Estrus synchronization in cattle and sheep using orally active progestogen
by Dharam Singh Dhindsa

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science (Animal Physiology)
Montana State University

Abstract:
An investigation was made to synchronize estrus in beef cows by feeding a progestogen (6 a-methyl 17 a-hydroxy-progesterone acetate). A total of 284 cattle were used in three trials at three different locations. The hormone was mixed with the concentrate at the rate of 90 mg. per pound and the hormone fed cattle (groups1-C and 2-C) were group fed 2 pounds of this concentrate per cow per day for 18 days. The control animals in Trials I and II received 2 pounds of the same concentrate free of hormone at the same rate and in Trial III the control cows received no concentrate.

Standing estrus was exhibited by 62.4 percent of the cows in a 3% day period following hormone withdrawal in the progestogen fed groups as compared to 9.9 percent in the controls. Hormone feeding significantly ($P<.01$) increased the number of cows showing estrus during the 3% day period following treatment but the number of feedings (1-S vs 2-S) had no significant ($P>.05$) effect.

Conception to first service pbst feeding was 32.8 and 36.7 percent for the hormone fed and control groups, respectively. Total conception rate to breeding during three cycles was 96.6 and 83.3 percent for the progestogen fed and control groups, respectively. These results indicate that progestogen feeding lowered conception rate to first estrus following hormone withdrawal and possibly enhanced overall conception rate.

One trial was conducted with 108 ewes in an attempt to synchronize estrus and to determine the effect of progestogen feeding on reproductive performance. Two groups, 1-S and 2-S, were fed one pound of grain per ewe per day which contained 50 mg, of the progestogen and feeding continued for 18 days. The control group, S, was fed the same ration without the progestogen at the same rate and for the same period of time. The ewes in groups S and 1-S were placed in the breeding pens immediately following hormone withdrawal and the ewes in group 2-S were placed in the breeding pens 9 days after hormone withdrawal.

During a 3 day period beginning 24 hours after hormone withdrawal, 94.7 and 39.4 percent of the ewes exhibited estrus in groups 1-S and S, respectively. These differences due to treatment were highly significant ($P<.01$) . The ewes remained synchronized during the second cycle as indicated by 94,8 percent of the ewes in group 2-S exhibiting estrus during the three day period beginning 18 days following hormone withdrawal.

Conception to first service was 67.7, 61.8 and 80.0 percent for groups S, 1-S and 2-S, respectively. Overall conception rates were 90.0, 100.0 and 97.1 percent for groups S, 1-S and 2-S, respectively. Progestogen feeding slightly reduced the conception rate following breeding at first post treatment estrus but overall lambing performance was higher in the two hormone fed groups. Progestogen feeding had no significant ($P>.05$) effect on lambing rate or type of birth distribution.

ESTRUS SYNCHRONIZATION IN CATTLE AND SHEEP USING ORALLY ACTIVE PROGESTOGEN

by

DHARAM SINGH DHINDSA

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science
(Animal Physiology)

Approved:

Fred S. Willson
Head, Major Department

Art Hovesland
Chairman, Examining Committee

Dean, Graduate Division

MONTANA STATE COLLEGE
Bozeman, Montana

August, 1963

ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to Professor A. S. Hoversland for encouragement and guidance throughout the entire graduate program, and for helpful suggestions in conducting the experimental work and writing the manuscript; to Dr. E. P. Smith for his suggestions pertaining to the manuscript and his guidance and assistance in statistical analysis of data; to Dr. D. W. Blackmore for helpful suggestions in the preparation of the manuscript and to Professor J. L. Van Horn for making adequate numbers of sheep available for the trial.

Appreciation is also expressed to Professor F. S. Willson for awarding the author a research assistantship which made it possible for him to pursue graduate work at Montana State College.

Greatful acknowledgement is made to livestock producers who cooperated on this trial, Mr. Jess Kilgore, Mr. Nick Muir and Mr. George Brewster, who made their cattle available for the trial and for their assistance in collecting the data.

Appreciation is extended to Dr. Roland Gessert of the Upjohn Co., Kalamazoo, Michigan who made the Repromix available for the trial.

Sincere appreciation is expressed to my wife, mother and brothers who made it possible for me to attain this goal through their sacrifices, work and encouragement.

TABLE OF CONTENTS

Page

INDEX TO TABLES

INDEX TO FIGURES

ABSTRACT

An investigation was made to synchronize estrus in beef cows by feeding a progestogen (6 a-methyl 17 a-hydroxy-progesterone acetate). A total of 284 cattle were used in three trials at three different locations. The hormone was mixed with the concentrate at the rate of 90 mg. per pound and the hormone fed cattle (groups1-C and 2-C) were group fed 2 pounds of this concentrate per cow per day for 18 days. The control animals in Trials I and II received 2 pounds of the same concentrate free of hormone at the same rate and in Trial III the control cows received no concentrate.

Standing estrus was exhibited by 62.4 percent of the cows in a 3½ day period following hormone withdrawal in the progestogen fed groups as compared to 9.9 percent in the controls. Hormone feeding significantly ($P<.01$) increased the number of cows showing estrus during the 3½ day period following treatment but the number of feedings (1-S vs 2-S) had no significant ($P>.05$) effect.

Conception to first service post feeding was 32.8 and 36.7 percent for the hormone fed and control groups, respectively. Total conception rate to breeding during three cycles was 96.6 and 83.3 percent for the progestogen fed and control groups, respectively.* These results indicate that progestogen feeding lowered conception rate to first estrus following hormone withdrawal and possibly enhanced overall conception rate.

One trial was conducted with 108 ewes in an attempt to synchronize estrus and to determine the effect of progestogen feeding on reproductive performance. Two groups, 1-S and 2-S, were fed one pound of grain per ewe per day which contained 50 mg. of the progestogen and feeding continued for 18 days. The control group, S, was fed the same ration without the progestogen at the same rate and for the same period of time. The ewes in groups S and 1-S were placed in the breeding pens immediately following hormone withdrawal and the ewes in group 2-S were placed in the breeding pens 9 days after hormone withdrawal.

During a 3 day period beginning 24 hours after hormone withdrawal, 94.7 and 39.4 percent of the ewes exhibited estrus in groups 1-S and S, respectively. These differences due to treatment were highly significant ($P<.01$). The ewes remained synchronized during the second cycle as indicated by 94.8 percent of the ewes in group 2-S exhibiting estrus during the three day period beginning 18 days following hormone withdrawal.

Conception to first service was 67.7, 61.8 and 80.0 percent for groups S, 1-S and 2-S, respectively. Overall conception rates were 90.0, 100.0 and 97.1 percent for groups S, 1-S and 2-S, respectively. *Progestogen feeding slightly reduced the conception rate following breeding at first post treatment estrus but overall lambing performance was higher in the two hormone fed groups. Progestogen feeding had no significant ($P>.05$) effect on lambing rate or type of birth distribution.

INTRODUCTION

The word synchronization means to bring together or to cause to happen at the same time. Synchronized estrus is when all the females exhibit estrus either at the same time or over a much shorter interval than occurs under natural conditions.

It has long been the goal of reproductive physiologists to control reproductive phenomena of animals. Artificial insemination is one method of reproductive control which has been developed and successfully applied in the livestock industry and particularly with cattle. In the western range area there are some limitations in applying artificial insemination due to the necessity of congregating large numbers of cows in a small area for a 25 to 35 day period. If some means were available to effectively synchronize estrus in cattle it would alleviate this problem and make possible the application of artificial insemination to a much larger segment of the cattle industry.

Many new developments have appeared in the area of reproductive control the past decade. Fertilized ova have been successfully collected and transferred from one female to another in mice, rabbits, sheep and cattle. Fertilized ova recovered from bred cows and ewes a few days post breeding have been collected, transferred into rabbits and shipped from one country to another and later recovered and transferred to the appropriate species and have resulted in live calves and lambs born. Media have been developed to store fertilized ova for an extended period of time. Semen has been successfully frozen and rodent ova have also been successfully frozen and later capable of development. Research efforts are now underway to control the sex of the offspring. Considerable work has been in progress in

in attempts to find methods of synchronizing estrus which will be easy to apply, very effective, cheap and with little or no side effects. The production of a very effective method of synchronizing heat is a necessity before many of the above mentioned procedures can be effectively applied in the livestock industry.

Much of the synchronization work reported to date has involved small numbers of animals and many trials have not been under western range conditions. It was therefore considered important to study synchronization of estrus in cattle and sheep under conditions as they prevail on the farm.

LITERATURE REVIEW

SOME BASIC CONCEPTS OF HORMONE ACTION AND INTERACTION

An application of recent advances made in our knowledge of the mechanism regulating ovarian function in cattle is essential to a clear understanding of the developments now occurring in the field of estrous cycle regulation.

The basic interactions between the pituitary and the ovary, shown schematically in a somewhat over simplified form appear in Figure 1 (Hansel 1961).

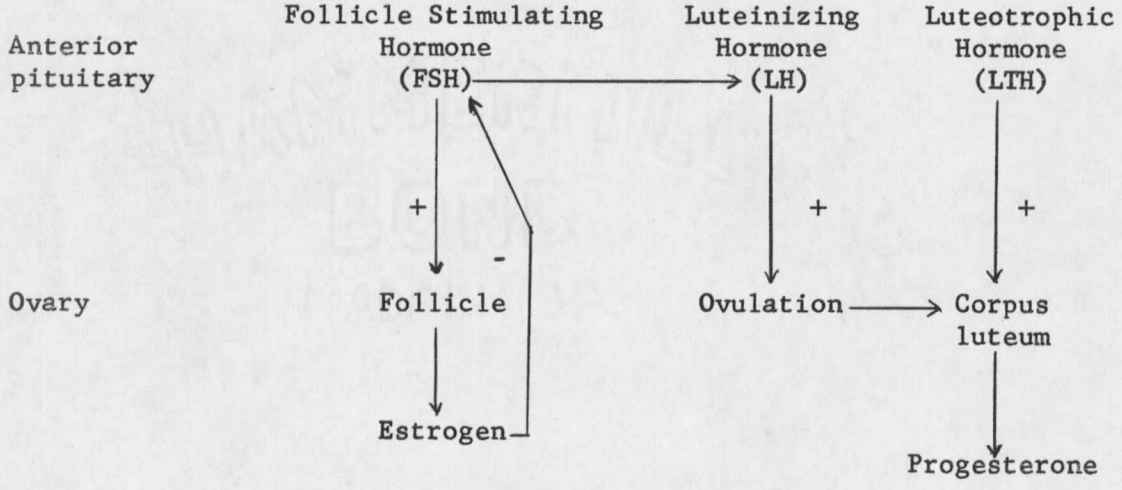

Figure 1. Interrelations between the anterior pituitary and the ovary.

Follicle growth and ovum maturation occur primarily under the influence of FSH, although small amounts of LH undoubtedly synergize the reaction. This rapid follicular growth, which occurs between the 10th day of the cycle and the beginning of the estrus in the cow is associated with increased estrogen production. Estrogen is thought to inhibit the pituitary production of FSH and to facilitate the release of sufficient amounts of pituitary LH to cause ovulation in addition to bringing the animal into estrus. The gonadotrophins producing basophils (delta cells) in the bovine pituitary

undergo a very rapid process of degranulation just before the beginning of estrus, which may reflect the final FSH release which causes preovulatory swelling of the follicle (Hansel et al., 1958). LH release in the cow probably occurs at some later time, during the 18 hour period of estrus, and ovulation results, on the average, 11 hours after the end of estrus (Hansel et al., 1958).

Following ovulation, the corpus luteum begins to develop and the third pituitary gonadotrophin, luteotrophic hormone (LTH) is thought to cause it to secrete progesterone. However, it should be emphasized that it has not yet been possible to specifically demonstrate a luteotrophic hormone in the bovine. The corpus luteum normally increases in size and progesterone content until the 16th day of the cycle, at which time it begins to regress rapidly. Simpson (1959) has given an excellent detailed account of these interrelationships between the gonadal and gonodotrophic hormones.

Since 1950 a great deal of information has accummulated to show that the hypothalmus, and higher nerve centers as well, exert marked influences over the secretion of the pituitary gonadotrophins. Results of experiments conducted in this area have given rise to a neurohumoral concept of the mechanism of ovulation and corpus luteum formation. It appears that the release of the pituitary gonodotrophins necessary for follicle maturation, ovulation and corpus lueum formation is controlled by one or more neurohumors produced by hypothalmic nuclei, or higher nerve centers, and transported to the anterior lobe of the pituitary by way of the specialized hypophyseal portal vascular system. Evidence of this concept, especially as related to the bovine, was reviewed recently by Hansel (1959).

Little is known of the nature of these hypothalmic mediators, or of the exteroceptive pathways to the hypothalmus and the stimuli which activate them in various species. Although incompletely understood, this mechanism is of particular importance because it changes our concepts of how gonadal hormones influence gonodotrophic hormone secretion and provides a partial answer to the question of how changes in an animal's environment are converted into changes in the nature and quantities of gonodotrophins secreted.

Studies aimed at finding the nature of the hypothalmic neurohumors and the environmental factors which influence their release in the cow have provided additional methods for altering the cycle.

METHODS OF ALTERING THE ESTROUS CYCLE

A great deal of experimental work has been conducted in an attempt to find a simple and dependable method for regulating estrus and ovulation in animals, without impairing fertility. There are several potential practical applications for such a method in all the farm animals, especially in beef and dairy cattle operations. Such a method will not only treat several types of infertility but the predetermination of estrus and ovulation will allow large numbers of animals to be bred within a period of a few days. Development of such a method is necessary before artificial insemination can be applied on a large scale in the livestock.

Enucleation of Corpus Luteum

It has been known for some time that the corpus luteum regulates the time of estrus. Loeb (1918) reported that the removal of the corpus luteum shortened the estrous cycle in the guinea pig. Hammond (1927) and McKenzie and Terrill (1937) found this to be true also for the cow and the ewe.

Dowling (1949) removed the corpora lutea from 76 dairy cows and reported 90 percent stood for service 2 to 4 days later. He also pooled data of 232 animals and showed a variation of onset of estrus after the expression of the corpus luteum to be 1 to 7 days with a mean of 4 days. Dowling further reported that the first ova occurring after expression of the corpus lutea were readily fertilized. This method has the disadvantage that the corpora cannot be easily removed at all stages of the cycle. Hemorrhage after the removal of the corpora lutea may also be a problem.

A simple method for altering the estrous cycle consists of manual removal of corpus luteum through the rectal wall. This method is only practicable in large species of animals like cattle, buffalo, camel, horse, and elephants. The expression of corpus luteum results in the female coming into estrus as it removes the inhibitory effect of progesterone, thus new follicles develop and estrus occurs. Hammond and Bhattacharya (1944) mentioned that on the average the cow will come in heat four days after the removal of the corpus luteum. Jakobson and Teige (1956) found much variation in a large group of cows in Denmark (Table I). Roberts (1956) reported observable estrus occurred in 50 to 80 percent of the cows within 2 to 7 days after corpora lutea removal. Conception occurred in 50 to 55 percent of the animals bred.

Hammond Jr. (1950) treated cows with various doses of pregnant mare serum, either towards the end of the normal cycle, or prior to expression of the corpus luteum. The object of the treatment was to obtain twin ovulations and calving. The results were not consistent and did not justify commercial application.

Table I. The occurrence of estrus in cows after removal of corpora lutea from the ovaries. 1/

Group	No. CL removed		Estrus not induced	Estrus induced	Interval in days, from enucleation to 1st insemination 1 - 7	8-21	22-28	29-50
Cows not bred since calving	1,509	No.	183	1326	811	129	269	117
		%	12.1	87.9	82.0	9.7	20.3	8.8
Cows inseminated since claving	597	No.	43	554	351	59	104	40
		%	7.2	92.8	63.2	10.7	19.0	7.2
Heifers not previously bred	400	No.	57	383	256	29	72	28
		%	12.9	87.0	66.3	7.6	18.8	7.3
Heifers previously inseminated	200	No.	10	190	116	20	37	17
		%	5.0	95.0	61.1	10.5	19.5	9.0
Total		No.	293	2453	1532	237	482	202
Average		%	10.7	89.3	62.5	9.7	19.7	8.2

1/ (Jakobsen and Teige, 1956).

Regardless of the skill of the person performing the operation, an occasional cow will die after corpus luteum removal as a result of excessive hemorrhage (Hansel, 1959). The incidence of adhesions in and around the oviduct also appears to be increased as a result of corpus luteum removal. Such adhesions may lead to infertility in subsequent years. These factors have lead to a decline in the popularity of manual corpus luteum removal as a treatment for "retained" corpora lutea and almost preclude the routine use of this method to regulate estrus and ovulation.

Progestogen Injection or Feeding

Progesterone is a steriod hormone produced mainly in the corpus luteum which develops cyclically in the ovary. It is also produced by placental membranes during pregnancy. Progesterone has other functions in addition to suppressing estrus and ovulation and maintaining pregnancy (Turner, 1961).

Several compounds have been synthesized in the last decade, which have progestogenic activity. Scientific workers have attempted to control estrus in several mammalian species using some of these synthetic compounds.

Laboratory animals

Selye et al. (1936) reported that injection of 4 mg. of synthetic progesterone caused the cessation of estrous cycles. There was ovarian atrophy, hypertrophy of pituitary and slight atrophy of thymus. Everett (1940) reported that administration of small sub-inhibitory quantities of progesterone restored the regular cycles in persistant estrus rats. The effective amount ranged from 0.25 mg. to 1.0 mg. per day.

Crystalline progesterone in doses 1.5 mg. and greater inhibited estrous cycles in normal female rats, but were resumed 3 to 5 days following the

last injection. Smaller doses, less than 1.5 mg. failed to do so (Phillips, 1937). An injection of extract of sows' corpora lutea and progesterone prepared from stigmasterol inhibited ovulation after mating in rabbits. In many rabbits injection of progesterone inhibited post partum estrus and they did not accept the buck (Makepeace et al., 1937).

An injection of 0.5 to 1 mg. of progesterone usually caused atresia of large follicles and appearance of diestrous in persistant estrus rats (Everett, 1943). Ring (1944) reported that estrogen and progesterone administered in sequence were more effective than estrogen alone in inducing sexual receptivity in the spayed female mouse. The optimum amount of progesterone was 0.05 mg. and the optimum time interval between injections was 48 hours. The mating behavior induced by the synergistic action of progesterone and estrogen appeared to be more normal in character than that induced by estrogen alone.

Murray and Eden (1952) reported that an injection of progesterone (1 to 15 mg./lb. body weight) delayed estrus in bitches for a protracted period. The injection was repeated at 2 to 3 weeks interval. There was no evidence of toxicity. Similar results for bitches were also reported by Candlin (1955) by injecting progesterone (1 to 2 mg./lb. body weight) at weekly intervals. The animals showed normal estrus about 10 days to two weeks followed by withdrawal of the hormone.

Cochrane and Meyer (1957) reported high doses of progesterone, when injected into the bred female rats, delayed nidatin. Karnofsky et al. (1952) reported that newly born mice were highly susceptible to the toxic and lethal action of progesterone, but within 2 to 3 days, resistance developed

rapidly. Progesterone given during the last three days of gestation caused death of embryos.

Dziuk (1960a) indicated that diets containing either progesterone or 6 a-methyl 17 a-hydroxyprogesterone acetate completely inhibited mating in mice. Inhibition was effective after a preliminary treatment period of 4 days and extended beyond end of treatment for at least 64 hours later. Eighty-nine percent of the mice which mated were pregnant. The pregnant females had a mean of 10.4 living fetuses. Ingestion of treated diets by males did not affect their fertility or libido.

Cattle

When heifers were injected with progesterone for a varying number of days starting near the end of the estrous cycle, it was observed that all the heifers exhibited estrus 5 to 6 days after the last injection (Christian and Casida, 1948; Willet, 1950; Graham, 1952; Donker, 1952; Trimberger and Hansel, 1955; and Greenstein et al., 1958). These workers also reported that 50 mg. of progesterone injected daily was capable of inhibiting estrus and ovulation during the treatment period. When lower levels were injected daily it prevented estrus during the treatment period but did not suppress ovulation.

Other workers have indicated that animals exhibit estrus 3 to 8 days after the last injection of progesterone. The majority of the animals will come in heat from the fourth to the sixth day post treatment (Donker, 1952; Dziuk, 1955; Nichols, 1957; Hansel and Malvin, 1960; Hansel et al., 1961; and Ulberg et al., 1951). When animals were given a single injection of progesterone they exhibited estrus 15 to 30 days later (Foote, 1962; Fosgate

et al., 1962; Nellor and Cole, 1954; 1956; and Ulberg et al., 1954).

It has been reported by several people that by feeding synthetic progestogen (Provera) for several days, cows exhibited heat 2 to 5 days after the last feeding (Collins et al., 1961; Nellor et al., 1960; and Zimbleman et al., 1961). Some workers have indicated that the length of the first estrous cycle after treatment was prolonged for several days beyond normal time (Trimberger and Hansel, 1955; and Willet, 1950).

Several scientists have reported that conception on the first estrus after treatment with progestogens is lowered. This decrease in fertility is temporary and overall conception is not affected (Graham, 1952; Foote, 1962; Trimberger and Hansel, 1955; and Zimbleman, 1961).

Nelms and Combs (1961) conducted a trial by feeding Provera at the rate of 0.8 mg./lb. body weight. Ninety percent of the cows showed estrus on the third, fourth, and fifth day following the removal of the hormone. Sixty percent of the cows calved as a result of breeding at the synchronized estrus. In the second trial cows received 220 mg. of Provera per day on twice a day feeding for 15 days. Estrus occurred in all cows, on the second and third day after the removal of the hormone. Sixty-seven percent of the cows were diagnosed pregnant to breeding on the first estrus following treatment.

Sheep

It has been indicated by several workers that when the ewes were injected with 10 mg. of progesterone for several days estrus and ovulation was suppressed during the treatment period. Seventy to 95 percent of the ewes exhibited estrus within 3 to 7 days of the last injection (O'Mary, 1950;

Dutt and Casida, 1948; Hunter, 1954; Raeside and Lamond, 1956; Robinson, 1959; and Foote and Matthews, 1962).

Some workers used only a single injection of progesterone to synchronize estrus in ewes and it was reported that estrus and ovulation was suppressed for 12 to 16 days. This inhibition depends upon the stage of breeding season (Robinson, 1956(a) and 1956(b) and Wagner et al., 1960).

Several injections of progesterone followed by an injection of pregnant mare serum (PMS) in various dose levels have been used in normal ewes. This combination of hormones has been reported to be more effective in controlling estrus and ovulation than the use of progesterone alone (Hunter, 1955; Dutt, 1953; Robinson, 1956(b) and 1958; Raeside and Lamond, 1956; Denny and Hunter, 1958; Edgar and Ronaldson, 1958; Gordon, 1958; Davies and Dun, 1957; Lishman and Hunter, 1961; and Bradon and Radford, 1960).

Ewes fed 50 to 100 mg. of Provera (6 a-methyl 17 a-hydroxy progesterone) mixed with their feed, did not exhibit estrus during hormone feeding. Two to five days after the last feeding of Provera, 75 to 98 percent of the ewes exhibited estrus (Hinds, 1961; Hinds et al., 1962(a) and 1962(b); Evans et al., 1961; Combs et al., 1961; and Hague et al., 1961 and 1962). Similar results have been reported by feeding 50 mg. of Provera per ewe per day for 14 days by Evans et al., (1962).

It has been reported by several workers that fertility on the synchronized estrus of ewes is lowered. This decrease in fertility was temporary as the optimum fertility was regained by the second post-treatment estrus (Foote and Matthews, 1962; and Hogue et al., 1961 and 1962).

On the other hand Evans et al., (1961 and 1962) reported that 63 to

84.2 percent of the ewes lambed to the first post treatment breeding. Hinds et al., (1962(a)) mentioned that there was no evidence to indicate any influence of hormone treatment on percent of ewes lambing, number of lambs born per ewe lambing, or performance of lambs.

It has been shown that ewes treated with Provera remained synchronized up to the third estrus post feeding (Foote and Matthews, 1962; and Hogue et al., 1961).

Swine

When gilts were injected with 50 to 100 mg. of progesterone for several days, both estrus and ovulation were suppressed. Doses lower than 50 mg. suppressed heat but ovulation was not prevented (Ulberg et al., 1951(b); Baker et al., 1954; and Sammelwitz and Nalbandov, 1958).

It has been observed that estrus and ovulation were successfully suppressed when gilts were fed Provera once daily at varying dose levels above 50 mg. for several days. The gilts exhibited estrus 3 to 6 days after the last feeding of Provera (Nellor, 1959 and 1960). Eighty nine percent of the gilts showed heat 4 to 5 days after progestogen feeding (0.5 mg. per pound body weight daily, for 15 days) was terminated. Similar results have been reported by First et al., (1960 and 1961).

Dziuk (1960(b)) fed gilts either crystalline progesterone or Provera (1000 mg. or 20 to 30 mg. respectively) for 14 to 21 days. Estrus was inhibited in 62.5 percent of gilts receiving 1000 mg. of progesterone per day but ovulation was not inhibited. Estrus was inhibited by all levels of Provera feeding but ovulation was inhibited only by levels exceeding 50 mg. per day per gilt. On the fourth, fifth, sixth, or seventh day after