Breeding heifers by appointment with PGF□2a and GnRH
by Richard Arnold Kinkie

A thesis submitted in partial fulfullment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
Montana State University

Abstract:
The purpose of this study was to determine the feasibility of inseminating a group of heifers without estrous detection at a predetermined time after injections of prostaglandin Fga and GnRH. Sixty-eight cycling Hereford heifers were divided randomly into five groups. Groups 1 through 4 were given 2 injections (IM) 11 days apart of 30 mg of PGF2α -THAM salt (PGF2α) . Groups 1 and 3 were injected with 100 ug IM of GnRH 60 hr after the second PGF2α injection.

Groups 1 and 2 were inseminated at 72 and 96 hr after the. second PGF injection and groups 3 and 4 were inseminated once at 80 hr. Group 5 was a control, and they were bred at 12 hr after estrus. Forty of the 56 PGF2α treated heifers (71%) expressed estrus after the second injection. The average interval from the first and second PGF2α injection to estrus was 53 and 51 hr, respectively. Conception at the synchronized breeding in the 72 and 96 hr and 80 hr groups was 43 and 14% (P=.04), respectively, for all heifers bred and 63 and 19% (P=.01), respectively, for those that were observed in estrus. The heifers that were inseminated twice at 72 and 96 hr all conceived to the 72 hr breeding except one which conceived to the 96 hr breeding. The first service conception rates for all heifers bred and those showing estrus were 21 and 32% for the GnRH groups and 36 and 48% for the no GnRH groups, respectively. There were no significant differences in conception rates between the treated heifers bred for 25 days by AI (63%) and the control heifers (66%) bred for 38 days- by AI. The conception rates for the entire breeding season AI and 45 days of clean up were 100, 100, 100, 93, 100% for groups 1 through 5, respectively.

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ABSTRACT

The purpose of this study was to determine the feasibility of inseminating a group of heifers without estrous detection at a predetermined time after injections of prostaglandin $F_{2\alpha}$ and GnRH. Sixty-eight cycling Hereford heifers were divided randomly into five groups. Groups 1 through 4 were given 2 injections (IM) 11 days apart of 30 mg of $PGF_{2\alpha}$ -THAM salt ($PGF_{2\alpha}$). Groups 1 and 3 were injected with 100 ug IM of GnRH 60 hr after the second $PGF_{2\alpha}$ injection. Groups 1 and 2 were inseminated at 72 and 96 hr after the second PGF injection and groups 3 and 4 were inseminated once at 80 hr. Group 5 was a control, and they were bred at 12 hr after estrus. Forty of the 56 $PGF_{2\alpha}$ treated heifers (71%) expressed estrus after the second injection. The average interval from the first and second $PGF_{2\alpha}$ injection to estrus was 53 and 51 hr, respectively. Conception at the synchronized breeding in the 72 and 96 hr and 80 hr groups was 43 and 14% (P=.04), respectively, for all heifers bred and 63 and 19% (P=.01), respectively, for those that were observed in estrus. The heifers that were inseminated twice at 72 and 96 hr all conceived to the 72 hr breeding except one which conceived to the 96 hr breeding. The first service conception rates for all heifers bred and those showing estrus were 21 and 32% for the GnRH groups and 36 and 48% for the no GnRH groups, respectively. There were no significant differences in conception rates between the treated heifers bred for 25 days by AI (63%) and the control heifers (66%) bred for 38 days by AI. The conception rates for the entire breeding season AI and 45 days of clean up were 100, 100, 100, 93, 100% for groups 1 through 5, respectively.

INTRODUCTION

Under modern management of beef cattle operations, labor conservation is a very important factor. In order to utilize the advantages of artificial insemination within a herd, previous systems have required many man-hours of observation for signs of estrus. In addition to this labor, long breeding periods have resulted in additional labor requirements for extended calving periods. If estrus could be controlled so that a large number of animals could be bred at one time without estrous detection, a considerable amount of labor could be saved.

Various investigators have recently reported that estrous cycles in cattle could be controlled with prostaglandins (Lauderdale et al., 1973; Stellflug et al., 1973; Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972a; Louis et al., 1972b; Rowson et al., 1972; McCracken et al., 1970). It has been shown that fertility of cattle synchronization with prostaglandins is comparable to that of cattle which are inseminated without estrous control (Lauderdale et al., 1974).

The purpose of this study was to evaluate different systems which might enable an operator to artificially inseminate a large group of cattle after estrous synchronization without estrous detection. An evaluation was made of inseminating at different predetermined times after estrous synchronization with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The effects of using GnRH after $PGF_{2\alpha}$ treatment for better control of ovulation time were also studied. These various effects

were evaluated by determining first service conception rates in the heifers and by looking at their subsequent calf's performance up to weaning time.

REVIEW OF LITERATURE

General

The reproductive or estrual cycle is regulated by hormonal interactions (Niswender et al., 1974). The hypothalamus secretes releasing hormones (GnRH) which act to release the gonadotropic hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), from the anterior lobe of the pituitary. FSH is secreted from the pituitary into the blood, where it travels to the ovary to stimulate follicular development. LH (also from the anterior pituitary) travels to the ovary and acts synergistically with FSH to stimulate secretion of estrogen by the follicle. As the follicle grows, its increasing secretion of estrogen triggers the release of a surge of LH from the pituitary. This high level of estrogen from the follicle acts on the central nervous system to bring the cow into behavioral estrus, by which time the estrogen secretion is already falling again (Short, 1972). The LH surge is responsible for final maturation and rupture of the follicle, resulting in the release of the ovum (ovulation). Ovulation occurs from 24 to 30 hours after the onset of the LH peak. After ovulation, the secretion of estrogen and gonadotropins is greatly reduced. At this reduced level, LH is partially responsible for the transformation of the granulosa cells from the ruptured follicle into luteal cells which secrete progesterone. Pituitary LH (Hansel et al., 1973) or possibly LH acting synergistically with prolactin from the anterior pituitary (Niswender et al.,

1974; Short, 1972) causes maintenance and secretion of progesterone by the corpus luteum (ruptured follicle). Progesterone exerts a negative feedback on the hypothalamus and/or adenohypophysis to inhibit the LH surge (Niswender et al., 1974) or to block the positive response of the hypothalamus to estrogen and so inhibit the LH surge (Short, 1972).

The secretion of progesterone increases as the corpus luteum (CL) grows, until it is at a maximum size on approximately day 12 of the cycle. It remains fairly constant until day 16 (Hansel et al., 1973) at which time, in the nonpregnant animal, the CL regresses rapidly.

The CL life is prolonged in several species which have been hysterectomized (LaVoie et al., 1975; Wiltbank and Casida, 1956; Loeb, 1923). Furthermore, it has been found that the endometrium controls CL regression (review by Melampy and Anderson, 1968). It has been postulated that the endometrium secretes a substance or unknown luteolytic factor (ULF) which causes luteal regression. The CL essentially stops functioning as measured by progesterone secretion within 12 to 24 hours following the onset of luteal regression. Once this luteal regression has occurred, the animal is ready to repeat the estrous cycle.

At any given time, it is quite likely that most of the animals within a group will be at different stages of the estrous cycle. The goal of estrous synchronization is to put all animals at the same

stage so that artificial insemination can occur at one time.

In early synchronization experiments, exogenous progesterone was given to animals for a period and then was withdrawn so that the LH surges would occur at approximately the same time.

Progesterone

Christian and Casida (1948) first reported that daily injections of progesterone suppressed estrus and prevented ovulation during the entire treatment period. Treatment was started on the fourteenth day of the estrual cycle and consisted of 14 daily injections of progesterone in corn oil. All heifers treated with 50 mg of progesterone per day came in estrus 5 to 6 days after the end of treatment.

It was later shown (Ulberg and Lindley, 1960; Ulberg et al., 1951) that lower daily dosages of 25 or 12.5 mg of progesterone usually prevent estrus and ovulation. However, Ulberg et al. (1951) found that follicles in the 20 to 30 MM range will develop during treatment. Estrus will occur with these follicles ovulating when the injection period is stopped. It was shown that follicles under the influence of prolonged daily injections of 12.5 mg would regress and be replaced by another follicle at 2 to 3 week intervals. Dosages lower than 12.5 mg had little if any effect. The authors concluded that progesterone inhibited LH from acting on the ovary. Loy et al. (1960) later postulated that progesterone inhibits the release of LH from the pituitary, thereby preventing its action upon the ovary.

Other reports (Woody et al., 1967; Nellor and Cole, 1956; Trimberger and Hansel, 1955) have shown that progesterone injections are effective in suppressing estrus and ovulation when they are given regardless of the stage of the estrous cycle when begun.

Willett (1950) injected 50 to 100 mg of progesterone into heifers daily for 13 to 17 days. After termination of injections, the heifers were observed for estrus. They were artificially inseminated once when observed in estrus and again 24 hours later. Eleven pregnancies resulted from the 22 breedings. Other research did not show the high first-service conception rate that Willett reported. Trimberger and Hansel (1955) reported settling 3 of 24 (12.5%) of the cows from natural service after dosages of 50, 75 or 100 mg of progesterone for 14 days compared to 64% first-service conception in the control group.

Ulberg and Lindley (1960) used progesterone levels varying from 12.5 mg to 50 mg. Estrus occurred 2.5 to 9.5 days post treatment in 86% (291/333) of the animals treated. They reported that administration of 14 daily injections of progesterone had a depressing effect upon the rate of pregnancy in animals inseminated during estrus subsequent to treatment, with the higher dosages being more detrimental. They also observed that an injection of 0.5 to 1.0 mg of estradiol benzoate 3 days after the last injection of progesterone initiated estrus and caused ovulation, as determined by rectal palpation and

subsequent slaughter, without a further reduction in pregnancy rate from those animals given only progesterone. The estrogen also eliminated much of the variation in time in the onset of estrus, after progesterone administration. They reported that there were no indications that any treatment affected productivity of the cow (calf weaning weight, gestation length, birth weight, or sex ratio of calves). This work suggested that estrogen, when used in sequence with progesterone, facilitated the release of LH to cause ovulation in beef cattle.

Wiltbank et al. (1961) postulated that estrogen causes LH release after finding that varying levels of estradiol valerate, estrone or a natural estrogenic compound caused early regression of the CL in more than 50% of the heifers treated. This supported earlier work done by Greenstein et al. (1958). However, estrus and ovulation were not consistent following this early regression. Later, Wiltbank et al. (1965) conducted trials using injections of progesterone alone or in combination with an estrogen compound. Two hundred-twenty cycling heifers were synchronized with 24 daily injections of 20 mg of progesterone alone or in combination with 10, 20 or 40 ug of estradiol or 40 mg of progesterone with 20, 40 or 80 ug of estradiol. Two other groups received the progesterone plus three injections of estradiol on the 23rd, 24th and 25th days. Synchronization varied from 70 to 100% of the heifers exhibiting estrus in a 4-day period. However, fertility was significantly lower in most of the heifers

receiving treatment as compared to the control groups. Wiltbank et al. (1965) concluded that the injection of estrogen with 40 mg of progesterone increased synchronization, but it did not with the lower 20 mg dose of progesterone. However, this may have been due to the time of estrogen injection.

Under range livestock management systems, it is not practical to inject heifers daily for the duration of the treatment period. Nellor and Cole (1956) found that a single injection of 540 to 1120 mg of crystalline progesterone in starch emulsion was capable of preventing estrus and ovulation in 179 treated heifers regardless of the stage of estrus when treatment was given. Estrus occurred 15 to 19 days after the progesterone injection in 89% of the heifers receiving 540 to 560 mg of progesterone and 15 to 23 days after the injection in heifers given 700 to 1120 mg of progesterone. Ovulation occurred in 95% of the heifers following this estrus. This treatment is of questionable practicality because of the wide range of time (8 days) that the treated heifers in a group would come into estrus.

Avery et al. (1961) used different treatments on three groups of cows. One group received daily injections of 50 mg of progesterone. Another group received injections of 150 mg of progesterone every third day and the third group had their corpus lutea expressed or removed manually via rectal palpation. There was no significant difference in the first service conception rate of any of the three

groups after breeding. Thirty-four percent of the 59 treated cows conceived to first service as compared to 32% of the 53 in the control group. It should be noted that corpus luteum expression is not recommended because of possible excessive hemorrhage in the animal.

Oral Progestogens

Significant progress was made in practical estrus synchronization in 1960 when it was found that estrus could be inhibited by adding a synthetic orally active progestogen to the cattle feed. Nellor et al. (1960) fed 6α-methyl-17αacetoxyprogesterone (medroxyprogesterone acetate or MAP) to 77 heifers at various stages of the estrous cycle. Heifers were treated individually or in groups to receive 0.44 to 1.76 mg of MAP per kg of body weight daily for 15 to 20 days. Estrus was inhibited at all levels of treatment, regardless of the stage of the cycle when treatment was started. Ovulation without behavioral estrus occurred at the 0.44 mg level and levels above 0.88 mg resulted in complete inhibition of follicular growth during treatment. Estrus occurred 4 to 5 days after the end of treatment with 0.88 mg and the period from end of treatment to the start of estrus increased as dosage increased.

Nelms and Combs (1961) fed 60 heifers at a level of 250 mg per day for 14 days. Each heifer was inseminated on the third, fourth and fifth days following removal of MAP from the feed. They were

inseminated without observing estrus, with a 40% conception rate. This was not significantly different from the 60% first service conception rate for the controls that were inseminated over 21 days.

Hansel and Malven (1960) also inseminated without regard to estrus after feeding MAP. They fed 968 mg per animal per day for 10 days, then fed an additional 10 days at a rate of 500 mg per head per day. No animals came in estrus during the 20 day feeding period. Sixteen of 32 heifers came in estrus on the third and fourth days following treatment termination. All of the animals were bred by AI regardless of estrus and half of them were injected with 0.5 mg of estradiol at the time of breeding. Thirteen of the 16 heifers not showing estrus ovulated. Only 22 animals were pregnancy tested after insemination and 8 were found pregnant (5 of 9 showing estrus and 3 of 13 not showing estrus conceived). The conception rate was equal in the estradiol treated group and in the group receiving only MAP.

Hansel et al. (1961) also used estradiol injections at the time of insemination on half of 32 cows that were fed MAP for 20 days. Sixteen of the cows came in heat and an additional 13 ovulated without estrus. All cows were inseminated 3 to 4 days after treatment and 25% conceived the first service. Again, the estradiol did not improve conception.

Fahning et al. (1966) reported 18 of 20 heifers fed 0.88 mg per kg body weight per day for at least 11 days showed estrus 2 to 4 days

following last feeding. They were inseminated starting at estrus, once every 12 hours until ovulation. The control group were inseminated three times at 12 hour intervals, starting at estrus. First service conception was significantly lower in the MAP treated heifers (26.3%) compared to the control group (81%).

Dhindsa et al. (1967) reported a 33% first service conception rate in 119 cows fed 180 mg MAP for 18 days. This was not significantly different from the 37% first service conception among 60 control cows. Eighty-seven percent of the treated cows exhibited estrus between 18 and 78 hr after treatment ended. Dhindsa et al. (1967) also inseminated three groups of heifers a different number of times after MAP treatment. One group was inseminated once at 12 hr after estrus, another group was inseminated at 48 hr and again at 72 hr and a third group was inseminated three times, at 48, 60 and 72 hr. The conception rates were 25, 38 and 21%, respectively, and were not significantly different. No explanation was given for the overall low conception rates.

Zimbelman (1963) studied different dose levels of MAP, 93% of the heifers that exhibited estrus of the 170 on test were in estrus on the second, third and fourth days after last feeding. The average conception rate of all treated animals bred within 7 days after last feeding was 51%, but there was great variation in conception from trial to trial. The conception rates of treated animals at second

service and of untreated animals were 76 and 74%, respectively. No apparent effect of MAP treatment was noted on the average gestation length or birth weight of the calf.

The high cost and the varied results of MAP caused the search for more potent drugs which could be effective when used in smaller quantities than MAP. Researchers found that halogenating certain adrenocortical steroids produced a number of halogenated progestins (Van Blake et al. 1963).

One of the halogenated progestins is 6-chloroΔ^6-17 acetoxyprogesterone (CAP). Van Blake et al. (1962) first reported that CAP fed at levels of from .055 mg to .66 mg per kg of body weight per day for 15 or 20 days suppressed estrus during the feeding period. They found that estrus occurred 4 to 7 days after feeding CAP for 15 days at the .055 mg and .11 mg levels. Seven of eight animals conceived to first service insemination. Van Blake et al. (1963) later confirmed their earlier results with a test using 20 untreated heifers and 69 heifers treated with varying levels of CAP. They found the hormone extremely potent in inhibiting estrus and ovulation. Heifers fed .044 mg per kg of body weight were synchronized in a period of 4 to 6 days after hormone removal. First service conception rates in the 57 treated heifers was 61% compared to 65% in the 20 controls. Subsequent cycles were of normal length and fertility.

Wagner et al. (1963) also tested dosage levels on different groups of heifers. They used dosages without regard to heifer weight and found that 5.0 mg of CAP per day did not inhibit cyclic activity in 60% of the heifers treated. However, 10 mg and 25 mg levels did inhibit estrus. The lower the CAP dosage was, the shorter the average interval was between the end of treatment and the onset of estrus. However, 90 to 100% of the heifers were synchronized within a 4 day period, and the first service conception rates were 42 and 50%. There was no control group in this study. They also gave supplemental exogenous estradiol (0.5 mg) two days after the last CAP feeding to one group of 16 heifers. These heifers exhibited estrus within a 24 hr period on the third day after CAP withdrawal. First service conception on these heifers was 50% which was not significantly different from the 42% first service conception rate in the other 40 heifers given only CAP.

As with progesterone injections, CAP treatments began showing lowered fertility in later tests. Wagner et al. (1968) reported lower conception rates in 187 CAP treated heifers compared to controls (33 vs. 55%, respectively). Hansel et al. (1966) used MAP or CAP treatments on 832 cows and found that fertility of MAP treated cows approximated that obtained in the normal controls. However, CAP fed cows had significantly lowered fertility. Hansel et al. (1966) went on to report that fertility with natural or artificial

breeding did not differ significantly and that fertility of MAP and CAP treated cows was uniformly high at the second post synchronization estrus.

Melengestrol acetate (MGA), another synthetic progestogen, was orally 300 to 900 times as potent as MAP and was found to be effective in inhibiting estrus and ovulation in heifers (Zimbelman and Smith, 1966). Dose levels over 0.4 mg per heifer per day were successful in suppressing estrus (Young et al., 1967; Zimbelman and Smith, 1966). Zimbelman and Smith (1966) fed different levels of MGA to different groups of heifers for 15 to 18 days. They reported first service conception rates in the various groups ranging from 25 to 88%, with an overall average of 42% on 72 heifers. There was no control group in this study. As with the other progestogens, they found that the average interval from the last feeding of MGA to estrus increased as the dose level increased.

Roussel et al. (1969) found that after feeding 1.0 mg of MGA per day for 14 days to 15 heifers, the first service conception rate (47%) was not significantly different from that of 15 untreated controls (40%). Roussel and Beatty (1969) treated 30 dairy cows for 14 days on the same 1.0 mg level. The occurrence of estrus after MGA withdrawal was 93%. However, the first service conception rate was only 26%. First service conception for the controls was not given. They reported that the total conception rate after the second estrus

was 60 and 53% for the treated and control cows, respectively. This indicated that MGA had no detrimental effect on the second service conception. The mean interval from first to second estrus after MGA was 21.9 days, and there was no significant difference in calving the MGA treated cows (no multiple births or prenatal losses, calving difficulty and retained placenta not significant).

Chakraborty et al. (1971) settled only one of twelve heifers treated with MGA on the first service. They fed 1 mg of MGA for 14 days irrespective of the stage of estrus when treatment was started. Synchronized estrus followed MGA termination from 3 to 6 days. This low conception rate was compared to 7 of 12 control heifers settling first service.

Hill et al. (1971) also reported significantly lowered fertility in heifers treated with MGA. They also found that the time of the estrous cycle when feeding MGA started influenced synchronization and fertility. When treatment started on day 4, 20% of the heifers failed to exhibit estrus and when started on day 15 of the cycle, 47% of the treated heifers failed to show estrus. Laparotomy following insemination yielded 9 cleaved ova from 14 heifers starting treatment on day 4 and 5 cleaved ova from 12 heifers starting treatment on day 15. These compared to 8 cleaved ova from 9 heifers in the control group.

Smith and Zimbelman (1968) gave injections of 2, 5 or 10 mg of

estradiol cypionate to 63 heifers during the last feeding or on day 1 or 2 after the last feeding of MGA. Thirty-five heifers served as controls and were given only MGA. Estradiol cypionate increased overall incidence of estrus (93 vs. 76%), but it had a detrimental effect on first service conception (20% in estradiol cypionate treatment vs. 32% in those given MGA alone).

Another progestogen, 16-alpha-17-dihydroxyprogesterone acetophenomide (DHPA) was tested in the late 1960's. As with the other progestogens previously described, varying fertility resulted.

Wiltbank et al. (1967) successfully synchronized 96% of a group of heifers in a 48 hr period after feeding 500 mg of DHPA daily for 20 days. The first service conception rate for this group was 26% as compared to 54% in the control group ($P<.05$). When 400 mg DHPA was fed for 9 days with 5 mg of estradiol valerate injected on the second day, 84% of the treated heifers were in estrus during a 96 hr period. First service conception rates in heifers treated were not significantly affected.

Wiltbank and Kasson (1968) furthered their study with 400 mg DHPA daily for 9 days and 5 mg of estradiol valerate on the second day of feeding. Estrus was synchronized in 95% of the 66 treated heifers with 54% conceiving. Fifty-two percent of the 33 control heifers conceived. They went on to report that the same treatment in lactating cows significantly reduced conception at the synchronized

estrus.

Progestogen Implants

Although feeding progestational compounds aided estrous synchronization from a practical standpoint, workers felt that the amount of progestational agent given to individual animals needed to be gauged more accurately. Two major routes have been used to administer drugs in more accurate amounts.

Sponges or pessaries which have been saturated with a measured amount of drug may be placed in the vagina of the cow, where the drug will be slowly absorbed. The sponges may be manually removed at any time for termination of drug administration. This method is used effectively quite often for estrous synchronization in ewes. Flurogestone acetate (SC9880 or Cronolone), a progestogen that is often used in sheep, has been used in bovine pessaries. Carrick and Shelton (1966) first showed that Cronolone blocked estrus and ovulation for the 18 to 20 days during which pessaries were intact in a majority of the animals treated; however, fertility was low.

Shimizu et al. (1967) reported estrus in cows occurring within two days of withdrawal of 18 day pessaries saturated with 100 or 200 mg of Cronolone. They reported no significant difference in first service conception between control cows (20/29) and those treated with either level of Cronolone (6/12). They also reported no significant difference between treatments started on the fifth, tenth or fifteen day of the

estrual cycle.

Wishart and Hoskin (1968) reported lowered first service fertility in 55 heifers showing estrus of 66 retaining pessaries from 81 treated with 200 mg Cronolone pessaries for 21 days (43.7% compared to 61.9% in the controls). Sreenan (1975) and Sreenan and Mulvhill (1975) verified these low fertility results after using Cronolone pessaries on heifers for 20 days. However, when treatment was reduced to 10 days and progestoerone plus estradiol valerate was given intramuscularly at the start of treatment, conception was increased to and above the normal rate.

Ayalon and Marcus (1975) reported the use of MAP in vaginal pessaries. They put pessaries with 250 mg MAP in 12 cows and heifers for 14 days. First service conception was 60%.

The major drawback in the use of vaginal pessaries is that problems arise in their retention. There is a difference in the retention of different sizes and shapes of sponges (Carrick and Shelton, 1966) and it has been found that retention is higher in cows treated for shorter periods of time (10 vs. 20 days) (Ayalon and Marcus, 1975; Wishart and Hoskin, 1968; Sreenan, 1957).

The other major means of administering progestogens in accurate amounts is through the use of subcutaneious implants. The implants are made of a material that will absorb and release the drug, but which will not be absorbed themselves once inside the animals.

Implants have been placed in the flank and brisket areas of animals. Recently, most implants have been placed in the ear.

Dziuk et al. (1966) used silicone implants with MGA in cows to test for effectiveness of inhibition of estrus. They implanted 70 cows and after five days introduced five fertile bulls to the herd. Implants were removed at various intervals up to 64 days from implantation. Estrus was observed in 45 cows (64%) between 36 and 72 hr after implant removal. Three cows conceived while implanted.

Curl et al. (1968) showed that subcutaneous implants of another progestogen (norethandrolone, SC-5914, or NE) controlled estrus and ovulation in cattle. Thirty-two cows were divided into six treatment groups. The different groups were treated with varying levels of NE impregnated polyhydroxy polymer implants. Ten cows either lost their implants or exhibited estrus during treatment. Of the 22 treated cows which did not lose their implants and/or exhibit estrus during treatment, 18 (81.8%) exhibited estrus within 48 hr after the 16 day implant removal. Of the 22 treated cows which were inseminated, 15 (68.2%) conceived at first breeding. Treatment levels of 153.7 and 168.0 mg NE appeared to be most effective for synchronization as compared to either higher or lower dosage levels.

Woody and Pierce (1974) implanted heifers subcutaneously behind the shoulder with norethandrolone and injected with estradiol valerate starting on various days of the cycle. They found that heifers

implanted prior to 10 days postestrus had longer ($P<.01$) intervals to estrus after implant removal than those implanted after 10 days postestrus. They also found in another trial that there were more heifers in estrus when implanted on day 14 than those implanted on day 2, and more were in estrus after a 16 day treatment than after a nine day treatment. Conception at first service was 50 to 83% in all 9-day treatment groups (averaging 73%) and was 83% in the 16-day treatment group in which implant insertion was 2 days after estrus. However, when implants were inserted 14 days after estrus and removed 16 days later, no heifers conceived to first service. No statistical analysis was reported for these data.

Liang and Fosgate (1970) showed that hydron plastic implants with 300 mg of 17-alpha-ethyl-19 nortestosterone (Nilevar) successfully inhibited estrus and ovulation in cows implanted subcutaneously for 17 days.

Wiltbank et al. (1971) used Nilevar implants on heifers for either 16 days or for 9 days. Estradiol valerate was injected on the day of implanting. Eighty-seven percent of 15 heifers implanted for 16 days exhibited estrus in 96 hr as compared to 93% of the 42 heifers implanted for nine days. First service conception was 38 and 61%, respectively, for those implanted for 16 and 9 days. This compares to 65% first service conception in the untreated controls. In a second

group of heifers implanted for 9 days, 50% were pregnant at first service as compared to 69% in the controls ($P>.05$).

Roche (1974a) also found a difference in conception rates between heifers implanted for different lengths of time. In heifers given progesterone implants and estradiol benzoate for either 9 or 12 days, there was no significant difference in conception, but conception in these groups was significantly higher than in those treated for 18 or 21 days. Estrous response was low in animals implanted on days 3 and 17, but high between 5 and 15. In another test, Roche (1974b) found that reducing progesterone administration from 20 to 10 days and giving 5 mg of estradiol benzoate on the day of implantation resulted in increased first service conception from 57% in 15 heifers treated 20 days to 82% in 15 treated for 10 days but also resulted in reduced estrous response (93% in 20 day treatment vs. 73% in 10 day treatment). Injection of 400 ug of estradiol benzoate 16 hr after implant removal on a 10 day treatment did not increase estrous response and lowered first service conception (40% in 23 treated vs. 80% in 18 controls).

Ear implants of 19 alpha-acetoxy 11 beta-methyl 19 norpreg 4ene 3, 2 dione (SC21009, Syncro-mate B or Norgestamet) have also been shown effective in synchronizing estrus in cycling heifers. Burrell et al. (1972) reported 93 to 98% of heifers treated for 9 days with 5 mg SC21009 implants plus 5 mg estradiol valerate showing estrus

within 4 days of implant removal. The number of heifers treated and percent conceiving for the control and treated groups, respectively, were 77 (65%) and 79 (55%). There was no significant difference in conception between treated groups and control groups. Wishart and Young (1974) also reported good synchronization in heifers given 9 day SC21009 implants along with 3 mg of SC21009 and 5 mg of estradiol valerate at the time of implantation. They inseminated at 48 and 60 hr after implant removal and reported fertility comparable to controls. Twenty cleaved ova were obtained from the 25 treated heifers as compared to 21 cleaved ova from the 25 control heifers.

Burrell et al. (1972) observed that when heifers were implanted for 16 days or implanted for 9 days and given 7.5 mg of estradiol valerate, first service conception was significantly lower than controls. The number of heifers and conception rate in the 16 day treatment group compared to the control group was 76 (32%) and 77 (65%) ($P<.05$). The group of 55 heifers treated with 7.5 mg estradiol valerate and a 9 day implant had a conception rate of 40%. This is compared to 55 control heifers with 64% first service conception. Whitman et al. (1972) found that when cows were implanted for 9 days and estradiol valerate levels were increased from 5 mg to 7.5 mg, synchronization increased but fertility decreased. They treated five groups of cows with 5 mg SC21009 implants. The groups received different levels of estradiol valerate at the time of implantation. Of the cows in the

groups receiving 5 mg estradiol valerate 79 and 74%, respectively, exhibited estrus within 4 days of implant removal. With 6 mg estradiol valerate, 75% of the cows exhibited estrus within 4 days, 84% with 6.5 mg and 92% and 100% with 7.5 mg. The conception rate at first service for those cows bred at synchronized estrus in treated groups compared to cows bred for 21 days in control groups were 39% vs. 47% and 74% vs. 63% at 5 mg of estradiol valerate, 56% vs. 66% at 6.5 mg of estradiol valerate and 45% vs. 53% and 43% vs. 66% at 7.5 mg of estradiol valerate. No conception data was given on the 6 mg estradiol valerate level group.

Woody and Abenes (1975) also concluded that the 9 day treatment was adequate to synchronize estrus in heifers and went on to report that estradiol valerate aided in causing early luteal regression. This supported work done by Shelton and Casida (1970) and Kaltenbach et al. (1964), who showed that low levels of estradiol may be luteolytic when administered late in the cycle.

Woody and Abenes (1975) found that fertility of heifers implanted for 16 days was lower when implanted on day 14 of the cycle as compared to those implanted 2 days postestrus. This may be explained by the observation that progesterone can induce luteal regression in heifers when injected early in the cycle, with its influence apparently decreasing with an increased interval postestrus (Woody and Ginther, 1968).

Ear implants of SC21009, as with other injected or fed progestogens (Wiltbank and Kasson, 1968), do not seem to be as effective in cows as they are in heifers. Whitman et al. (1972) showed that poor synchronization resulted in cows suckling calves which were given implants plus 5 mg of estradiol.

Ear implants seem to be a very effective way of giving progestogens. There is only one drawback that may or may not cause a problem. Woody and Abenes (1975) postulated that implants may not work completely when they are used during extremely cold weather. There may be less progestogen absorbed into the blood due to a reduced blood supply in the capillary beds of the ear. During cold weather, blood is shunted by some capillary beds to allow greater blood flow through the larger vessels in the ear.

It has been known for many years that the corpus luteum plays an important role in controlling the length of the estrous cycle and the time of ovulation because of its major secretory product, progesterone, which inhibits the ovulatory surge of LH. All of the synchronization experiments previously discussed have dealt with the administration of exogenous progesterone compounds which act to suppress the LH surge until the corpora lutea have regressed in each animal within a group.

Prostaglandin $F_{2\alpha}$

An alternate approach to synchronization is to remove or regress the corpora lutea simulataneously in a group of cattle so that the natural progesterone is stopped and the LH surge can occur without altering follicular growth. Probably the first technique using this theory was the manual removal of the corpora lutea. However, this was found to be time consuming and somewhat dangerous, so it found very limited use (Inskeep, 1973). Various methods of chemical regression of the corpus luteum have been found. Injections of oxytocin from days 2 to 6 of the estrous cycle, injections of estrogen, the presence of foreign bodies in the uterus, irrigation of the uterus with iodine solutions or administration of LH antibodies all cause premature luteolysis in cattle (Roche, 1974b). Few of these methods have been incorporated into synchronization systems.

The use of estrogen for regression of the corpus luteum (Shelton and Casida, 1970; Wiltbank et al., 1961; Greenstein et al., 1958) has been used effectively along with progestens so that treatment time can be reduced to as low as 9 days (Woody and Abenes, 1975). Oxytocin is luteolytic in some species at specific times during the estrous cycle (Review by Melampy and Anderson, 1968) and has been used to a limited extent in combination with progestogens for estrous synchronization (Hansel et al., 1961). However, it did not prove to be beneficial.

In the 1930's, a group of biologically-active lipids made up of linolenic, arachidonic and pentaenoic acids were found (Speroff and Ramwell, 1970). These compounds were first found in seminal fluid and were thought to originate in the prostate; therefore, they were named prostaglandins. In later research, prostaglandins were detected in or found to be released from most mammalian body tissue (Lauderdale, 1974).

Loeb (1923) observed in the guinea pig that complete or almost complete hysterectomy is followed by luteal maintenance for a period of 60 to 80 days. He also noted uterine removal in young animals did not interfere with subsequent maturation of follicales or ovulation.

In 1956, Wiltbank and Casida reported that hysterectomy prolonged the life span of the corpus luteum in cows and ewes. Since that time, it has been demonstrated clearly that the uterus plays a role in the regression of the corpus luteum (Reviews by Caldwell et al., 1969; Melampy and Anderson, 1968).

It was first suggested by Babcock (1966) that a prostaglandin might be the agent from the uterus which has a luteolytic effect. Subsequent work showed that prostaglandin ($PGF_{2\alpha}$) injections induced luteolysis in a variety of species, including cattle (Lauderdale et al., 1973; Stellflug et al., 1973; Lauderdale, 1972; Liehr et al., 1972; Louis et al., 1972a; Louis et al., 1972b; Rowson et al., 1972;

McCracken et al., 1970). The mode of action by which $PGF_2\alpha$ induces luteolysis is not known, however, Pharris et al. (1972) postulates five possible mechanisms by which prostaglandin may work. The possible areas were $PGF_2\alpha$ could be exerting its primary luteolytic effect are depicted in Figure 1 from Pharris et al. (1972).

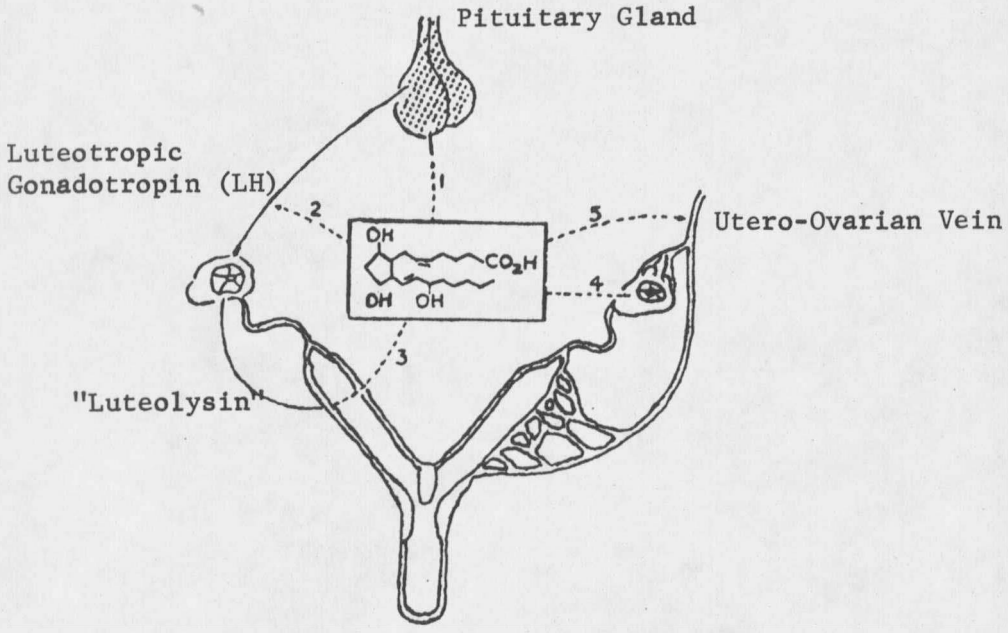
Since the pituitary gland is important in maintenance of luteal activity, it was first thought to be the target for $PGF_2\alpha$. The theory was that $PGF_2\alpha$ either totally blocked the pituitary or had the ability to inhibit the luteotropin (Figure 1, #1). Luteotropins vary in different animal species. For example, LH is necessary in the rabbit, prolactin in the rat and prolactin and FSH in the hamster. $PGF_2\alpha$ is not effective for luteolysis until corpora lutea have reached a certain stage of maturity. $PGF_2\alpha$ given prior to day 4 of pregnancy in the rat is not luteolytic. However, after day 6, it is 100% luteolytic. It was found that ergocorine, which is luteolytic in the rat by blocking prolactin release from the pituitary, is more effective prior to luteal maturity.

It has also been shown through hormone assay that LH levels are unaffected by $PGF_2\alpha$ treatment (Review by Pharris et al. 1972). Also, ovarian arterial infusions of $PGF_2\alpha$ induce luteolysis at doses which are ineffective when given systemically. Thus it is suggested that the hypothalamus and pituitary are not directly involved in $PGF_2\alpha$-induced luteolysis.

Another mechanism by which $PGF_{2\alpha}$ could induce luteolysis is by way of the uterus (Figure 1, #3). Prostaglandin is a potent smooth-muscle stimulator and could cause the uterus to contract and release endogenous uterine luteolysin, much as does oxytocin in the bovine. However, treatment of hysterectomized animals with $PGF_{2\alpha}$ still causes luteal regression (LaVoie et al., 1975).

A third possible method of $PGF_{2\alpha}$ action would be a direct toxic effect on the corpus luteum itself (Figure 1, #4). Reports indicate that $PGF_{2\alpha}$ inhibits secretion of progesterone by luteal tissues in vitro (Henderson and McNatty, 1975; O'Grady, 1972). This suggests that $PGF_{2\alpha}$ is capable of exerting a direct biochemical effect on the luteal cell to directly inhibit progesterone synthesis. It has been postulated that $PGF_{2\alpha}$ is not effective in bovine CL regression up to day 5 (Rowson et al., 1972) because the pre-ovulatory LH surge saturates the regulatory units of the luteal cells and that it is this bound hormone that protects the young CL (Henderson and McNatty, 1975).

Another possibility is that $PGF_{2\alpha}$ exerts an antigonadotropic effect (Figure 1, #2). Even though the pituitary had been eliminated as a site of action, the interaction could take place in the circulation or at the receptor site on the corpus luteum. It has been found that $PGF_{2\alpha}$ does inhibit FSH-like and LH-like activity of pregnant mares' serum (PMS) and human chorionic gonadotropin (HCG) respectively. Studies have indicated that $PGF_{2\alpha}$ may cause a decreased hormone binding



1 = direct feedback on pituitary gland
2 = antigonadotropic effect
3 = stimulation of uterus to produce luteolysin
4 = direct toxicity of corpus luteum
5 = constriction of utero-ovarian vein.
From Behrman et al., Ann. N.Y. Acad. Sci. 180, 437 (197). In. Pharris et al., (1972).

Figure 1. Possible mechanisms of prostaglandin $F_{2\alpha}$ in luteolysis.

capacity at the CL binding sites (Hichens et al., 1974). It is suggested by this antagonism to luteotropins that this is a possible mechanism whereby $PGF_{2\alpha}$ exerts its luteolytic effect.

The final possible mechanism is the alteration of ovarian blood flow (Figure 1, #5). Studies have been reported which suggest that ovarian perfusion is reduced after $PGF_{2\alpha}$ treatment. In rats, after a single dose of $PGF_{2\alpha}$, there is an immediate drop in blood flow of 50 to 60% of the control levels and lasting about 25 minutes (Review by Pharris, 1970).

The mechanism whereby prostaglandins are luteolytic is still unknown, but three hypothesis are supportable. These are gonadotrophin antagonism, alteration of ovarian blood flow or a direct toxic effect on the CL.

It has been found that $PGF_{2\alpha}$ is not effective in regression the bovine corpus luteum during the first 4 days of the estrous cycle (Rowson et al., 1972). However, it is effective from days 5 to 16 of the cycle (Lauderdale, 1972).

When $PGF_{2\alpha}$ is given from day 5 to 16, there is an immediate drop in blood progesterone levels to about 50% within 12 hr (Hafs et al., 1974). Significant elevations of plasma-estrone and estradiol-17B occur during the first 24 hr after treatment. Luteinizing hormone peaks at about 70 hr and estrus begins at approximately 72 hr with ovulation around 95 hr (Hafs et al., 1974).

The commonly used dosage is 30 to 33.5 mg of $PGF_{2\alpha}$ THAM salt

intramuscular. However, it has been shown that single dosages of 60 mg, 30 mg or double dosages of 15 mg at 6 hr intervals do not significantly affect the time of ovulation after drug administration (Stellflug et al., 1975; Stellflug et al., 1973).

In early experimental work, prostaglandins were usually given by intra-uterine infusions. A lower dosage (usually 5 mg) was required for this method as compared to intramuscular or subcutaneous injection. It was found that intra-uterine infusion requires some degree of skill to pass the cervix and is sometimes impossible in heifers and may result in more variable response. The treatment is time consuming and involves risk of uterine infection (Hearnshaw et al., 1974; Henricks et al., 1974). Therefore, it was suggested that the best means of $PGF_2\alpha$ administration was through either subcutaneous or intramuscular injection.

Lauderdale et al. (1974) were probably the first to use prostaglandins for estrus synchronization. In their experiment, cattle were divided into three treatment groups at four locations. Treatment one was the control group, where the animals were observed for estrus and inseminated during an 18 to 25 day interval. The second group was injected with 30 mg of $PGF_2\alpha$ THAM salt if a CL was detected through palpation or assumed to be present. These cows were then observed for estrus and inseminated during days 1 through 7 after $PGF_2\alpha$. Cattle in the third group received the same $PGF_2\alpha$ treatment

as group two and were inseminated twice at 72 and 90 hr after $PGF_2\alpha$ without regard to estrus. After insemination, the percent pregnant was based on the number inseminated for treatment one and two and on the number either observed in estrus or having a CL formed and detected by palpation from days 1 to 7 after $PGF_2\alpha$ for treatment three. The percent pregnant and number inseminated for treatment one through three, respectively, were: 53.3% - 122, 52.2% - 69 and 55.8% - 86. It was concluded that there was no significant difference in fertility among the groups.

These successful results prompted other workers to use $PGF_2\alpha$ for estrus synchronization. Roche (1974c) assigned 33 heifers between days 5 and 20 of their cycle to three groups. One group was the control which was untreated and inseminated at estrus. The number and percent pregnant was 8 and 73%, respectively. The second group received 30 mg of $PGF_2\alpha$, and animals were inseminated after observed estrus with 6 (75%) conceiving. The third group was treated as was group two, but they received only 20 mg $PGF_2\alpha$. The number conceiving was 7 (70%). There was no significant difference in fertility between groups. The majority of the heifers were detected in estrus within 4 days of injection. Neither the dosage nor the stage of the cycle when given influenced the estrus response.