961).

A study was undertaken by Staigmiller et al. (1979) to examine the effects of undernutrition on ovarian response to exogenous gonadotropin. Mature cows were fed a high or low level (130% and 70% of NRC requirements for TDN) ration for 92 days prior to being superovulated with FSH. The number of large follicles and CL at 3 days post estrus was correlated with estimated body condition, being higher in cows with more condition. However, neither ovulation rate nor fertilization rate differed between cows in the high or low TDN groups.

Summary

A major factor which limits success of embryo transfer is large variation in numbers of ovulations after treatments imposed to cause superovulation. The most widely used gonadotropin has been PMSG. A batch-to-batch variation has been suggested to exist but evidence to support this is not conclusive. Prior to the development of $PGF_{2\alpha}$, PMSG was administered as a single injection on day 16 of the natural cycle. However, since then it has been shown that initiation of gonadotropin treatment during the mid-luteal phase, after day 8, of the cycle followed by $PGF_{2\alpha}$ 48 to 72 hrs later gives superior results to treatments begun earlier or during the follicular phase of the cycle. This appears to be the treatment of choice.

Recently there has been much interest in the use of pituitary extracts. Limited studies comparing PMSG to FSH have suggested that FSH may be superior to PMSG for induction of multiple ovulations.

A dose-response relationship has been shown to exist. As dose levels of gonadotropin increase, ovulation rate increases. However the variation from animal to animal also increases. Furthermore, as ovulation rate increases, recovery and fertilization rates tend to decrease. An optimum response has been described by Seidel et al. (1978) as one in which each ovary has five to ten ovulations.

A relationship has been shown to exist between the mean percentage of follicles ovulating and the time interval separating PMSG injection and estrus. A time period of 5 days has been suggested as optimal to allow follicular maturation prior to the LH surge. High

progesterone levels are important during the first 3 days of gonadotropin treatment to block LH release and prevent premature ovulations. An immediate decrease of progesterone is then necessary to insure rapid onset of estrus and endogenous release of LH.

Estrogen concentration has been shown to be correlated with ovarian response, however extremely high levels have been suggested to be detrimental to egg transport and fertilization. Progesterone concentration several days following estrus has been positively correlated with ovulation rate.

Differences in populations of ovarian follicles at the time of initial gonadotropin treatment could explain much of the variation in response to superovulation. However, at present, methods of determining populations of ovarian follicles are impossible, and as a consequence, little can be done to incorporate knowledge of their status into a superovulation regimen.

Development of an immunological response to repeated injections of gonadotropins has been demonstrated. It has been suggested that this problem may be overcome by allowing a cow to carry a pregnancy after several superovulation treatments.

Further variation in superovulation response can result due to breed differences, although this is probably not a major factor. A seasonal variation in response has been suggested but evidence to support this is inconclusive.

Ovulation rate in sheep and swine can be increased with elevated energy intake. Even though this relationship has not been

shown in cattle; past experience with the effects of nutrition on reproduction dictates that properly balanced rations adequate in TDN be fed.

Numerous factors have been shown to affect the response to superovulation but individual variation always remains. At the present time, conditions can be defined to optimize the ovarian response but precise control over ovulation rate is not possible.

MATERIALS AND METHODS

Experimental Animals

The cows used in Trial 1 originated from the Lake City Experiment Station breeding project. Cattle in this herd are of four types: Herefords--unselected or selected for growth rate, Charolais x Hereford x Angus, and Holstein x Hereford x Angus. This herd is managed on a spring calving basis. Twenty-seven cows diagnosed as non-pregnant at the end of the breeding season in 1978 with anatomically normal reproductive organs ranging in age from 2 to 9 years were selected for Trial 1. Five were heifers which had failed to conceive and 22 were parous cows 5 to 8 months post-partum. All animals utilized were in good body condition and had been vaccinated for brucellosis, leptospirosis, vibriosis and IBR.

Charolais crossbred virgin heifers originating from northern Michigan were purchased in April 1979 at approximately 12 months of age for use in Trial 2. They were in thin body condition and many had not reached puberty at the time of their purchase. All had been calfhood vaccinated and upon arrival were vaccinated for leptospirosis, vibriosis and IBR. Serum progesterone concentrations were monitored until 26 heifers were found to be cycling. Reproductive organs of the heifers were palpated to eliminate any with genital abnormalities. These heifers were approximately 16 months of age at the start of the experiment.

In Trial 3, eight Charolais crossbred virgin heifers were used. They had previously been involved in a nutrition study at the MSU Beef Cattle Research Center. Age, origin and vaccination history were unknown.

Feeding

All cattle were housed at the Beef Cattle Research Center (BCRC). Daily feeding and management were performed by BCRC personnel.

Cattle on Trial 1 were fed a 100% corn silage ration. The silage had been treated with anhydrous ammonia at the time of ensiling and was balanced for calcium, phosphorous and salt with a mineral supplement. Vitamins A and D were provided. The ration contained 10.9% crude protein (DM basis). Additionally, cows received 200 mg monensin per head per day.

For 3 months prior to the start of Trial 2, Charolais cross heifers received a 60% corn silage, 40% concentrate ration containing 13.3% crude protein (DM basis). One week before starting the experiment, the ration was increased to 85% concentrate, 15% corn silage (13.0% crude protein, DM basis). The rations were balanced for calcium, phosphorous and salt. Vitamins A and D were provided. All heifers received 200 mg monensin per head daily.

Heifers in Trial 3 were fed a 60% corn silage, 40% concentrate ration containing 11.3% crude protein (DM basis). The ration was balanced for calcium, phosphorous, salt and Vitamins A and D were provided. No monensin was fed.

Products Used

Equine pituitary extract (EPE) was obtained from Biological Specialties, Middleton, Wisconsin and originated from one lot. The manufacturer's description is given in Appendix Table A1. Further, a bioassay (Fevold and Hisaw, 1934) is completed by the manufacturer for each lot processed. The equivalent of 125 Fevold-Hisaw Rat Units of gonadotropic hormones are packaged per 5 ml vial. The results of the bioassay for Lot No. 1803 are presented in Appendix Table A2.

Human Chorionic Gonadotropin (HCG) was also obtained in a single lot from Biological Specialties. The manufacturer's description is given in Appendix Table A3.

Pituitary FSH and LH of domestic animal origin were purchased from Reheis Chemical Company, Kankakee, Illinois. The manufacturer's analysis for FSH used in this experiment is given in Appendix Table A4, while analysis for LH is reported in Appendix Table A5.

Prostaglandin $F_{2\alpha}$ (Lytalyse®) was obtained through the courtesy of Dr. James Lauderdale, The Upjohn Company, Kalamazoo, Michigan. A dose of 25 mg of $PGF_{2\alpha}$ was injected intramuscularly.

EPE, HCG and FSH-LH are packaged in sterile, lyophilized form. Reconstitution was completed no more than 2 hr prior to administration.

Preliminary Treatment and Allotment

Estrous cycles of cattle within each trial were synchronized using two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) given 11 days apart. At the time of the second $PGF_{2\alpha}$ injection, a blood sample was taken from each animal in Trials 1 and 2 for determination of serum

progesterone concentration. Cattle in all trials were then observed for estrus three times daily during the 6 day period following injection of $PGF_{2\alpha}$.

Cattle in Trial 1 were blocked by age and randomly allotted to treatment groups. Cattle in Trials 2 and 3 were randomly allotted to treatments. Due to the large number of animals in Trials 1 and 2, equal numbers from each treatment were grouped for slaughter on each of three successive days. This was necessary since a maximum of 10 reproductive tracts could be processed in one day. Cattle in Trial 3 were slaughtered on the same day.

Slaughter dates were as follows:

Trial 1: November 7, 8, 9, 1978

Trial 2: August 13, 14, 15, 1979

Trial 3: October 31, 1979.

Experimental Design

In order to establish a starting point for dosage of equine pituitary extract (EPE), a preliminary study was conducted. Two cows treated with 750 Fevold-Hisaw Rat Units of EPE responded with 2 to 4 ovulations based on rectal palpation. Since one of the objectives of Trial 1 was to find the upper limit of a workable dose range for EPE, dosages were set higher than perceived necessary. Total doses of EPE were: 2250 and 4500 Fevold-Hisaw Rat Units.

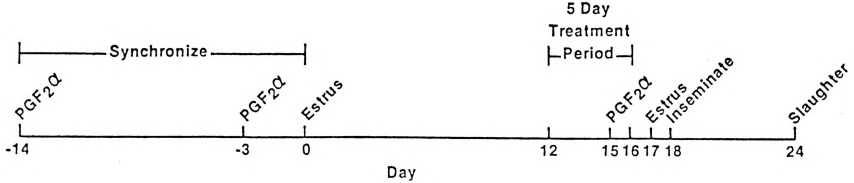
Trial 1 consisted of four groups of cows given the low or high dose of EPE with or without HCG. A fifth group served as controls. Daily doses of EPE (Fevold-Hisaw Rat Units) and HCG (IU) were divided

into two equal amounts and injected subcutaneously at 12 hr intervals for five days beginning on day 12 postestrus (0 = day of estrus). Daily doses were: low EPE = 750, 500, 500, 250, 250; high EPE = 1500, 1000, 1000, 500, 500; and HCG = 0, 1000, 1000, 500, 500 for days 12, 13, 14, 15 and 16, respectively.

Prostaglandin $F_{2\alpha}$ was injected intramuscularly at 60 hr in control and 72 hr in EPE-treated cows, after initiation of gonadotropin injections on day 12. Two, three, and one straws of frozen semen were inseminated 12, 24, and 36 hrs after estrus was first detected but starting no later than 60 hrs after prostaglandin $F_{2\alpha}$ was injected. Cows were slaughtered seven days postestrus and reproductive tracts were removed for study. The experimental design for Trial 1 is shown graphically in Figure 2.

Trial 2 consisted of four groups of heifers (n = 4/group) receiving a low or high dose of EPE for 3 days injected subcutaneously once daily (1X) or divided equally and administered at 12 hr intervals (2X). A fifth group received the low dose for 2 days injected once daily (n = 5). A sixth group received a 5:1 mixture of follicle stimulating hormone and luteinizing hormone (FSH-LH) of domestic animal origin, injected in equal daily doses at 12 hr intervals for 5 days (n = 5).

Injections began on day 12 of the estrous cycle. Daily doses of EPE (Fevold-Hisaw Rat Units) were: low EPE--3 days = 375, 250, 125; high EPE--3 days = 750, 500, 250; and low EPE--2 days = 375, 375. The dose rate of FSH-LH was based on mg of FSH (Armour standard) and was



Treatments	n
1. Low dose EPE	6
2. Low dose EPE + HCG	6
3. High dose EPE	6
4. High dose EPE + HCG	5
5. Controls	4

Dosages			
	EPE (Fevold-Hisaw Rat Units)		
Day	Low	High	HCG (IU)
12	750	1500	0
13	500	1000	1000
14	500	1000	1000
15	250	500	500
16	250	500	500

Figure 2. Experimental design for Trial 1.

given subcutaneously twice daily as follows: 5, 5, 4, 4, 3, 3, 2, 2, 2, 2 mg.

Prostaglandin $F_{2\alpha}$ was injected 72 hr after initiation of gonadotropin injections on day 12. Insemination and necropsy procedures were the same as those used in Trial 1. The experimental design for Trial 2 is shown graphically in Figure 3.

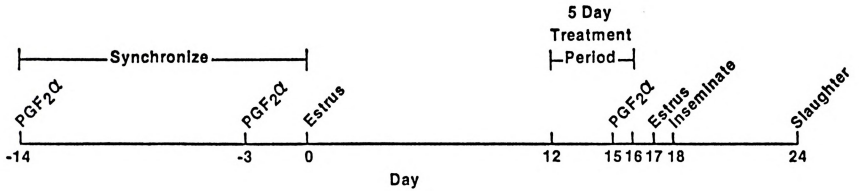
Trial 3 consisted of 2 groups of heifers receiving equal total doses of EPE administered at constant or variable daily doses. EPE was injected once daily for 5 days beginning on day 12 of the estrous cycle. Daily doses of EPE (Fevold-Hisaw Rat Units) were: constant EPE = 250, 250, 250, 250, 250 (n = 4); and variable EPE = 375, 250, 125, 125, 375 (n = 4).

Syncro-Mate-B[1] was administered on day 11 postestrus, 24 hr prior to initiation of gonadotropin treatments. Implants were removed 72 hr following the initial EPE injection. Insemination and necropsy procedures were the same as those described for Trial 1. The experimental design for Trial 3 is shown in Figure 4.

Blood Collection Procedures

In Trials 1 and 2, 20 ml blood samples were taken for quantification of progesterone and estradiol-17β. All samples were taken from the jugular vein using an 18 gauge needle and 20 ml disposable syringe. Blood was transferred to 16 x 100 mm disposable culture

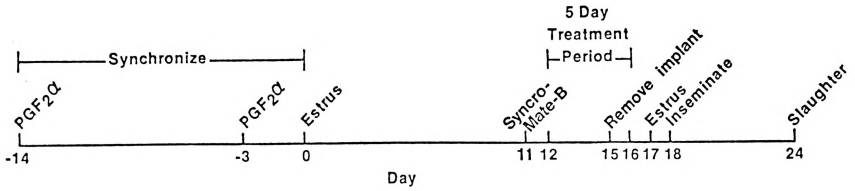
[1]Syncro-Mate-B is a product of Searle Agriculture, Inc., which consists of an implant containing 6 mg of norgestomet, placed subcutaneously on the back of the ear, and an intramuscular injection of 3 mg norgestomet and 6 mg estradiol valerate.



Treatments	n
1. Low dose EPE - 1X - 3 days	4
2. Low dose EPE-2X - 3 days	4
3. High dose EPE-1X - 3 days	4
4. High dose EPE-2X - 3 days	4
5. Low dose EPE-1X-2 days	5
6. FSH-LH (5:1 mixture)	5

	Dosages			
	EPE (Fevold-Hisaw Rat Units)			
Day	Low-3 day	High-3 day	Low-2 day	FSH (mg)
12	375	750	375	10
13	250	500	375	8
14	125	250	0	6
15	0	0	0	4
16	0	0	0	4

Figure 3. Experimental design for Trial 2.



Treatments	n
1. Constant dose EPE	4
2. Variable dose EPE	4

	Dosages	
	EPE (Fevold-Hisaw Rat Units)	
Day	Constant	Variable
12	250	375
13	250	250
14	250	125
15	250	125
16	250	375

Figure 4. Experimental design for Trial 3.

tubes and left at room temperature for 1 to 2 hr. Tubes were then stored in a refrigerator for 24 to 48 hr before being centrifuged (3000 g) for 15 min. Serum was poured into 12 x 75 mm disposable culture tubes. The tubes were capped and stored at -20°C until ready for hormone assays.

A sample of jugular venous blood was taken at the time of the second $PGF_{2\alpha}$ injection during synchronization of estrus. Blood samples were taken every 12 hr for a 132 hr period starting at the time of the first gonadotropin injection. An additional blood sample was taken at the time of slaughter. Figure 5 shows the schedule for sampling of blood.

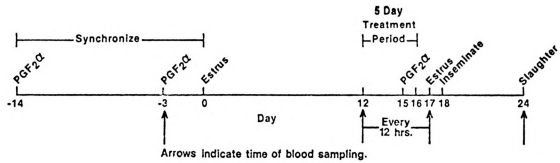


Figure 5. Blood sampling schedule for Trials 1 and 2.

Necropsy Procedures

On day 7 postestrus cattle were hauled 52 kilometers to Milligan Pack at Parma, Michigan. Slaughter began, under Federal inspection, at 9:00 a.m. Reproductive tracts were removed, identified, placed on ice and returned to campus for data collection. Processing of tracts began at 1:00 p.m.

Ovarian and Embryonic Data Collection

All ovarian and embryonic data were collected and recorded separately for the right and left side. Ovaries were removed, weighed individually and the number of corpora lutea counted. The diameter of unovulated follicles at the surface of the ovary was measured and recorded. Follicular fluid was drained and, following blotting, the weight of stromal and luteal tissue was taken. Follicular fluid weight was obtained by subtracting stromal and luteal tissue weight from total ovarian weight.

Flushing procedures for Trial 1 were as follows. Each oviduct was dissected free from the mesovarium. Each uterine horn was then cut 15 cm posterior to the utero-tubular junction (UTJ). A two-way Foley catheter (Bard, 16 Fr.) was placed into a uterine horn and the cuff inflated. Using a blunt needle attached to a 50 ml syringe, 20 ml of phosphate buffered saline (PBS) was flushed through the oviduct, uterine horn and Foley catheter into a 200 x 38 mm test tube. The media had previously been sterilized by a 0.20 micron membrane filter (Nalge Sybron Corporation, Rochester, New York). The oviduct was then removed at the UTJ and 50 ml of PBS were flushed

through the uterine horn and collected. The uterine horn was allowed to balloon with fluid several times by clamping the Foley catheter. Of 70 ml used per horn, 67 to 68 ml were recovered.

Following a settling period of not less than 15 min, two 10 ml aliquots of fluid were pipetted from the bottom of each collection tube and placed in 100 x 15 ml petri dishes. Using a binocular dissecting microscope each aliquot was examined for embryos. The number of embryos recovered was recorded.

Procedures for Trials 2 and 3 were the same as those described for Trial 1 with the following exceptions. Each uterine horn was dissected 10 cm posterior to the UTJ. This was necessary due to small size of the reproductive tracts from the virgin heifers. Forty ml of media were used on the second step of flushing the uterine horn. The flushing media used was Dulbecco's PBS (Grand Island Biological Company, Grand Island, New York) to which 100 ml heat inactivated fetal calf serum plus 100,000 units penicillin, 100,000 mcg streptomycin and 250 mcg Fungizone® had been added per liter. Flushing fluid was collected directly into a 90 x 50 mm dish (Fisher Scientific Company) for observation. This eliminated the pipetting step used in Trial 1. A microscope with 100X magnification was used for determining condition of embryos.

Quantification of Progesterone

Serum progesterone concentration was determined by radioimmunoassay similar to that of Louis et al. (1973).

Depending upon expected concentration, duplicate aliquots (50-100 µl) of each unknown were placed in 16 x 100 mm disposable culture tubes. About 2000 cpm of ^{3}H-1,2,6,7-progesterone (104 Ci/m mole, repurified by column chromatography) was added to six randomly selected samples to estimate procedural losses. These were mixed 10 sec and allowed to equilibrate for 30 min before extracting. For comparison among assays, duplicate aliquots of standard sera (3/assay) with high and low progesterone concentrations and blank extraction tubes (4/assay) were assayed with each set of unknown samples.

Each tube was mixed with 2 ml benzene-hexane (1:2) for 30 sec, then stored at -20°C for at least 1 hr to freeze the aqueous phase. The organic solvent from tubes with ^{3}H-progesterone was decanted into a scintillation vial for quantification of the recovered radioactivity. The solvent in the extraction tubes of unknowns was decanted into 12 x 75 mm disposable culture tubes for radioimmunoassay as follows.

Three sets of standard tubes containing 0, 1, 2, 5, 10, 25, 50, 75, 100, 150 and 200 µl of stock progesterone (10 ng/ml in methanol, Sigma Chemical Company) were included in each assay and treated similarly to the unknowns. Standard progesterone and serum extracts were dried in a vacuum (-29 lbs) oven (50°C) with a dry ice

trap between the oven and vacuum pump. Antibody (MSU #74 produced in rabbits against progesterone-20-oxim-human serum albumin, diluted 1:2000) diluted to 200 µl phosphate-buffered saline (0.1 M), pH 7.4 containing 0.1% gelatin (PBS-G) was added. Crossreaction of rabbit antiprogesterone (MSU #74) has previously been reported by Convey et al. (1977). After addition of antibody, each tube was mixed 2 sec. Then about 24,000 cpm ^{3}H-1,2,6,7-progesterone (104 Ci/m mole, repurified by column chromatography) diluted in 200 µl PBS-G was added to each tube. The tubes were mixed 2 sec and incubated at 5°C for 12 to 18 hr.

To separate free from antibody-bound progesterone, 0.5 ml of dextran-coated charcoal (0.5 g Carbon Decolorizing Neutral Norit, Fisher Scientific Company, and 1 g Dextran T-70, Pharmacia Inc., Uppsala, Sweden, in 100 ml distilled water) was added at 5°C. Each tube was mixed for 2 sec and centrifuged (3000 g) immediately for 15 min at 5°C. Antibody bound ^{3}H-progesterone in 0.5 ml of the supernatant fluid was measured in a liquid scintillation spectrometer and recorded on a cassette tape. Using the CDC 6500 computer, the unknown progesterone concentrations were calculated.

Procedural losses resulted in 88.5 ± 2.0% extraction efficiency (n = 9) for Trial 1 samples and 86.9 ± 2.1% extraction efficiency (n = 7) for Trial 2 samples. Values for all blank extraction tubes were negligible. Intra- and inter-assay coefficients of variation are presented in Table 2.

TABLE 2. PROGESTERONE INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION

Standard Sera (3/assay)	Intra-Assay (%)	Inter-Assay (%)
- - - - - - - - - - - - - - Trial 1 (n = 9) - - - - - - - - - - - - - -		
Low (no. 4009--estrus cow)	25.54	34.72
High (no. 4010--pregnant cow)	8.38	14.21
- - - - - - - - - - - - - - Trial 2 (n = 7) - - - - - - - - - - - - - -		
Low (no. 4016--estrus cow)	11.54	21.86
High (no. 4008--diestrus cow)	6.19	7.65

Quantification of Estradiol-17β

Serum estradiol-17β concentration was determined by radioimmunoassay similar to that of Butcher et al. (1974).

a. Extraction. One milliliter of each unknown was placed in a 16 x 100 mm disposable culture tube. To account for procedural losses, 2000 cpm of ^{3}H-2,4,6,7,16,17-estradiol (100 Ci/m mole repurified by column chromatography) was added to two randomly selected samples for each assay. These were mixed 10 sec and allowed to equilibrate for 30 min before extracting. Duplicate aliquots of distilled water, ovariectomized cow serum (ovex) and ovex to which 5, 40 and 100 pg of stock estradiol (100 pg/ml in benzene, Sigma Chemical Company) had been added, were included in each assay.

Estradiol was extracted by mixing with 3 ml of freshly opened anesthesia grade ether (Mallinckrodt, Inc., St. Louis, Missouri) for 2 min. Following centrifugation for 10 min at 3000 g, the aqueous

phase was frozen on dry ice and the ether decanted into 12 x 75 mm disposable culture tubes. Extracts were then dried in a vacuum (-29 lbs) oven (no heat) with a dry ice trap between the oven and vacuum pump.

b. Chromatography. Glass 5 ml disposable pipettes (Kimble #72120, Toledo, Ohio) were used for columns. A 4 mm glass bead was placed in the column and a slurry of 0.8 g of Sephadex LH-20 (Pharmacia, Inc., Piscataway, New Jersey) soaked overnight in methylene chloride: methanol (90:10, Budrick and Jackson Laboratories, Inc., Muskegon, Michigan) was added. A disc of glass filter paper (2.1 cm GF/A, Whatman Ltd., England) was placed on top of the column. Columns were rinsed with 10 ml of the 90:10 eluting solution which had been allowed to equilibrate for 30 min. Samples were added to the columns in 0.2 ml of the eluting solution and allowed to enter. Then 3.7 ml of the solution was added and the elute discarded. A final 4.0 ml of eluting solution was placed on the column and collected into 12 x 75 mm disposable culture tubes. Samples for procedural losses were collected into scintillation vials.

c. Radioimmunoassay. Three sets of standard tubes containing 0, 1, 2, 4, 6, 10, 20, 40, 60, 100 and 200 pg of stock estradiol were included in each assay and treated similarly to the unknowns. Standard estradiol and serum extracts were dried in the vacuum (-29 lbs) oven (50°C). Assay tube walls were rinsed down with 250 µl of methanol, and the rinse methanol was evaporated. PBS-G was used to dilute the estradiol antiserum to 1:40,000. MSU anti-estradiol #74 was prepared in rabbits against estradiol-6-oxim-human serum albumin.

Crossreactivity has previously been reported by Oxender et al. (1977). Antibody (200 µl) was added and tubes were mixed 2 sec. PBS-G (200 µl) with 5000 cpm of ^{3}H-2,4,6,7,16,17-estradiol (100 Ci/m mole, repurified by column chromatography) was added to each tube and mixed 2 sec. All tubes were incubated 12 to 18 hr at 5°C.

To separate free from antibody-bound estradiol, 0.5 ml of dextran coated charcoal was added at 5°C. Each tube was mixed for 2 sec and centrifuged (3000 g) immediately for 15 min at 5°C. Antibody-bound ^{3}H-estradiol in 0.5 ml of the supernatant fluid was quantified in a liquid scintillation spectrometer and recorded on a cassette tape. Using the CDC 6500 computer, unknown estradiol concentrations were calculated.

Procedural losses resulted in 79.3 ± 5.5% extraction efficiency (n = 4). Blank tubes containing distilled water (2/assay) averaged 1.84 ± 2.77 pg/ml. Intra- and inter-assay coefficients of variation are presented in Table 3.

TABLE 3. ESTRADIOL 17β INTRA- AND INTER-ASSAY COEFFICIENTS OF VARIATION (n = 4)

Standard Sera (2/assay)	Intra-Assay (%)	Inter-Assay (%)
Ovex (no. 4015--ovariectomized cow)	9.91	22.79
Ovex + 5 pg E_2 17β	7.74	11.00
Ovex + 40 pg E_2 17β	13.29	30.31
Ovex + 100 pg D_2 17β	14.16	33.52

Data Calculations and Statistical Analysis

Analysis of variance was used to examine main effects and interactions of ovarian and embryonic data within each trial (Snedecor and Cochran, 1967). Hormone data were analyzed by split plot analysis of variance (Gill and Hafs, 1971). Significant differences between hormone profiles were determined using Bonferroni's t-test (Miller, 1966), modified for serial correlation according to Albers (1978). Comparison of slopes was used to detect differences between hormone regression lines (Gill, 1978).

RESULTS

Estrus Synchronization

To facilitate collection of ovarian and embryonic data, estrous cycles of all animals within each trial were synchronized. Results are presented in Table 4.

Progesterone concentrations > 1 ng/ml were assumed to represent presence of a functional corpus luteum (CL). In Trial 1, 24 of 27 cows had progesterone levels in excess of 1 ng/ml. Even though the remaining 3 animals had less than 1 ng/ml of progesterone, standing estrus was observed within 56 hours following $PGF_{2\alpha}$ injection. At the time of the second $PGF_{2\alpha}$ injection in Trial 2, all heifers had progesterone concentrations in excess of 1 ng/ml, indicating a CL was present.

Eighty-one percent of cows in Trial 1 were observed in standing estrus with the remainder showing definite estrual behavior. Similarly, in Trial 2, 25 of 26 heifers displayed standing estrus while the remaining heifer exhibited estrual behavior. In Trial 3, 5 of 8 heifers manifested standing estrus with the remaining 3 demonstrating estrual behavior.

The length of time from the second $PGF_{2\alpha}$ injection to estrus was nearly equal across all 3 trials and averaged close to 60 hr. However the variation was quite high with a range from 28 to 120 hr.

TABLE 4. ESTROUS SYNCHRONIZATION OF EXPERIMENTAL ANIMALS

Trial		Progesterone Concentration[a] (ng/ml)	Hours to Estrus[b]	Body Weight[c] (kg)
1	mean	2.34 ± 1.22	58.2 ± 19.7	518.5 ± 74.2
	range	6.02 to 0.38	120 to 28	658 to 376
2	mean	4.91 ± 1.34	58.9 ± 17.2	405.7 ± 24.5
	range	7.49 to 1.87	96 to 34	447 to 356
3	mean	--[d]	61.4 ± 7.0	467.3 ± 46.0
			72 to 52	526 to 386

[a]Serum progesterone concentration at time of 2nd $PGF_{2\alpha}$ injection.

[b]Hours to standing estrus or estrus behavior following 2nd $PGF_{2\alpha}$ injection.

[c]Weight taken at time of 2nd $PGF_{2\alpha}$ injection.

[d]No blood samples were taken from cattle in Trial 3.

Trial 1

The objective of Trial 1 was to test two doses of EPE with and without the addition of HCG for effectiveness to induce multiple ovulations (Table 5).

EPE treated animals had a greater number of anovulatory follicles ≥ 10 mm relative to controls ($P < .05$) demonstrating that EPE is a very potent stimulus of follicular growth. As a result of large variation, number of corpora lutea observed (0 to 96) and number of embryos recovered (0 to 21) in cows treated with EPE did not differ from controls. Compared to EPE treatments without HCG, EPE treatments with HCG resulted in fewer corpora lutea and a greater number of anovulatory follicles ≥10 mm ($P < .05$). HCG was effective at suppressing ovulation at both low and high dose of EPE as no interaction between dose of EPE and HCG was present.

Total ovary weight, stromal and luteal tissue weight and follicular fluid weight increased as dose of EPE increased. The addition of HCG reduced the weight of stromal and luteal tissue and increased follicular fluid weight which is consistent with the decreased number of corpora lutea and increased number of anovulatory follicles ≥10 mm observed in HCG treated cows.

Trial 2

The objective of Trial 2 was to determine the effect of once or twice daily injections of 2 different doses of EPE. A comparison between the same dose of EPE injected once daily for 2 or 3 days was made. In addition, a five day, twice daily treatment of FSH-LH used

TABLE 5. OVARIAN RESPONSE--TRIAL 1

		Low Dose EPE[a]		High Dose EPE[b]		Significance Level		
	Control	w/o HCG	w HCG[c]	w/o HCG	w HCG[c]	Dose EPE	HCG	Interaction
No. unovulated follicles ≤ 9 mm[d]	12.0 ± 11.0	27.3 ± 12.7	13.2 ± 9.0	36.5 ± 41.2	8.4 ± 7.7	.691	.034	.472
No. unovulated follicles > 10 mm[d]	0.5 ± 0.6	7.8 ± 4.0	25.8 ± 9.8	16.3 ± 15.5	25.0 ± 8.5	.016	.010	.309
No. corpora lutea[d]	1.0 ± 0.0	31.5 ± 19.1	7.4 ± 10.2	43.2 ± 41.5	13.8 ± 22.0	.194	.019	.824
No. embryos recovered[d]	0.5 ± 0.6	5.0 ± 4.1	0.8 ± 1.6	7.3 ± 7.7	3.4 ± 5.1	.284	.130	.834
Total ovary wt., g[c]	16.1 ± 3.5	122.6 ± 48.7	146.1 ± 94.0	179.2 ± 93.7	212.6 ± 114.8	.007	.446	.762
Stromal and luteal tissue wt., g[e]	13.0 ± 2.5	76.2 ± 41.9	38.9 ± 16.3	86.0 ± 64.8	53.0 ± 11.9	.067	.044	.913
Follicular fluid wt., g[e]	3.1 ± 1.4	46.4 ± 19.3	107.2 ± 82.2	93.2 ± 80.9	159.6 ± 11.1	.035	.058	.768

[a]Low Dose EPE = 750, 500, 500, 250, 250 (Fevold-Hisaw Rat Units) on days 12, 13, 14, 15 and 16, respectively.

[b]High Dose EPE = 1500, 1000, 1000, 500, 500 (Fevold-Hisaw Rat Units) on days 12, 13, 14, 15 and 16, respectively.

[c]HCG = 0, 1000, 1000, 500, 500 I.U. on days 12, 13, 14, 15 and 16, respectively.

[d]Values are expressed as the mean number per cow (both ovaries combined) followed by the standard deviation.

[e]Values are expressed as the mean weight per ovary followed by the standard deviation.

by Colorado State University (Elsden et al., 1976) was included to provide a positive control.

The data for cows treated with EPE for 3 days is included in Table 6. None of the parameters measured were affected by dose of EPE or number of injections per day and no interaction was detected.

A single daily injection of EPE for 3 days resulted in more corpora lutea and embryos recovered than the same dose of EPE given once daily for 2 days (Table 7). The number of anovulatory follicles ≥10 mm was not affected by treatment.

Cows treated with FSH-LH had more corpora lutea and embryos than cows treated with EPE for 3 days (Table 8). Further reduction in the number of anovulatory follicles ≥10 mm was observed with the FSH-LH treatment relative to EPE treatments.

Trial 3

An objective of Trial 3 was to ovulate anovulatory follicles ≥10 mm incurred on EPE treatments. The EPE treatment period was extended from 3 to 5 days and estrous cycles were synchronized with progestogen (Syncro-Mate B®). Results of comparing an equal total dose of EPE given by 2 regimens are presented in Table 9.

Number of corpora lutea and number of embryos recovered were greater for constant dose EPE than for variable dose EPE. Number of unovulated follicles ≥10 mm were significantly higher for variable dose EPE than constant dose EPE ($P < .05$). The large dose of EPE given on day 5 of the variable dose treatment appeared to be detrimental to the number of ovulations. Many follicles were stimulated on this treatment but a low percentage ovulated.

TABLE 6. OVARIAN RESPONSE FOR 3 DAY EPE TREATMENTS--TRIAL 2

	Low Dose EPE[a]		High Dose EPE[b]		Significance Level		
	1 X	2 X	1 X	2 X	Dose EPE	Inj/Day	Interaction
No. unovulated follicles ≤ 9 mm[c]	14.3 ± 10.7	19.0 ± 16.9	4.8 ± 5.6	14.3 ± 10.7	.099	.099	.574
No. unovulated follicles ≥ 10 mm[c]	3.5 ± 2.4	3.3 ± 3.4	2.3 ± 1.3	3.3 ± 2.6	.522	.700	.522
No. corpora lutea[c]	7.5 ± 8.1	7.0 ± 2.7	12.0 ± 12.9	10.0 ± 7.9	.235	.689	.810
No. embryos recovered[c]	5.3 ± 6.8	6.0 ± 3.2	9.0 ± 11.2	4.5 ± 4.4	.665	.471	.315
Total ovary wt., g[d]	19.5 ± 10.0	15.0 ± 4.5	22.7 ± 23.3	22.2 ± 13.5	.318	.630	.698
Stromal and luteal tissue wt., g[d]	15.2 ± 8.7	13.0 ± 4.3	17.6 ± 14.5	17.8 ± 10.3	.318	.786	.749
Follicular fluid wt., g[d]	4.4 ± 3.5	2.0 ± 2.0	5.1 ± 10.2	4.4 ± 5.2	.463	.484	.692

[a]Low Dose EPE = 375, 250, 125 (Fevold-Hisaw Rat Units).

[b]High Dose EPE = 750, 500, 250 (Fevold-Hisaw Rat Units).

[c]Values are expressed as the mean number per cow (both ovaries combined) followed by the standard deviation.

[d]Values are expressed as the mean weight per ovary followed by the standard deviation.

TABLE 7. OVARIAN RESPONSE FOR LOW DOSE EPE--1X--3 DAYS VS. LOW DOSE EPE--1X--2 DAYS--TRIAL 2

	Low Dose EPE[a] 1X--3 Days	Low Dose EPE[b] 1X--2 Days	Significance Level
No. unovulated follicles ≤ 9 mm[c]	14.3 ± 10.7	15.6 ± 10.6	.792
No. unovulated follicles ≥ 10 mm[c]	3.5 ± 2.4	3.0 ± 3.1	.753
No. corpora lutea[c]	7.5 ± 8.1	2.0 ± 2.5	.067
No. embryos recovered[c]	5.3 ± 6.8	1.4 ± 2.2	.165
Total ovary wt., g[d]	19.5 ± 10.0	11.5 ± 9.1	.104
Stromal and luteal tissue wt., g[d]	15.2 ± 8.7	7.9 ± 5.4	.047
Follicular fluid wt., g[d]	4.4 ± 3.5	3.6 ± 6.1	.756

[a]Low dose EPE = 375, 250, 125 (Fevold-Hisaw Rat Units).

[b]Low dose EPE = 375, 375 (Fevold-Hisaw Rat Units).

[c]Values are expressed as the mean number per cow (both ovaries combined) followed by the standard deviation.

[d]Values are expressed as the mean weight per ovary followed by the standard deviation.

TABLE 8. OVARIAN RESPONSE FOR 3 DAY EPE TREATMENTS VS. FSH-LH--TRIAL 2

	Combined 3 Day EPE Treatments	FSH-LH	Significance Level
No. unovulated follicles ≤ 9 mm[a]	13.1 ± 11.7	21.2 ± 16.3	.111
No. unovulated follicles ≥ 10 mm[a]	3.1 ± 2.3	0.8 ± 0.4	.009
No. corpora lutea[a]	9.1 ± 8.0	13.0 ± 7.8	.211
No. embryos recovered[a]	6.2 ± 6.6	11.2 ± 6.7	.056
Total ovary wt., g[b]	19.9 ± 14.2	24.5 ± 9.6	.342
Stromal and luteal tissue wt., g[b]	15.9 ± 9.8	22.6 ± 10.5	.074
Follicular fluid wt., g[b]	4.0 ± 5.9	1.9 ± 1.7	.280

[a]Values are expressed as the mean number per cow (both ovaries combined) followed by the standard deviation.

[b]Values are expressed as the mean weight per ovary followed by the standard deviation.

TABLE 9. OVARIAN RESPONSE--TRIAL 3

	Constant Dose EPE[a]	Variable Dose EPE[b]	Significance Level
No. unovulated follicles ≤ 9 mm[c]	15.0 ± 5.7	25.3 ± 15.6	.165
No. unovulated follicles ≥ 10 mm[c]	3.0 ± 2.9	22.0 ± 24.9	.047
No. corpora lutea[c]	11.3 ± 9.0	4.5 ± 8.3	.143
No. embryos recovered[c]	8.3 ± 6.1	3.3 ± 6.5	.155
Total ovary wt., g[d]	17.9 ± 10.6	19.0 ± 9.9	.850
Stromal and luteal tissue wt., g[d]	15.2 ± 10.1	10.1 ± 7.8	.295
Follicular fluid wt., g[d]	4.0 ± 5.9	8.8 ± 8.2	.212

[a]Constant dose EPE = 250, 250, 250, 250, 250 (Fevold-Hisaw Rat Units).

[b]Variable dose EPE = 375, 250, 125, 125, 375 (Fevold-Hisaw Rat Units).

[c]Values are expressed as the mean number per cow (both ovaries combined) followed by the standard deviation.

[d]Values are expressed as the mean weight per ovary followed by the standard deviation.

Constant dose EPE, which produced a greater number of ovulations possessed more total weight in stromal and luteal tissue. Variable dose EPE, which resulted in a greater number of large unovulated follicles, possessed more total ovary weight and follicular fluid weight.

Hormonal Profiles

Throughout the period from initiation of gonadotropin injections to estrus, blood samples were taken twice daily. Samples were assayed for concentration of progesterone and estradiol 17β. Following collection of ovarian and embryonic data, animals were classified according to number of ovulations as good or poor ovulatory response (Table 10).

TABLE 10. OVULATORY RESPONSE OF EXPERIMENTAL ANIMALS

No. Ovulations	Classification	Number of Animals	
		Trial 1	Trial 2
1 CL	Control	4	0
3 CL	Poor	9	9
5 CL	Good	14	17

Prior to injection of $PGF_{2\alpha}$ cows having a poor ovulatory response in Trial 1 tended to increase in progesterone concentration compared to good responders and control cows (Figure 6), but this difference was not significant ($P > .1$). In Trial 2, heifers with a poor ovulatory response had significantly lower progesterone levels

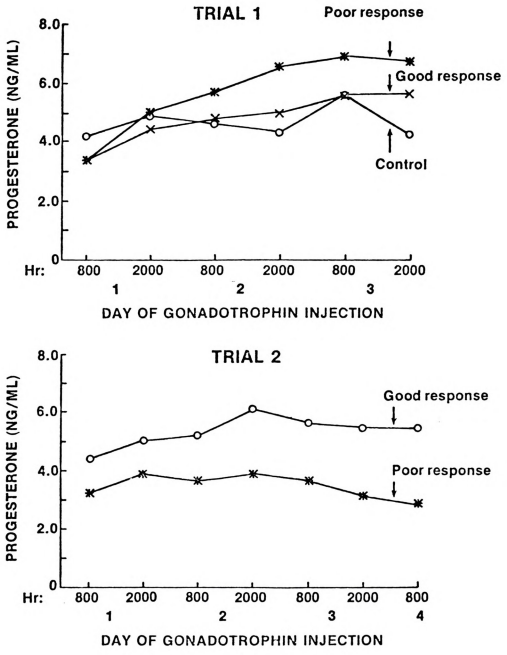


Figure 6. Progesterone profiles during the period from initiation of gonadotropin injection to $PGF_{2\alpha}$ injection.

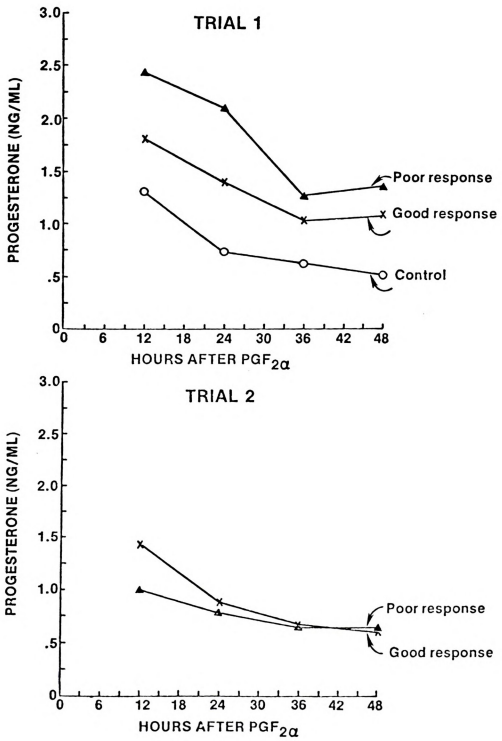


Figure 7. Progesterone profiles during the period from 12 to 48 hrs after $PGF_{2\alpha}$ injection.

throughout the period of gonadotropin treatment prior to injection of $PGF_{2\alpha}$ ($P < .01$).

Following injection of $PGF_{2\alpha}$ (Figure 7), both cows with good and poor ovulatory response in Trial 1 maintained elevated progesterone concentrations relative to controls ($P < .05$). However, the progesterone profiles did not differ between good and poor responders. Similarly, in Trial 2 animals having good or poor ovulatory response did not differ in progesterone level following $PGF_{2\alpha}$ injection.

Concentration of estradiol 17β in jugular serum is plotted in Figure 8 with regression lines drawn. In Trial 1 the rate of increase in estradiol 17β for cows with good and poor ovulatory response was significantly higher than that for control cows ($P < .01$). Cows with good ovulatory response reached peak estradiol 17β vluaes on day 5 while cows with poor ovulatory response did not reach peak values until day 6. Comparison of animals with good and poor ovulatory response revealed no difference in the slope of regression lines for both Trials 1 and 2.

As seen in Figure 9, a significant positive correlation existed between the number of corpora lutea and serum progesterone concentration on day 7 postestrus in both Trials 1 and 2 ($P < .01$). The correlation in Trial 1 was 0.52 while a correlation of 0.77 was observed in Trial 2.

Embryo Recovery and Condition of Embryos

Cows within each trial were grouped according to the ovulation rate and the mean within each group was determined (Table 11). The number of embryos recovered was then expressed as a percentage of the

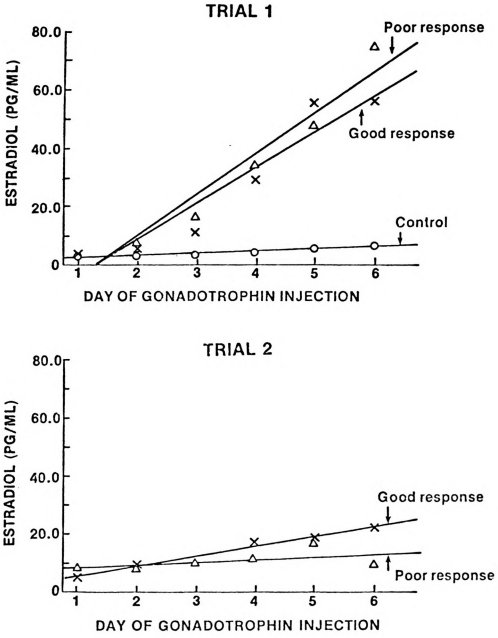


Figure 8. Estradiol 17β concentrations during period of gonadotropin injection with regression lines.

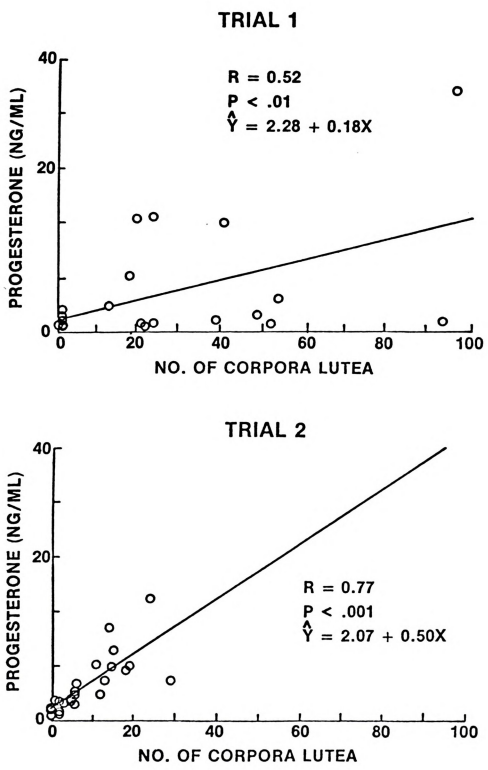


Figure 9. Relationship between number of corpora lutea and serum progesterone concentration on day 7 postestrus.

TABLE 11. PERCENTAGE OF POTENTIAL EMBRYOS RECOVERED

Number of Ovulations	Trial 1			Trial 2			Trial 3		
	Mean	N	Embryos Recovered (%)	Mean	N	Embryos Recovered (%)	Mean	N	Embryos Recovered (%)
1-5	1.2	6	29	2.6	8	43	2.5	2	20
6-10		0		6.0	5	93	6.0	1	100
11-15	12.0	1	0	13.3	6	71	11.0	1	100
16-20	18.7	3	29	18.5	2	68	17.0	1	76
21-30	22.3	3	27	26.5	2	83	24.0	1	63
31-60	46.0	5	14		0			0	
>60	94.5	2	14		0			0	

ovulation rate for each group. The highest recovery percentage occurred when the ovulation rate was between 6 and 10 while the lowest recovery percentage was for ovulation rates in excess of 30.

In Trials 2 and 3, embryos were examined in detail and their condition determined. Normal embryos were defined as those having normal cell division and cleavage for their expected stage of development. No assessment of viability was made. Embryos not considered normal were primarily at the one cell stage and may or may not have been fertilized. The percentage of embryos at the normal stage of development is presented in Table 12.

Table 12. Occurrence of Normal Embryos

Treatment	Mean No. Embryos	Mean No. Normal Embryos[a]	% Normal
	Trial 2		
Low dose EPE--1X--3 days	5.3	3.3	59
Low dose EPE--2X--3 days	6.0	5.5	92
High dose EPE--1X--3 days	9.0	8.0	91
High dose EPE--2X--3 days	4.5	4.3	94
Low dose EPE--1X--2 days	1.4	1.4	100
FSH--LH	11.2	9.8	88
	Trial 3		
Constant dose EPE	8.3	6.5	79
Variable dose EPE	3.3	2.3	69

[a]Normal embryos were defined as those having normal cell division and cleavage for their expected stage of development. There was a delay period of up to 12 hrs from the time cows were slaughtered until embryos were examined.

DISCUSSION

Synchronization of estrous cycles was successfully accomplished in all trials. The time from $PGF_{2\alpha}$ injection to estrus averaged close to 60 hr which was somewhat less than reported by other workers. Oxender et al. (1974) reported a mean interval from $PGF_{2\alpha}$ to onset of estrus of 72.5 ± 5 hr for 18 cows receiving an intrauterine infusion of 5 mg $PGF_{2\alpha}$ and 74.3 ± 3 hr for 5 cows injected intramuscularly with 30 mg $PGF_{2\alpha}$. Similarly, Peters et al. (1977) reported the average interval from $PGF_{2\alpha}$ to estrus in 150 cows and heifers was 69.5 ± 1.8 hr. In lactating cows, Burfening et al. (1978) observed the average interval from $PGF_{2\alpha}$ to estrus was 73.5 hr but in yearling heifers the interval was 52 hr.

Ovarian response by animals to an equine pituitary extract (EPE) in Trial 1 demonstrated EPE to be a very potent stimulator of follicular growth. The large variation observed is consistent with other investigators using PMSG and FSH-LH (Elsden et al., 1978; Hasler, 1978; Bellows et al., 1969). Anovulatory follicles ≥10 mm are also reported to be common in superovulated cows (Moore, 1975a; Hafez et al., 1963).

Addition of HCG to superovulation treatments in Trial 1 was detrimental to the number of follicles ovulating. The reason for this detrimental effect is not clear. HCG has been shown to resemble pituitary LH in both biologic activity and chemical structure (Swaminathan and Bahl, 1970). LH synergizes with FSH in promoting follicular

development and is required for the production and secretion of the follicular hormones (Goodman, 1974). In both experimental and commercial embryo transfer work at Colorado State University, 20% LH is added to FSH for induction of superovulation (Seidel et al., 1978). This treatment has been shown to be effective for superovulation (Elsden et al., 1976; Hasler, 1978) and was effective in Trial 2 of the present study. Recently, however, Humphrey et al. (1979) found preparations of high FSH/LH ratio to be more conducive to induction of ovulation than preparations of low FSH/LH ratio. They observed the addition of HCG to PMSG treatments reduced ovulatory success by 37%. In Trial 1 the addition of HCG to EPE treatments reduced the percentage of follicles ≥ 10 mm which ovulated by 48%. Clearly, treatments with EPE plus HCG were inferior to treatments with EPE alone.

The most effective treatment for inducing multiple ovulations in Trial 2 was FSH-LH yielding an average of 11.2 embryos per cow treated. Additionally, a lower number of anovulatory follicles ≥ 10 mm resulted from FSH-LH (0.8) when compared to 3 day EPE treatments (3.1). Similar results were observed by Moore (1975) who reported that horse anterior pituitary (HAP) and PMSG were equally effective in superovulating both cows and heifers, but in heifers, HAP produced more anovulatory follicles than did PMSG.

The same total dose of EPE given for 3 days produced an average of 5.3 embryos per cow treated while 1.4 embryos resulted when the dose was given for 2 days. Once daily injections of EPE produced results equal to those from twice daily injections. Based on these results,

a total dose of 750 units given as a single daily injection for 3 days would be the program of choice for EPE.

Attempts to reduce the number of anovulatory follicles ≥10 mm by giving EPE at a constant rate for five days and by giving a large dose of EPE on the fifth day were unsuccessful in Trial 3. A method to ovulate residual large follicles would improve the usefulness of EPE. A potential method would be to extend the interval from the first injection of gonadotropin to $PGF_{2\alpha}$ injection which would delay the onset of estrus. It is possible that unovulated follicles were not matured to the point where they could respond to the LH surge. Delaying estrus would allow greater time for follicles to develop and mature. Longer intervals to estrus have been shown to produce a higher percentage of follicles ovulating (Sreenan and Beehan, 1976; Henricks and Hill, 1978; Hafez et al., 1963).

Based on the results of these trials, the primary advantage for use of EPE would be that injections need only be given once a day. Once daily injections would reduce stress to the donor cow and lessen labor requirements on the part of a manager. Under certain circumstances, this could increase the value of EPE as a practical treatment for superovulation in cows.

Progesterone profiles during the period from initiation of gonadotropin injections to estrus showed inconsistency between Trials 1 and 2. Concentration of progesterone in serum of animals with a poor ovulatory response increased in progesterone prior to estrus but did not differ from animals with good ovulatory response or control animals.

One cause of the elevated progesterone level of cows with poor ovulatory response could be due to the HCG treatment. Seven of nine cows having poor ovulatory response received HCG treatment which is known to be luteotropic and could have stimulated an increase in progesterone secretion from the corpus luteum. A further possibility exists that some of the follicles of cows exhibiting poor ovulatory response may have become luteinized, secreted progesterone and were incapable of ovulation.

In Trial 2, however, animals with poor ovulatory response had significantly lower progesterone levels than cows with good ovulatory response. Since HCG treatments were not included in Trial 2, this could not have affected the progesterone profiles. The lowered progesterone concentration during the gonadotropin treatment period prior to $PGF_{2\alpha}$ could have contributed to the reduction in ovulatory response. Gengenbach et al. (1978) observed heifers with high progesterone concentrations during treatment with 2000 IU PMSG averaged 10.7 more corpora lutea than similarly treated heifers with low progesterone. Further, in reviewing superovulation of calves, Betteridge (1977) noted that inserting vaginal sponges impregnated with 60 mg fluorogestone acetate (FGA) at the time of PMSG treatment and left in place for 4 days improved the superovulatory response and shortened the period over which ovulations occurred. Calves treated with FGA displayed a much greater LH release with higher peak values than calves not treated with FGA. High progesterone levels during the time of gonadotropin treatment may be necessary to block LH release from the

pituitary and prevent premature ovulations. This theory is consistent with the difference in ovulatory response between animals having high or low progesterone levels prior to $PGF_{2\alpha}$ in Trial 2. If low progesterone concentration at the time of superovulation leads to a poor ovulatory response, then use of exogenous progesterone may offer a means of increasing the number of ovulatory follicles.

Following $PGF_{2\alpha}$ injection, both cows with poor and good ovulatory response in Trial 1 maintained elevated progesterone levels relative to controls. As with the progesterone levels prior to $PGF_{2\alpha}$, this could again be explained in part by the luteotropic effect of HCG or the presence of luteinized follicles. The fact that there was no difference between good and poor responders in either Trial 1 or 2 would appear to eliminate the progesterone level following $PGF_{2\alpha}$ as a cause of the difference in ovulatory response.

The rate of increase in estradiol 17β showed no relationship to the ovulatory response observed in Trials 1 and 2. This would eliminate estradiol 17β concentration as a possible cause of the variation seen in ovulatory response.

A positive relationship between the number of CL and plasma progesterone concentration in superovulated cattle has been reported previously (Gengenbach et al., 1978; Booth et al., 1975; Spilman et al., 1973; Henricks et al., 1973). The results from Trials 1 and 2 would support this.

The highest percentage of embryos recovered occurred when the ovulation rate was between 6 and 10 while the lowest recovery was

observed for ovulation rates in excess of 30. This finding is consistent with the theory of Seidel et al. (1978) who suggest that ovaries producing 20 or 30 ovulations have mechanical difficulties in ovum pickup and transport through the fimbria. Furthermore, they note that the extremely high progesterone output of multiple corpora lutea probably alters sperm and ovum transport in the oviducts. In addition, the blood from such a large number of ovulations and increased stress to the reproductive tract from manipulating a grossly enlarged ovary promotes problems. Consistent with this theory are Bellows et al. (1969) who observed the percent ova recovered decreased from 88 to 58 as the mean ovulation rate increased from 1 to 18.

The percentage of embryos determined to be normal ranged from 59 to 100 among different treatments. These percentages are similar to those reported by other workers (Bellows et al., 1969; Betteridge, 1977).

Being a foreign protein, the possibility of EPE injection inducing anaphylaxis exists. Fifty-two animals were treated with EPE in these trials and no problems with anaphylaxis were encountered. Further, all animals were slaughtered in federally inspected meat plants with no abnormalities in lymph glands, livers, or kidneys detected.

SUMMARY

Various schedules for administration and dosages of an equine pituitary extract (EPE) were examined for effectiveness to induce multiple ovulations in beef cows. Results demonstrated that EPE could be used to superovulate cows but responses were highly variable. Unovulated follicles ≥10 mm diameter were encountered with EPE treatments. The addition of HCG to EPE treatments reduced the ovulatory success further. A treatment of FSH-LH resulted in a greater number of ovulations and embryos than any of the EPE treatments. The primary advantage for use of EPE would be that injections need only be given once daily.

Hormonal analysis revealed that high progesterone concentration during the gonadotropin treatment prior to $PGF_{2\alpha}$ injection was associated with a good ovulatory response. No relationship between progesterone level following $PGF_{2\alpha}$ injection or estradiol 17β concentration during gonadotropin treatment and the resulting ovulatory response was observed. A positive correlation between the progesterone level on day 7 postestrus and the number of corpora lutea present was observed.

APPENDIX

APPENDIX

TABLE A.1 MANUFACTURER'S DESCRIPTION OF EPE

ANTERIOR PITUITARY GONADOTROPIN
For Veterinary Use Only

DESCRIPTION: This Anterior Pituitary Gonadotropin in a sterile lyophilized form contains primarily Follicle Stimulating Hormone (FSH) and the natural balance of Luteinizing Hormone (LH). Each 5 ml. vial contains 125 biologically assayed Fevold-Hisaw Rat Units* of gonadotropic hormones extracted from equine anterior pituitary glands. Each 5 ml. vial of Sterile Diluent contains 0.9% Sodium Chloride and Water for Injection, q.s.

ACTIONS: Anterior Pituitary Gonadotropins are hormones of the anterior pituitary which stimulate the germinal epithelium of both the male and female. In the female FSH and LH synergistically stimulate maturation of the follicle so that it will produce estrogen. Estrus and its associated physiological and behavioral phenomena are secondary actions of the gonadotropic complex. In the male the gonadotropic hormones stimulate growth of the epithelium of the seminiferous tubules and interstitial cells, which produce testosterone, of the testes.

INDICATIONS: Anterior Pituitary Gonadotropin may be used to treat breeding difficulties of animals who have abnormal function of the ovaries or testes, including cases of functional impotence of the male and failure of the female to come into heat or conceive, when caused by a general deficiency of gonadotropic hormones.

DOSAGE AND ADMINISTRATION: Anterior Pituitary Gonadotropin is available in 5 ml. vials, containing the equivalent of 125 Fevold-Hisaw Rat Units* of gonadotropic hormones as a lyophilized powder. A 5 ml. vial of Sterile Diluent is supplied with each package.

Under sterile precautions, reconstitute the lyophilized powder with the accompanying 5 ml. vial of Sterile Diluent. Agitate until in solution, then withdraw the required amount. The reconstituted material, being a protein, should be used immediately. The remainder may be stored in the frozen state and thawed at room temperature.

Administer subcutaneously or intramuscularly according to the following schedule:

CATTLE

COWS – 50-100 Units (2-4 ml., intramuscularly) on successive days to retard cyst formation and stimulate normal ovulation. Breeding should be withheld until the second heat period to avoid possible twinning.

BULLS – 50-100 Units (2-4 ml., intramuscularly) on successive days.

HORSES

MARES – 75-125 Units (3-5 ml.). Repeat in 3 weeks if necessary.

STALLIONS – 125 Units (5 ml.). Repeat when necessary.

SHEEP

EWES – 25 Units (1 ml.). Repeat in 16 days if necessary.

RAMS – 50 Units (2 ml.). Repeat when necessary.

HOGS

SOWS – 50 Units (2 ml.). Repeat in 16 days if necessary.

BOARS – 75 Units (3 ml.). Repeat when necessary.

GOATS

DOES – 25 Units (1 ml.). Repeat in 19 days if necessary.

BUCKS – 50 Units (2 ml.). Repeat when necessary.

DOGS

BITCHES UNDER 25 POUNDS – 25 Units (1 ml.). Repeat in 10 days if necessary.

BITCHES OVER 25 POUNDS – 50 Units (2 ml.). Repeat in 10 days if necessary.

MALES UNDER 25 POUNDS – 37½ Units (1-1½ ml.). Repeat when necessary.

MALES OVER 25 POUNDS – 75 Units (3 ml.). Repeat when necessary.

Note: Animals suffering from concurrent uterine or tubal pathology will not respond to this therapy.

PRECAUTIONS: Pituitary fractions are complex proteins. If a reaction due to protein sensitivity occurs, epinephrine should be administered intravenously.

CAUTION: Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

*Fevold, H. L., and Hisaw, S. L. Am. J. Physiol., 109:655 (1934).

April, 1977

TABLE A.2 MANUFACTURER'S BIOASSAY OF EPE

VETERINARY DIVISION
THE MOGUL CORPORATION

FP-13
1/76

QUALITY CONTROL REPORT
for Anterior Pituitary Gonadotropin
125 Fevold-Hisaw Rat Units = 5 ml

Our Lot No. 1803 Expiration Date April 1981 P/O No. _____
Your Lot No. 1803 Invoice No. _____

A. Injection Data:

Dose: 0.25 ml/injection (Total dose = 4 Fevold-Hisaw Rat Units).

Date	4/4/78		4/5/78		4/6/78		Comments:
	time	by	time	by	time	by	
AM	8 15	909	8 30	909	8 12	909	
PM	4 05	909	4 15	909	4 00	909	

B. Ovarian Weight Data: Tare weight 4.522069.

Rat No.	TREATED Gross g.	Net mg	Remarks	CONTROL Gross g.	Net mg	Remarks
1	4.572107	50.01		4.538858	17.02	
2	4.595123	73.01		4.538331	16.25	
3	4.567170	45.69		4.536456	13.54	
4	4.588100	64.94		4.535548	13.43	
5	4.576115	54.09		4.536440	14.34	
6	4.579772	27.66		4.535595	13.86	
7	4.573947	51.31		4.537422	12.36	
8	4.587191	65.95		4.533786	15.85	
	Σ	462.51		Σ	117.09	
	×	57.81		×	14.64	
		98.7%	% of 4x control	×4	58.56	= 100%

Weights and calculations by 909 Checked by BDH Date 4/7/78
Results: 50% - 100% increase in ovarian weight equivalent to 125 Fevold-Hisaw Rat Units per vial.

C. Sterility Data:

Test Dates Started	Concluded	Thioglycollate broth tubes 30°-35°C	Tryptic soy broth tubes 20°-25°C	Results (S)/U/I
3/23/78	4/7/78	30/30 tubes negative	30/30 tubes neg.	Sat.

Test conducted by 909 Released 4/8/78

D. Safety:

Test Dates Started	Concluded	Results (S)/U/I
4/6/78	4/8/78	Sat.

Test conducted by 909

TABLE A.3 MANUFACTURER'S DESCRIPTION OF HCG

CHORIONIC GONADOTROPIN FOR INJECTION U. S. P

DESCRIPTION: Human chorionic gonadotropin (HCG), a polypeptide hormone produced by the human placenta, is composed of an alpha and a beta sub-unit. The alpha sub-unit is essentially identical to the alpha sub-units of the human pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as well as to the alpha sub-unit of human thyroid-stimulating hormone (TSH). The beta sub-units of these hormones differ in amino acid sequence. Chorionic Gonadotropin is derived from the urine of pregnant women. It is standardized by a biological assay procedure.

ACTIONS: The action of HCG is virtually identical to that of pituitary LH, although HCG appears to have a small degree of FSH activity as well. It stimulates production of gonadal steroid hormones by stimulating the interstitial cells (Leydig cells) of the testis to produce androgens and the corpus luteum of the ovary to produce progesterone. Androgen stimulation in the male leads to the development of secondary sex characteristics and may stimulate testicular descent when no anatomical impediment to descent is present. This descent is usually reversible when HCG is discontinued. During the normal menstrual cycle, LH participates with FSH in the development and maturation of the normal ovarian follicle, and the mid-cycle LH surge triggers ovulation. HCG can substitute for LH in this function.

During a normal pregnancy, HCG secreted by the placenta maintains the corpus luteum after LH secretion decreases, supporting continued secretion of estrogen and progesterone and preventing menstruation. HCG HAS NO KNOWN EFFECT ON FAT MOBILIZATION, APPETITE OR SENSE OF HUNGER, OR BODY FAT DISTRIBUTION.

INDICATIONS: HCG HAS NOT BEEN DEMONSTRATED TO BE EFFECTIVE ADJUNCTIVE THERAPY IN THE TREATMENT OF OBESITY. THERE IS NO SUBSTANTIAL EVIDENCE THAT IT INCREASES WEIGHT LOSS BEYOND THAT RESULTING FROM CALORIC RESTRICTION, THAT IT CAUSES A MORE ATTRACTIVE OR "NORMAL" DISTRIBUTION OF FAT, OR THAT IT DECREASES THE HUNGER AND DISCOMFORT ASSOCIATED WITH CALORIE-RESTRICTED DIETS.

1. Prepubertal cryptorchidism not due to anatomical obstruction. In general, HCG is thought to induce testicular descent in situations when descent would have occurred at puberty. HCG thus may help predict whether or not orchiopexy will be needed in the future. Although, in some cases, descent following HCG administration is permanent, in most cases, the response is temporary. Therapy is usually instituted between the ages of 4 and 9.

2. Selected cases of hypogonadotropic hypogonadism (hypogonadism secondary to a pituitary deficiency) in males.

3. Induction of ovulation and pregnancy in the anovulatory, infertile woman in whom the cause of anovulation is secondary and not due to primary ovarian failure, and who has been appropriately pre-treated with human menotropins.

CONTRAINDICATIONS: Precocious puberty, androgen-ostatic carcinoma or other androgen dependent neoplasm, prior allergic reaction to HCG.

WARNINGS: HCG should be used in conjunction with human menopausal gonadotropins only by physicians experienced with infertility problems who are familiar with the criteria for patient selection, contraindications, warnings, precautions, and adverse reactions described in the package insert for menotropins. The principal serious adverse reactions during this use are: (1) Ovarian hyperstimulation, a syndrome of sudden ovarian enlargement, ascites (with or without pain, and/or pleural effusion, (2) Rupture of ovarian cysts with resultant Hemoperitoneum, (3) Multiple births, and (4) Arterial thromboembolism.

PRECAUTIONS: Induction of androgen secretion by HCG may induce precocious puberty in patients treated for cryptorchidism. Therapy should be discontinued if signs of precocious puberty occur.

2. Since androgens may cause fluid retention, HCG should be used with caution in patients with cardiac or renal disease, epilepsy, migraine, or asthma.

ADVERSE REACTIONS: Headache, irritability, restlessness, depression, fatigue, edema, precocious puberty, gynecomastia, pain at the site of injection.

DOSAGE AND ADMINISTRATION (Intramuscular Use Only): The dosage regimen employed in any particular case will depend upon the indication for use, the age and weight of the patient, and the physician's preference. The following regimens have been advocated by various authorities.

Prepubertal cryptorchidism not due to anatomical obstruction

1. 4,000 U.S.P. Units three times weekly for three weeks.
2. 5,000 U.S.P. Units every second day for four injections.
3. 15 injections of 500 to 1,000 U.S.P. Units over a period of six weeks.
4. 500 U.S.P. Units three times weekly for four to six weeks. If this course of treatment is not successful, another is begun one month later, giving 1,000 U.S.P. Units per injection.

Selected cases of hypogonadotrobic hypogonadism in males

1. 500 to 1,000 U.S.P. Units three times a week for three weeks, followed by the same dose twice a week for three weeks.
2. 4,000 U.S.P. Units three times weekly for six to nine months, following which the dosage may be reduced to 2,000 U.S.P. Units three times weekly for an additional three months.

Induction of ovulation and pregnancy in the anovulatory, infertile woman in whom the cause of anovulation is secondary and not due to primary ovarian failure and who has been appropriately pre-treated with human menotropins (See prescribing information for menotropins for dosage and administration for that drug product).

5,000 to 10,000 U.S.P. Units one day following the last dose of menotropins. (A dosage of 10,000 U.S.P. Units is recommended in the labeling for menotropins).

IMPORTANT: USE COMPLETELY WITHIN 60 DAYS AFTER RECONSTITUTION. REFRIGERATE AFTER RECONSTITUTION.

HOW SUPPLIED: The freeze-dried, stabilized active principle is supplied in either the two vial package including Bacteriostatic Water for Injection as diluent or in the Co-vial with the Benzene in the lower and Bacteriostatic Water for Injection in the upper chamber.

When reconstituted, each vial contains in U.S.P. Units:

VIAL SIZE:	10 ml.	10 ml.	30 ml.	10 ml.
Chorionic Gonadotropin	5,000 U.	10,000 U.	15,000 U.	20,000 U.
Mannitol, U.S.P.	50 mg.	100 mg.	150 mg.	200 mg.
Benzyl Alcohol, N.F.	0.9%	0.9%	0.9%	0.9%

with Sodium Phosphate Dibasic and Sodium Phosphate Monobasic to adjust pH.

The product is assayed in accord with the U.S.P. method and potencies refer to U.S.P. Units (International Units) defined in terms of the U.S.P. Chorionic Gonadotropin Reference Standard.

CAUTION: Federal law prohibits dispensing without prescription.

DIRECTIONS FOR RECONSTITUTION: TWO-VIAL PACKAGE: with draw sterile air from lyophilized vial and inject into diluent vial. Remove 10 ml. from diluent and add to lyophilized vial; agitate gently until solution is complete.

DIRECTIONS FOR RECONSTITUTION: Co-vial — remove protective white cap. Turn the red plunger-stopper through 90° and then press it down with thumb so that the liquid in the upper chamber and the red rubber center seal are forced into the lower compartment. Agitate slowly until complete solution. Sterilize the rubber stopper and insert syringe needle squarely into the center until the tip is just visible in the empty upper chamber. Invert vial and withdraw dose in usual manner.

Manufactured by LYPHO-MED, INC., Chicago, Illinois 60651
Revised: March, 1977 Insert No. 30-01

TABLE A.4 MANUFACTURER'S ANALYSIS OF FSH

REHEIS CHEMICAL COMPANY
DIVISION OF ARMOUR PHARMACEUTICAL COMPANY
BOX 811, KANKAKEE, ILL. 60901
AREA CODE 815 • 932-6771

July 6, 1979

A Certificate of Analysis

Invoice No. 217656 Order No. 330847

TO	Michigan State University, Stores Receiving, Animal Husbandry
PRODUCT	Follicle Simulating Hormone
LOT NO.	S16707 DATE OF ANALYSIS August 30, 1978

THIS CERTIFICATE IS A DECLARATION OF ANALYSIS AT TIME OF MANUFACTURE

Potency	51.8 A.U./vial
Moisture	1.4%
Average Fill	29.1 mg./vial
Parenteral Toxicity	Satisfactory
Sterility	Sterile

J.W. Bastian, Ph.D.
Director
Quality Control

JWB:rr

TABLE A.5 MANUFACTURER'S ANALYSIS OF LH

REHEIS CHEMICAL COMPANY
DIVISION OF ARMOUR PHARMACEUTICAL COMPANY
BOX 511, KANKAKEE, ILL. 60901
AREA CODE 815 • 932-5771

July 6, 1979

A Certificate of Analysis

Invoice No. 217656 Order No. 330847

TO	Michigan State University, Stores Receiving, Animal Husbandry
PRODUCT	Pituitary Luteinizing Hormone, Equivalent to 25 mg. Armour Standard /vial
LOT NO.	R16006 DATE OF ANALYSIS August 2, 1977

THIS CERTIFICATE IS A DECLARATION OF ANALYSIS AT TIME OF MANUFACTURE

Potency	22.0 A.U./vial
Moisture	2.6%
Average Weight	3.4 mg./vial
Sterility	Sterile
Parenteral Toxicity	Satisfactory

J.W. Bastian, Ph.D.
Director
Quality Control

JWB:rr

TABLE A.6 ESTROUS SYNCHRONIZATION OF EXPERIMENTAL ANIMALS (INDIVIDUAL DATA)

Trial 1				Trial 2				Trial 3[d]		
Animal No.	Progesterone Concentration[a] (ng/ml)	Hours to Estrus[b]	Body Weight[c] (kg)	Animal No.	Progesterone Concentration[a] (ng/ml)	Hours to Estrus[b]	Body Weight (kg)	Animal No.	Hours to Estrus[b]	Body Weight[c] (kg)
7001	2.95	72	621	1	3.55	78	431	3	52	386
7104	2.41	56	547	2	5.93	78	433	31	59	454
7250	1.97	51	549	4	5.46	48	408	344	72	513
7251	3.05	56	590	5	4.78	48	399	348	72	526
7312	2.82	72	599	6	4.11	48	406	354	59	494
7348	2.23	57	576	11	4.45	72	392	374	59	426
7425	1.15	57	587	12	4.70	72	404	436	59	463
7428	1.36	120	551	13	7.49	58	356	448	59	476
7436	3.21	72	519	15	1.87	96	447			
7450	0.84	48	429	16	3.03	34	404			
7454	3.20	52	499	17	7.16	78	422			
7457	1.73	56	658	19	5.80	78	392			
7525	1.56	28	510	20	4.75	54	379			
7552	3.29	99	465	21	3.88	54	413			
7571	4.37	48	542	23	5.49	48	415			
7607	2.76	80	481	24	4.53	96	424			
7620	1.59	51	440	25	4.55	48	395			
7630	0.69	56	601	26	3.37	58	424			
7637	2.01	76	608	27	5.58	48	408			
7639	0.38	33	454	28	4.03	58	406			
7659	6.02	52	376	29	5.62	58	356			
7663	3.30	52	490	30	6.05	34	404			
7720	3.56	51	417	34	7.12	48	361			
7724	1.37	28	513	35	5.36	48	390			
7730	1.99	48	494	37	3.25	34	438			
7733	2.01	48	376	38	5.67	58	442			
7744	1.32	52	508							

[a] Serum progesterone concentration at time of 2nd $PGF_{2\alpha}$ injection.

[b] Hours to standing estrus or estrus behavior following 2nd $PGF_{2\alpha}$ injection.

[c] Weight taken at time of 2nd $PGF_{2\alpha}$ injection.

[d] No blood samples were taken from cattle on Trial 3.

TABLE A.7 INDIVIDUAL OVARIAN AND EMBRYO DATA FOR TRIAL 1

Treatment	Anim. No.	Breed	Age	Side[a]	Total Ovary Wt., g	Stromal and Luteal Tissue Wt., g	Follicular Fluid Wt., g	No. Follicles ≤9 mm	No. Follicles ≥10 mm	No. Corpora Lutea	Total No. Eggs and Embryos Recovered
Control	7250	Hereford	7	L	10.0	7.5	2.5	6	0	0	0
				R	10.0	7.7	2.3	7	0	1	1
	7525	Hereford	4	L	11.2	9.4	1.8	12	1	1	0
				R	6.9	5.6	1.3	15	0	0	0
	7637	HoAnHf[b]	3	L	5.7	3.4	2.3	4	1	0	0
				R	7.6	6.8	.8	2	0	1	1
	7733	Hereford	2	L	8.0	7.3	.7	0	0	1	0
				R	5.1	4.4	.7	2	0	0	0
Low Dose EPE	7104	Hereford	8	L	35.5	29.4	6.1	4	0	18	3
				R	45.5	39.9	5.6	5	1	22	3
	7251	HoAnHf[b]	7	L	44.2	18.1	26.1	12	1	1	0
				R	38.8	14.8	24.0	13	8	1	0
	7552	Hereford	4	L	43.7	22.0	21.7	6	7	11	5
				R	49.5	24.6	24.9	13	4	12	3
	7571	HoAnHf[b]	4	L	41.7	28.1	13.6	15	3	16	0
				R	88.0	56.7	31.3	23	3	32	0
	7607	Hereford	3	L	75.8	34.5	41.3	19	7	13	4
				R	65.1	36.3	28.8	25	5	10	6
	7744	ChAnHf[c]	2	L	92.9	75.8	17.1	15	2	30	2
				R	115.1	77.0	38.1	14	6	23	4
Low Dose EPE + HCG	7348	Hereford	6	L	69.5	26.7	42.8	0	6	0	0
				R	105.1	35.7	69.4	0	20	20	4
	7450	Hereford	5	L	54.9	16.0	38.9	11	12	0	0
				R	65.3	16.2	49.1	4	15	0	0
	7457	Hereford	5	L	147.9	27.9	120.0	13	21	1	0
				R	165.1	28.3	136.8	6	19	0	1
	7630	ChAnHf[c]	3	L	22.6	12.5	10.1	6	4	12	0
				R	17.4	10.4	7.0	2	6	9	0
	7659	Hereford	3	L	50.9	16.6	34.3	7	14	0	0
				R	35.6	15.6	20.0	4	8	1	0
	7720	Hereford	2	L	75.1	12.0	63.1	15	18	0	0
				R	67.3	15.6	51.7	11	12	1	0

[a]Refers to left or right ovary and uterine horn.

[b]Holstein x Angus x Hereford

[c]Charolais x Angus x Hereford

TABLE A.7--CONTINUED

Treatment	Anim. No.	Breed	Age	Side[a]	Total Ovary Wt., g	Stromal and Luteal Tissue Wt., g	Follicular Fluid Wt., g	No. Follicles ≤9 mm	No. Follicles ≥10 mm	No. Corpora Lutea	Total No. Eggs and Embryos Recovered
High Dose EPE	7001	ChAnHf[c]	9	L	60.0	43.9	16.1	14	5	21	5
				R	50.2	31.0	19.2	14	6	17	4
	7312	Hereford	6	L	61.4	39.8	21.6	8	3	9	7
				R	53.1	37.9	15.2	1	7	10	1
	7425	Hereford	5	L	129.3	85.4	43.9	18	4	37	3
				R	174.4	129.9	44.5	9	4	59	18
	7620	Hereford	3	L	71.3	18.1	53.2	13	8	4	0
				R	115.8	27.2	88.6	12	14	8	0
	7663	Hereford	3	L	40.3	27.6	12.7	6	2	48	2
				R	41.0	28.7	12.3	5	0	45	4
	7730	ChAnHf[c]	2	L	124.0	20.5	103.5	52	17	0	0
				R	154.3	26.2	128.1	67	28	1	0
High Dose EPE + HCG	7428	ChAnHf[c]	5	L	53.5	36.7	16.8	5	3	9	2
				R	52.1	27.5	24.6	10	8	8	2
	7436	ChAnHf[c]	5	L	175.0	33.2	141.8	8	13	0	1
				R	166.8	32.4	134.4	5	21	1	0
	7454	Hereford	5	L	114.0	18.0	96.0	0	13	0	0
				R	196.1	34.6	161.5	0	15	0	0
	7639	ChAnHf[c]	3	L	78.1	14.6	63.5	0	11	0	0
				R	137.5	27.7	109.8	0	14	0	0
	7724	HoAnHf[b]	2	L	24.3	14.0	10.3	11	15	25	2
				R	65.5	26.1	39.4	3	12	26	10

[a]Refers to left or right ovary and uterine horn.

[b]Holstein x Angus x Hereford

[c]Charolais x Angus x Hereford

TABLE A.8 INDIVIDUAL OVARIAN AND EMBRYO DATA FOR TRIAL 2

Treatment	Anim. No.	Side[a]	Total Ovary Wt., g	Stromal and Luteal Tissue Wt., g	Follicular Fluid Wt., g	No. Follicles ≤9 mm	No. Follicles ≥10 mm	No. Corpora Lutea	Total No. Eggs and Embryos Recovered	No. Fertile Embryos[b]
High Dose	4	L	11.3	11.2	.1	2	0	4	4	5
EPE		R	9.2	8.0	1.2	3	1	2	2	
2 Inj./day	13	L	20.1	19.4	.7	5	1	8	1	2
3 Days		R	25.8	25.5	.3	3	0	7	1	
	20	L	29.6	18.5	11.1	20	4	1	0	0
		R	5.5	4.4	1.1	9	1	0	0	
	35	L	30.3	18.7	11.6	5	3	6	5	10
		R	46.0	36.7	9.3	10	3	12	5	
High Dose	5	L	13.4	12.2	1.2	0	3	2	0	0
EPE		R	6.1	4.5	1.6	2	1	0	0	
1 Inj./day	21	L	19.6	18.6	1.0	5	1	13	11	20
3 Days		R	25.2	23.8	1.4	8	1	16	13	
	24	L	13.5	8.6	4.9	0	2	2	1	1
		R	2.0	1.8	.2	1	0	0	0	
	37	L	76.7	46.6	30.1	0	1	10	8	11
		R	25.2	24.9	.3	3	0	5	3	
Low Dose	6	L	6.8	6.7	.1	0	1	1	1	3
EPE		R	19.3	18.5	.8	1	1	5	3	
2 Inj./day	16	L	11.4	8.3	3.1	3	2	1	2	6
3 Days		R	16.7	15.4	1.3	8	1	5	5	
	25	L	14.2	13.8	.4	12	0	5	4	10
		R	16.5	16.1	.4	12	0	6	6	
	26	L	20.9	15.9	5.0	25	4	4	2	3
		R	14.0	9.4	4.6	15	4	1	1	
Low Dose	15	L	12.7	4.8	7.9	12	3	0	0	0
EPE		R	10.9	6.2	4.7	16	2	0	0	
1 Inj./day	23	L	10.6	9.8	.8	5	0	1	0	0
3 Days		R	16.3	16.0	.3	3	0	4	1	
	27	L	34.1	23.7	10.4	1	3	7	3	7
		R	33.7	29.4	4.3	3	2	12	12	
	28	L	24.9	19.8	5.1	8	3	4	4	6
		R	12.8	11.5	1.3	9	1	2	2	

[a]Refers to left or right ovary and uterine horn.

[b]Left and right sides were combined prior to determining fertilization.

TABLE A.8--CONTINUED

Treatment	Anim. No.	Side[a]	Total Ovary Wt., g	Stromal and Luteal Tissue Wt., g	Follicular Fluid Wt., g	No. Follicles ≤9 mm	No. Follicles ≥10 mm	No. Corpora Lutea	Total No. Eggs and Embryos Recovered	No. Fertile Embryos[b]
Low Dose EPE	1	L	5.2	3.8	1.4	12	1	0	0	0
		R	7.8	4.8	3.0	14	3	0	0	
1 Inj./day	11	L	8.7	8.4	.3	3	0	2	2	5
2 Days		R	16.3	15.4	.9	4	1	4	3	
	17	L	32.0	11.9	20.1	14	6	0	0	0
		R	13.3	6.4	6.9	10	2	0	0	
	29	L	3.5	2.2	1.3	8	1	0	0	0
		R	6.9	6.6	.3	11	0	1	0	
	30	L	2.0	1.8	.2	0	0	0	0	2
		R	19.0	17.6	1.4	2	1	3	2	
FSH - P	2	L	13.1	9.1	4.0	4	1	1	1	2
2 Inj./day		R	13.2	8.0	5.2	4	0	1	1	
5 Days	12	L	21.4	21.0	.4	1	0	6	6	12
		R	28.4	28.3	.1	2	0	6	6	
	19	L	21.0	20.3	.7	6	1	7	3	5
		R	21.7	21.3	.4	14	0	7	5	
	34	L	16.2	13.5	2.7	13	1	5	6	13
		R	35.2	32.2	3.0	28	0	8	8	
	38	L	35.6	34.4	1.2	17	0	12	10	17
		R	39.5	38.0	1.5	17	1	12	10	

[a]Refers to left or right ovary and uterine horn.

[b]Left and right sides were combined prior to determining fertilization.

TABLE A.9 INDIVIDUAL OVARIAN AND EMBRYO DATA FOR TRIAL 3

Treatment	Anim. No.	Side[a]	Total Ovary Wt., g	Stromal and Luteal Tissue Wt., g	Follicular Fluid Wt., g	No. Follicles ≤9 mm	No. Follicles ≥10 mm	No. Corpora Lutea	Total No. Eggs and Embryos Recovered	No. Fertile Embryos[b]
Variable	3	L	19.8	6.1	13.7	13	18	0	0	0
Dose		R	25.2	8.9	16.3	30	18	0	0	
EPE	348	L	19.6	17.4	2.2	13	0	8	5	9
1 Inj./day		R	27.2	25.8	1.4	6	1	9	8	
5 days	374	L	4.5	3.4	1.1	4	1	1	0	0
		R	2.9	2.5	0.4	3	0	0	0	
	448	L	25.9	9.1	16.8	12	22	0	0	0
		R	26.5	7.8	18.7	20	28	0	0	
Constant	31	L	20.4	14.4	6.0	11	2	4	4	6
Dose		R	19.0	11.2	17.8	8	5	2	2	
EPE	344	L	8.5	8.3	0.2	1	0	7	7	8
1 Inj./day		R	7.7	7.4	0.3	6	0	4	4	
5 days	354	L	28.1	27.2	0.9	9	0	10	5	12
		R	37.5	34.1	3.4	10	2	14	10	
	436	L	8.8	6.0	2.8	10	3	1	1	0
		R	13.5	12.9	0.6	5	0	3	0	

[a]Refers tp left or right ovary and uterine horn.

[b]Left and right sides were combined prior to determining fertilization.

LITERATURE CITED

LITERATURE CITED

Albers, W. 1978. Testing the mean of a normal population under dependence. Annals. Statist. 6:1337.

Allen, D. M., and G. E. Lamming. 1961. Nutrition and reproduction in the ewe. J. Agr. Sci. 56:69.

Avery, T. L., M. L. Fahning and E. F. Graham. 1962. Investigations associated with the transplantation of bovine ova. II. Superovulation. J. Reprod. Fertil. 3:212.

Bellows, R. A., D. C. Anderson and R. E. Short. 1969. Dose-response relationships in synchronized beef heifers treated with follicle stimulating hormone. J. Anim. Sci. 28:638.

Betteridge, K. J. 1977. Embryo transfer in farm animals. A review of techniques and applications. Canada Dept. of Agriculture, Monograph 16.

Booth, W. D., R. Newcomb, H. Strange, L. E. A. Rowson and H. B. Sacher. 1975. Plasma oestrogen and progesterone in relation to super-ovulation and egg recovery in the cow. Vet. Rec. 97:366.

Brand, A., M. H. Aarts, D. Zaayer and W. D. Oxender. 1979. Recovery and transfer of embryos by non-surgical procedures in lactating dairy cattle. (In press.)

Burfening, P. J., D. C. Anderson, R. A. Kinkie, J. Williams and R. L. Friedrich. 1978. Synchronization of estrus with $PGF_{2\alpha}$ in beef cattle. J. Anim. Sci. 47:999.

Butcher, R. L., W. E. Colins and N. W. Fugo. 1974. Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinol. 94:1704.

Cahill, L. P., J. C. Mariana and P. Mauleon. 1979. Total follicular populations in ewes of high and low ovulation rates. J. Reprod. Fertil. 55:27.

Convey, E. M., T. W. Beck, R. R. Neitzel, E. F. Bostwick and H. D. Hafs. 1977. Negative feedback control of bovine serum LH concentration from completion of the preovulatory LH surge until resumption of luteal function. J. Anim. Sci. 45:792.

Danner, M. L., W. D. Oxender, R. L. Fogwell, and R. H. Douglas. 1979. Use of an equine pituitary extract with and without HCG to superovulate cows. Theriogenology 11:96. Abstr.

Dickman, Z. 1969. Shedding of the zona pellucida. In Advances in Reproductive Physiology. Vol. 4. Edit., A. McLaven.

Dowling, D. F. 1949. Problems of the transplantation of fertilized ova. J. Agric. Sci. Camb. 39:374.

Dunn, T. G., J. E. Ingalls, D. R. Zimmerman and J. N. Wiltbank. 1969. Reproductive performance of 2-year-old Hereford and Angus heifers as influenced by pre- and post-calving energy intake. J. Anim. Sci. 29:719.

Elsden, R. P., S. Lewis, I. A. Cumming and R. A. S. Lawson. 1974. Superovulation in the cow following treatment with PMSG and prostaglandin $F_{2\alpha}$. J. Reprod. Fertil. 36:455. Abstr.

Elsden, R. P., J. F. Hasler and G. E. Seidel (Jr.). 1976. Non-surgical recovery of bovine eggs. Theriogenology 6:523.

Elsden, R. P., L. D. Nelson and G. E. Seidel (Jr.). 1978. Superovulating cows with follicle stimulating hormone and pregnant mare's serum gonadotrophin. Theriogenology 9:17.

Ensminger, M. E. 1970. Breeding sheep. In Sheep and Wool Science. The Interstate Printers and Publishers, Danville, Illinois.

Fevold, H. L. 1939. Extraction and standardization of pituitary follicle-stimulating and luteinizing hormones. Endocrinol. 24:435.

Fevold, H. L., and S. L. Hisaw. 1934. Interactions of gonad stimulating hormones in ovarian development. Am. J. Physiol. 109:655.

Ford, S. P., and F. Stormshak. 1978. Bovine ovarian and pituitary responses to PMSG and GnRH administered during metestrus. J. Anim. Sci. 46:1701.

Gengenbach, D. R., N. Butendieck, P. M. Riek, R. L. Scipioni, E. B. Oltenacu and R. H. Foote. 1978. Controlled superovulation in dairy heifers using prostaglandin $F_{2\alpha}$ and pregnant mare serum gonadotropin. J. Anim. Sci. 46:1293.

Gill, J. L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Iowa State University Press, Ames.

Gill, J. L., and H. D. Hafs. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33:331.

Goodman, H. M. 1974. Reproduction. In Medical Physiology. Vol. 2. C. V. Mosby, St. Louis.

Gordon, I. 1975. Problems and prospects in cattle egg transfer. Irish Vet. J. 21:21 and 29:30.

Graham, E. F. 1974. Do you know these transplant facts? Simmental Shield. June 1974.

Greve, T., and H. Lehn-Jensen. 1979. Embryo transplantation in cattle. Acta Vet. Scand. 20:135.

Hafez, E. S. E. 1974. Physiology of reproduction. In Reproduction in Farm Animals. Lea and Febiger, Philadelphia.

Hafez, E. S. E., E. Rajakoski, P. B. Anderson, O. L. Frost and G. Smith. 1964. Problems of gonadotropin-induced multiple pregnancy in beef cattle. Am. J. Vet. Res. 25:1074.

Hafez, E. S. E., T. Sugie and I. Gordon. 1963. Superovulation and related phenomena in the beef cow. I. Superovulatory responses following PMS and HCG injections. J. Reprod. Fertil. 5:359.

Hallford, D. M., E. J. Turman, R. P. Wetteman and C. E. Pope. 1975. Plasma LH and estradiol in the bovine after PMSG. J. Anim. Sci. 41:356. Abstr.

Hallford, D. M., E. J. Turman, R. P. Wettemann and C. E. Pope. 1979. Endocrine and reproductive response of beef cows to PMSG. J. Anim. Sci. 49:1030.

Hallford, D. M., E. J. Turman, R. P. Wetteman, C. E. Pope and D. C. Meyerhoeffer. 1975. Reproductive response of the bovine to PMSG. J. Anim. Sci. 40:187. Abstr.

Harper, M. J. K., and M. C. Chang. 1971. Some aspects of the biology of mammalian eggs and spermatozoa. In Advances in Reproductive Physiology. Vol. 5. Edit., M. W. Bishop.

Hasler, J. F. 1978. Superovulation of the lactating dairy cow. Theriogenology 9:94.

Henricks, D. M., and J. R. Hill. 1978. Effects of PMSG and $PGF_{2\alpha}$ on gonadal hormones and reproduction in the beef heifer. J. Anim. Sci. 46:1309.

Henricks, D. M., J. R. Hill (Jr.), J. F. Dickey and D. R. Lamond. 1973. Plasma hormone levels in beef cows with induced multiple ovulations. J. Reprod. Fertil. 35:225.

Henricks, D. M., and D. R. Lamond. 1972. Hormonal interrelations in beef cows with induced multiple ovulations. Nature 235:222.

Humphrey, W. D., B. D. Murphy, D. Reiger, R. J. Mapletoft, J. G. Manns and P. B. Fretz. 1979. Effects of FSH/LH ratio of PMSG on ovulatory responses. Theriogenology 11:101.

Jainudeen, M. R., E. S. E. Hafez, P. D. Gollnick and L. A. Moustafa. 1966. Antigonadotropins in the serum of cows following repeated therapeutic pregnant mare serum injections. Am. J. Vet. Res. 27:669.

Laster, D. B. 1973. Ovulation, fertility and prenatal mortality in heifers treated with PMSG or porcine FSH. J. Reprod. Fertil. 33:275.

Lemon, M., and J. Saumande. 1972. Oestradiol-17β and progesterone after induction of superovulation by PMSG in cattle. J. Reprod. Fertil. 3:501. Abstr.

Lemon, M., and J. Saumande. 1974. The evolution of ovarian steroid hormones during luteolysis and folliculogenesis in the cow. Europ. J. Obstet. Gynecol. Reprod. Biol. 4, Suppl.:S69-S75.

Lopez-Barbella, S. R., A. C. Warnick, T. H. Wise and M. J. Fields. 1979. Endocrine response of the cow to PMSG and subsequent multiple corpora lutea regression by prostaglandin $F_{2\alpha}$. J. Anim. Sci. 48:1135.

Louis, T. M., H. D. Hafs and B. E. Seguin. 1973. Progesterone, LH, estrus and ovulation after prostaglandin $F_{2\alpha}$ in heifers. Proc. Soc. Exp. Biol. Med. 143:152.

Mariana, J. G., P. Mauleon, M. Benoit and D. Chupin. 1970. Variability of the number of ovulations obtained after injection of 1600 I.U. PMSG and 1500 I.U. HCG. Ann. Biol. Anim. Biochem. Biophys. 1:47.

Miller, R. G. (Jr.). 1966. Simultaneous Statistical Inference. McGraw-Hill, New York.

Moore, N. W. 1975a. The control of time of estrous and ovulation and the induction of superovulation in cattle. Aust. J. Agric. Res. 26:295.

Moore, N. W. 1975b. The use of prostaglandin $F_{2\alpha}$ given by either intrauterine infusion or by intramuscular injection for the control of oestrus and ovulation in cattle. Ann. Biol. Anim. Biochem. Biophys. 15:451.

Moore, N. W., and J. N. Shelton. 1962. Oestrus and ovarian response of the ewe to a horse anterior pituitary extract. Nature (Lond.) 194:1283.

Moore, N. W., and J. N. Shelton. 1964. Response of the ewe to a horse anterior pituitary extract. J. Reprod. Fertil. 7:79.

Nelson, L. D., R. A. Bowen, N. R. Homan and G. E. Seidel (Jr.). 1976. Seminal treatments for superovulated heifers. Proc. 89th Ann. Res. Conf. Colorado State University. Abstr. 109.

Newcomb, R., W. B. Christie, L. E. A. Rowson, D. E. Walters and W. E. D. Bousfield. 1979. Influence of dose, repeated treatment and batch of hormone on ovarian response in heifers treated with PMSG. J. Reprod. Fertil. 56:113.

Newcomb, R., and L. E. A. Rowson. 1976. Multiple ovulation, egg transplantation: towards twinning. In Principles of Cattle Reproduction, ed. H. Swan and W. H. Broster. University of Nottingham, Butterworths, London.

Oxender, W. D., P. A. Noden and H. D. Hafs. 1977. Estrus, ovulation and serum progesterone, estradiol and LH concentrations in mares after an increased photoperiod during winter. Am. J. Vet. Res. 38:203.

Oxender, W. D., P. A. Noden, T. M. Louis and H. D. Hafs. 1974. A review of prostaglandin $F_{2\alpha}$ for ovulation control in cows and mares. Am. J. Vet. Res. 35:997.

Peters, J. B., J. A. Welch, J. W. Lauderdale and E. K. Inskeep. 1977. Synchronization of estrus in beef cattle with $PGF_{2\alpha}$ and estradiol benzoate. J. Anim. Sci. 45:230.

Phillippo, M., and L. E. A. Rowson. 1975. Prostaglandins and superovulation in the bovine. Ann. Biol. Anim. Biochem. Biophys. 15:233.

Rajakoski, E. 1960. The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical and left-right variations. Acta Endocrinologica. Suppl. 52:7.

Salisbury, G. W., and N. L. Vandemark. 1961. Physiology of Reproduction and Artificial Insemination of Cattle. W. H. Freeman and Co., San Francisco.

Saumande, J., and J. Pelletier. 1975. Relationship of plasma levels of oestradiol 17β and luteinizing hormone with ovulation rate in superovulated cattle. J. Endocrinol. 64:189.

Seidel, G. E. (Jr.), R. A. Bowen, L. D. Nelson and N. R. Homan. 1975. Responses of cattle to superovulation treatments. Proc. 88th Ann. Res. Conf., Colorado State University. Abstr. 163.

Seidel, G. E. (Jr.), S. M. Seidel and R. A. Bowen. 1978. Bovine embryo transfer procedures. Colo. State Univ. Expt. Sta., General Series 975.

Self, H. L., R. H. Grummer and L. E. Casida. 1955. The effects of various sequences of full and limited feeding on the reproductive phenomena in Chester White and Poland China gilts. J. Anim. Sci. 14:573.

Shea, B. F., D. J. Hines, D. E. Lightfoot, G. W. Ollis and S. M. Olson. 1976. The transfer of bovine embryos. In Egg Transfer in Cattle; Commission of the European Communities, ed. L. E. A. Rowson.

Shelton, J. N., and N. W. Moore. 1967. The response of the ewe to PMS and to horse anterior pituitary extract. J. Reprod. Fertil. 14:175.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames.

Solti, L., T. Greve and H. H. Koefoed-Johnson. 1978. Plasma progesterone assays in superovulated cattle. Acta Vet. Scand. 19:298.

Sorenson, A. M. (Jr.), W. B. Thomas and J. W. Gosset. 1961. A further study of the influence of level of energy intake and season on reproductive performance of gilts. J. Anim. Sci. 20:347.

Spilman, C. H., G. E. Seidel (Jr.), L. L. Larson, G. R. Vukman and R. H. Foote. 1973. Progesterone, 20β-hydroxypregn-4-en-3-one, and luteinizing hormone levels in superovulated prepuberal and postpuberal cattle. Biol. Reprod. 9:116.

Sreenan, J. M. 1976. The effect of cycle stage and plasma progesterone level on ovarian response to PMSG stimulation in the bovine. Proc. Soc. Study Fertil., Sheffield.

Sreenan, J. M., and D. Beehan. 1976. Methods of induction of superovulation in the cow and transfer results. In Egg Transfer in Cattle, ed. L. E. A. Rowson, Commission of the European Communities.

Staigmiller, R. B., R. E. Short, R. A. Bellows and J. B. Carr. 1979 Effect of nutrition on response to exogenous FSH in beef cattle. J. Anim. Sci. 48:1182.

Stewart, F., W. R. Allen and R. M. Moor. 1976. Pregnant mare serum gonadotropin: ratio of follicle stimulating hormone and luteinizing hormone activities measured by radioreceptor assay. J. Endocrinol. 71:371.

Swaminathan, N., and O. P. Bahl. 1970. Dissociation and recombination of the subunits of human chorionic gonadotropin. Biochem. Biophys. Res. Commun. 40:422.

Turman, E. J., R. P. Wettemann and J. G. MaGee. 1978. Response of cows to repeated treatments with PMS. Okla. Agr. Expt. Sta. Res. Rpt., p. 215.

Willett, E. L., P. J. Buckner and W. H. McShan. 1953. Refractoriness of cows repeatedly superovulated with gonadotropins. J. Dairy Sci. 36:1083.

Wiltbank, J. N., C. W. Kassan and J. E. Ingalls. 1969. Puberty in crossbred and straightbred beef heifers on two levels of feed. J. Anim. Sci. 29:602.

Zimmerman, D. R., H. G. Spies, E. M. Rigor, H. L. Self and L. E. Casida. 1960. Effect of restricted feeding, crossbreeding, and season of birth on age at puberty in swine. J. Anim. Sci. 19:687.

This is to certify that the

thesis entitled

EFFICACY OF AN EQUINE PITUITARY

EXTRACT TO SUPEROVULATE COWS

presented by

Myron Lindle Danner

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Animal Science

David R. Hawkins

Major professor

Date July 21, 1981