

THE EFFECT OF STAGE OF THE ESTROUS CYCLE ON INTERVAL  
TO ESTRUS AND FERTILITY AFTER PGF<sub>2α</sub> INJECTION  
IN BEEF FEMALES

by

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A MASTER'S THESIS

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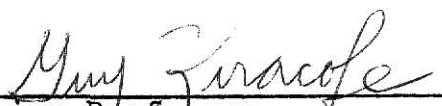
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## LITERATURE REVIEW

Properties of Prostaglandins

Prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) is an unsaturated monocarboxylic acid of 20 carbon atoms based on a 5-membered ring with two adjacent side-chains (figure 1). The free form is soluble in alcohol, and the sodium or tromethamine (THAM) salt is soluble and stable in water (Walpole, 1975).

$PGF_{2\alpha}$  is biosynthesized from arachidonic acid in many mammalian tissues.  $PGF_{2\alpha}$  is not stored in the tissues, but is released directly into the blood where it is rapidly metabolized by the lung and liver. Over 90% of the circulating  $PGF_{2\alpha}$  is converted to 15-keto-13,14-dihydro- $PGF_{2\alpha}$  (its main plasma metabolite) during a single passage through the lung (Vane, 1969; Kindahl, 1980).

Exogenous  $PGF_{2\alpha}$  causes regression of the corpus luteum (CL) in rats (Bishop and Flack, 1973), sheep (McCracken et al., 1970), swine (Hallford et al., 1974), horses (Douglas and Ginther, 1974) and cattle (Rowson et al., 1972; Lauderdale, 1972; Louis et al., 1972; King and Robertson, 1974; Roche, 1974; Hafs et al., 1975a).

Synthetic analogs of  $PGF_{2\alpha}$  have been developed. These analogs are potent luteolytic agents and are highly selective in their pharmacological activity (Cooper and Rowson, 1975). Two analogs, ICI 79,939 and ICI 80,996, cause luteal regression in cattle (Tervit et al., 1973; Cooper, 1974). Due to their luteolytic property,  $PGF_{2\alpha}$  and its analogs can be used as a method

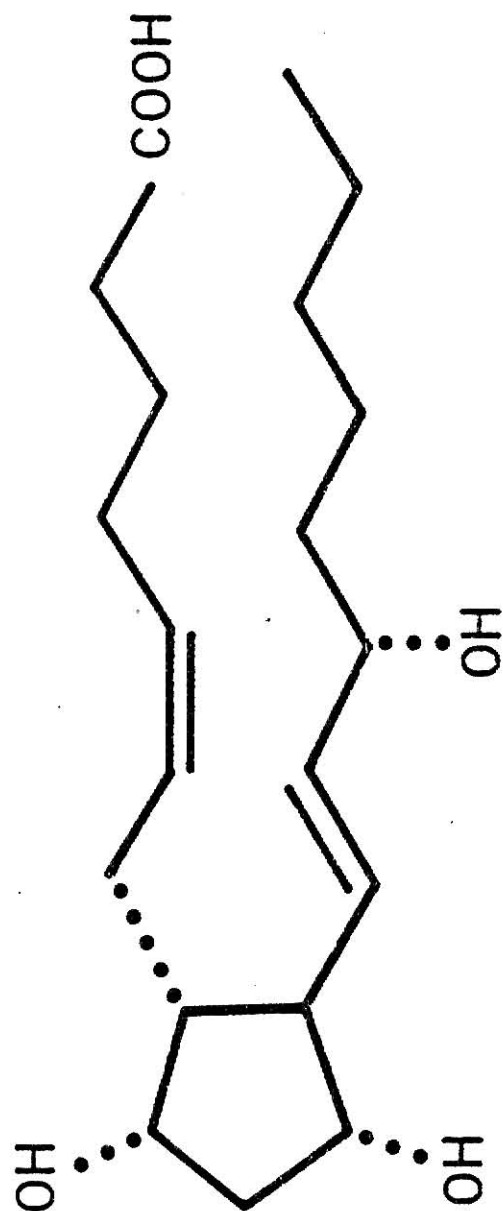


Figure 1. Chemical structure of PGF<sub>2α</sub>.

of estrous synchronization in beef and dairy females.

#### Luteolytic Ability of PGF<sub>2α</sub> During the Estrous Cycle

Prostaglandins (PGF<sub>2α</sub> and its analogs) will not initiate ovarian activity in cattle that are not cycling (Roche et al., 1978). Roche (1976) palpated ovaries of heifers before giving two intramuscular (im) injections of cloprostenol (ICI 80,996) 11 days apart. Heifers with inactive ovaries at the beginning of treatment did not show synchronized estrus.

In cycling cattle, prostaglandins when given on Days 0 to 4 of the estrous cycle (Day 0= day of estrus) are ineffective in causing CL regression (Lauderdale, 1972). Rowson et al. (1972) placed .5 mg PGF<sub>2α</sub> into the uterine horn ipsilateral to the ovary containing the CL on two consecutive days. This treatment caused luteal regression in cattle on Days 5 to 16 of the cycle but was ineffective in cattle on Days 1 to 4.

In Holstein heifers, none of the nine animals given a subcutaneous (sc) injection of 30 mg PGF<sub>2α</sub> on Days 0 to 4 showed estrus in response to the treatment (King and Robertson, 1974). Likewise, beef heifers given PGF<sub>2α</sub> on Days 1 to 4 did not return to estrus. Those treated on Days 5 to 16 returned to estrus in 2 to 4 days (Ellicott et al., 1974; Lauderdale, 1972).

Beal et al. (1980) reported that the corpus luteum of cattle can be regressed with twice daily injections of 25 mg PGF<sub>2α</sub> given on both Days 3 and 4 of the cycle. Two injections of 25 mg PGF<sub>2α</sub> given on Day 4 only or twice daily injections on two consecutive days earlier in the cycle did not reduce cycle

length.

During a normal bovine estrous cycle, the animal's own luteolytic mechanism causes the CL to regress beginning on Day 16 (Gomes and Erb, 1965). After luteolysis the animal will show estrus in 2 to 4 days. Thus, cattle treated with prostaglandins after Day 16 will show estrus after treatment, but this estrus is not a result of the exogenous prostaglandin.

It has been established that prostaglandins are ineffective in anestrus cattle; however, in cycling cattle, prostaglandins regress the corpus luteum on Days 5 to 16 of the estrous cycle. Prior to Day 5 the CL is insensitive to prostaglandin unless repeated treatments are given on Days 3 and 4. The CL is regressed by the cow's own internal luteolytic mechanism after Day 16 of the cycle.

#### Reproductive and Endocrine Events After Administration of Exogenous Prostaglandins

The sequence of reproductive and endocrine events which occurs after prostaglandin treatment is similar to that which occurs during the 3 days before estrus in untreated cattle (Louis et al., 1974; Swanson and Hafs, 1971; Wettemann et al., 1972; Chenault et al., 1975). Louis et al. (1974) gave intrauterine PGF<sub>2α</sub> treatment to cows on Days 7, 11 or 15 and found luteal diameter decreased from 2.5 to 1.6 and .9 cm by 24 and 48 hr post-treatment. Blood serum progesterone declined to less than 50% of the pre-treatment concentration by 12 hr and continued to decline through 72 hr. Estradiol doubled within 24 hr and continued to increase until the onset of estrus. Serum LH remained

low until its rapid increase to a peak shortly before estrus. Estrus was followed by ovulation and formation of a new CL. The subsequent cycle was normal. The reproductive and endocrine events following  $\text{PGF}_{2\alpha}$  were similar to control cycles in the same cows. Louis et al. (1973) also showed a similar sequence of events for cattle treated im with  $\text{PGF}_{2\alpha}$ . Blood serum progesterone fell 60% by 12 hr and reached basal levels by 24 hr. Blood serum LH remained at diestrous concentrations until 12 hr before estrus. LH then surged to a peak which lasted 6 to 8 hr. Estrus was followed by ovulation. Dobson et al. (1975) gave heifers ICI 79,939 im during diestrus. The corpora lutea showed rapid morphological regression; plasma progesterone levels fell significantly by 6 hr post-treatment and reached basal levels by 24 hr. Estrus was followed by ovulation. The corpora lutea formed were functional as judged by plasma progesterone concentrations and cycles of normal length.

The interval from the beginning of estrus to ovulation averaged 24 hr when Louis et al. (1975) infused  $\text{PGF}_{2\alpha}$  into the uterus of diestrous cows. This was similar to the interval in untreated heifers (Swanson et al., 1972). Refsal and Seguin (1980) monitored ovulation by rectal palpation in heifers given cloprostenol im. Ovulation occurred 24.4 hr after the onset of estrus. Wishart (1974) examined the ovaries of Friesian heifers at 4-hr intervals by endoscopy following  $\text{PGF}_{2\alpha}$  induced estrus. The interval from estrous onset to ovulation was 27.2 hr which was similar to 29.0 hr for untreated controls. Thus, estrus can be used as an indicator of the proper time to inseminate cattle

following prostaglandin treatment.

From the literature reviewed, it appears that treatment of diestrous cattle with prostaglandins causes a rapid regression of the CL with a corresponding decrease in plasma progesterone levels. Once the inhibitory effect of progesterone is removed, follicular development occurs and plasma estrogen concentrations increase. Threshold estrogen levels result in the expression of estrus and the pre-ovulatory release of LH. Ovulation follows estrus at an interval similar to that in spontaneous estrus.

#### Failure to Synchronize Estrus After Prostaglandin Treatment

Failure to synchronize estrus in cattle after treatment with prostaglandins during responsive periods of the estrous cycle have ranged from 0 to 40% (Adeyemo et al., 1979; Seguin et al., 1978). Several causes contributing to synchronization failure have been reported. Lauderdale (1975) observed seven cows that were not synchronized after two injections of  $\text{PGF}_{2\alpha}$  given 11 days apart. Those cows were detected in estrus a normal cycle interval after the synchronized period indicating that a CL was formed. Synchronization failure was due to failure in estrous detection and/or to cows having ovulations without estrus.

Cooper and Rowson (1975) reported that two heifers expressed inconclusive symptoms of estrus following treatment with cloprostenol. Those animals ovulated at the expected time after treatment. Thimonier et al. (1975) also found ovulation without estrus as a cause of non-synchronization.

Seguin et al. (1978) injected cows that had a mature CL



with cloprostenol. Sixty to 65% of the cows were detected in estrus within 5 days after treatment. They attributed this low response to failure to detect estrus. Lauderdale et al. (1974) determined that the rate of estrous detection during a 7-day period following  $\text{PGF}_{2\alpha}$  treatment ranged from 73 to 90% when estrus was checked twice a day.

King and Robertson (1974) found that three heifers on Days 7 or 8 at  $\text{PGF}_{2\alpha}$  treatment had normal length cycles from their last date of estrus indicating that luteolysis did not occur. Refsal and Seguin (1980) treated heifers with cloprostenol and monitored progesterone decline. One early cycle heifer (Days 5 to 8) had incomplete luteal regression. Serum progesterone decreased from 3.0 ng/ml to 1.0 ng/ml at 12 hr post-treatment and then rose to 1.7 ng/ml at 24 hr. This animal showed no signs of estrus. In a trial done by Chenault et al. (1976), one of seven dairy animals treated with  $\text{PGF}_{2\alpha}$  on Days 8 to 13 had incomplete luteal regression. Plasma progesterone declined following  $\text{PGF}_{2\alpha}$  but did not fall below 1.0 ng/ml. Toward the end of the sampling period, the CL appeared to recover as demonstrated by increasing progesterone concentration. This animal had a 21-day cycle. Bachlaus et al. (1980) found similar proportions of Day 9 and Day 15 Indian Water Buffalo heifers that failed to return to estrus after treatment with prostaglandins. The nonresponding heifers had a slow decline in progesterone concentrations which never fell below 1.0 ng/ml.

Jackson et al. (1979b) monitored progesterone levels in the

milk of dairy cows after both of two injections of cloprostenol given 11 days apart. Eighteen per cent of the cows did not show estrus after the second injection. Lack of synchronization was due to an extended period of 8 or more days of low progesterone following the first injection. Thus, the second injection was given to these cows when they had an immature corpus luteum. Roche and Prendiville (1979) reported that 16% of the dairy cows that showed estrus after the first injection of cloprostenol responded late (observed in estrus 6 to 11 days post-treatment). At the second injection, these cows were on Days 0 to 5 and failed to synchronize.

Unobserved estrus, ovulation without estrus, incomplete luteal regression and delayed CL regression following the first injection are all factors which result in failure to synchronize estrus in cycling cattle following prostaglandin treatment. In any particular trial, one or a combination of these factors may result in synchronization failure.

#### Degree of Synchrony After Prostaglandins

One objective of an estrous synchronization program is to synchronize estrus and ovulation such that a single time insemination will result in acceptable conception rates. However, the degree of synchrony of estrus after prostaglandin treatment has been variable. Lauderdale et al. (1974) gave a single injection of  $\text{PGF}_{2\alpha}$  to beef cows and heifers which had a palpable CL. Animals showed estrus over a 7-day period with 88% in estrus on Days 3, 4 and 5 post-treatment. Similar results were found in

dairy cows and heifers given a single injection of cloprostenol. Animals showed estrus from Day 1 to 7 after treatment with 83% in estrus on Days 3 to 5 (Seguin et al., 1978). The variation in time to estrus after a single injection of prostaglandin indicates that a single time insemination will not give maximum conception rates.

The degree of synchrony of estrus after the second of two injections of prostaglandins given 10 to 12 days apart has been greater than after a single injection. Johnson (1978) gave Hereford/Friesian heifers two injections of cloprostenol 11 days apart. Following the first and second injection, 40 and 70% of the heifers which expressed estrus were in estrus within a 24-hr period around the mean time to estrus. They suggested that the more precise onset of estrus after the second injection was due to the majority of animals being at a similar stage of the estrous cycle.

Refsal and Seguin (1980) divided cycling heifers into early diestrous (Days 5 to 8) and late diestrous (Days 9 to 17) groups at the first of two cloprostenol treatments given 11 days apart. At the second injection all heifers were at a similar stage of the estrous cycle. The degree of synchrony of the onset of estrus was significantly more precise after the second injection than the first. Cooper and Rowson (1975) obtained a high degree of synchronization after the second injection of cloprostenol. Ninety-one per cent of the heifers showed estrus during a 24-hr period. However, in a similar trial only 42% of the heifers and

48% of the cows were in estrus during a 24-hr period following the second injection (Hafs et al., 1975b).

Several methods have been used to increase the degree of synchronization of estrus and ovulation following prostaglandin treatment. Thimonier et al. (1975) gave two injections of ICI 80,996 10 days apart to one group of cattle. Another group was given a 9-day progesterone (SC 21,009) implant plus a single ICI 80,996 injection given 2 days before implant removal. The percent in estrus during a 24-hr period was greater (43 vs 71%) for the implant plus prostaglandin treatment. Inskeep et al. (1975) treated beef females with two injections of  $\text{PGF}_{2\alpha}$ . Half of the animals received 400 ug estradiol benzoate im 48 hr after the second  $\text{PGF}_{2\alpha}$  treatment. Estradiol benzoate increased the proportion of animals in estrus between 48 and 84 hr from 64 to 95%. Peters et al. (1977) found that estradiol benzoate given 48 hr after  $\text{PGF}_{2\alpha}$  increased the precision of synchronization. Onset of estrus within a period of 56 to 86 hr after  $\text{PGF}_{2\alpha}$  increased by 23 and 15% in cows and heifers by the estrogen treatment. Estradiol benzoate given 28 hr after the second ICI 80,996 injection reduced the variation in the time of estrous onset and LH peak in cows (Nancarrow and Radford, 1975).

Two thousand IU of pregnant mare serum gonadotropin (PMSG) given 1 or 2 days before ICI 79,939 reduced the interval to estrus and increased the synchrony of estrous onset in cattle (Tervit et al., 1973). However, Moore (1975) found that a lower dose of PMSG (1000 IU) given 2 days before  $\text{PGF}_{2\alpha}$  decreased

estrous synchrony.

Chipepa et al. (1977) gave LHRH/FSHRH analog to beef cows either 48 or 68 hr after the second  $\text{PGF}_{2\alpha}$  injection to synchronize ovulation. The LH surge was synchronized around 1 hr after LHRH/FSHRH treatment. However, the conception rate to the 72-hr insemination was not improved over  $\text{PGF}_{2\alpha}$  alone.

It appears that the degree of synchrony of estrus is low following a single prostaglandin treatment; however, a higher degree of synchrony is possible following a double prostaglandin injection regime, but results have been variable. The degree of grouping the animals into a similar stage of the estrous cycle at the time of treatment appears to be the major factor in the precision of synchronization. Other factors such as age and breed of the animals, type of prostaglandin and season of the year may affect the degree of synchronization but have not been investigated. Progesterone, estradiol benzoate, PMSG and LHRH/FSHRH analog each in combination with prostaglandin have improved the degree of synchrony over prostaglandin alone. However, not all of these compounds are commercially available, and their effect on fertility has not been thoroughly studied.

#### Factors Affecting the Interval to Estrus After Exogenous Prostaglandins

The mean time interval from prostaglandin treatment to the onset of estrus is variable and ranges from 48 to 92 hr (Hardin et al., 1980; Thimonier et al., 1975). Factors affecting this interval are the day of the estrous cycle at treatment, number

of prostaglandin treatments, season of the year, age and breed of animal. Time of day when prostaglandin is given and dose of prostaglandin do not appear to affect the interval to estrus.

The day of the estrous cycle when cattle were treated with prostaglandins affected the interval to onset of estrus. Ellicott et al. (1974) treated Hereford heifers with  $\text{PGF}_{2\alpha}$  on Days 6 to 15. The return to estrus was influenced by day of the cycle injected: Days 6 and 7, 47 hr; Days 8 and 9, 79.6 hr and Days 10 to 15, 74 hr. Dobson et al. (1975) observed that heifers injected with ICI 79,939 on Days 6 to 8 had a shorter interval to estrus than those injected on Days 9 to 13. Jackson et al. (1979a) also found an interaction between the day of the cycle and interval to estrus. Heifers on Days 7, 8, 15 and 16 at the time cloprostenol was injected responded significantly earlier than those on Days 12 to 14. However, when Louis et al. (1974) deposited  $\text{PGF}_{2\alpha}$  into the uterine horn ipsilateral to the corpus luteum on Days 7, 11 or 15 in Holstein cows, the day of the cycle had no effect on the interval to estrus. All cows showed estrus approximately 72 hr post-treatment.

Two mechanisms have been proposed to account for the day of the cycle effect on interval to estrus. First, Dobson et al. (1975) proposed that the shorter interval to estrus in cattle on Days 6 to 8 was due to mid-cycle follicles which were present at that time (Hancock, 1962) which continued to mature and ovulate. This agrees with the work of Edqvist et al. (1975). They gave  $\text{PGF}_{2\alpha}$  to heifers on Day 8 or 14 of the cycle. On Day 8 a

follicle could be palpated at the time of treatment. After luteolysis that follicle continued to grow and ovulate. On Day 14 no follicle could be palpated. A palpable follicle appeared within 2 days after treatment. Jackson et al. (1979a) found significantly higher FSH levels in animals treated with cloprostenol on Days 6 to 9 than in those treated on Days 11 to 15 thus giving indirect evidence of follicular development between Days 6 and 9. Chenault et al. (1976) found that animals with a higher concentration of plasma progestins at PGF<sub>2α</sub> treatment had a significantly longer interval to estrus. Refsal and Seguin (1980) found that the serum progesterone level at the time of cloprostenol administration was positively correlated to the interval to estrus. Thus, animals on Days 10 to 15 would have a higher progesterone level than those on Days 5 to 9 (Gomes and Erb, 1965) and their interval to estrus would be longer. Either of these mechanisms, and others, may be involved in determining the rapidity of response to prostaglandins.

The interval to estrus after the second of two prostaglandin injections given 10 to 12 days apart was significantly shorter than after the first injection (Johnson, 1978; Hardin et al., 1980; Thimonier et al., 1975). However, the earlier response after the second injection is confounded by the effect of day of the cycle since the two injection system groups the majority of the animals on Days 6 to 8 at the second injection (Johnson, 1978). Thimonier et al. (1975) gave cows two injections of ICI 80,996 10 days apart. When the first injection did not induce



luteolysis, the second injection was given after the 10th day of the same cycle and the interval to the onset of estrus was 72 to 96 hr. When the first injection induced luteal regression, the second was given between Days 6 and 8 of a new cycle. The time to onset of estrus was 48 hr. Thus, the overall mean time to estrus appears to depend on the animal distribution by day of the cycle at the second injection.

Britt et al. (1978) reported that the interval to estrus after the second  $\text{PGF}_{2\alpha}$  injection was not affected by the time of day that  $\text{PGF}_{2\alpha}$  was given. However, season of the year had a significant effect on time interval to estrus. They proposed that endocrine changes associated with season of the year altered the hormonal events leading to estrus after  $\text{PGF}_{2\alpha}$ . Burfening et al. (1978) compared the time to estrus of heifers and lactating cows. The interval to estrus was significantly shorter for the heifers than for the cows (52 vs 75 hr). Moore (1975) measured the time interval to estrus in Jersey, Friesian, Hereford, Santa Gertrudis and Brahman cows treated with  $\text{PGF}_{2\alpha}$ . The Brahman cows responded 24 hr later than the other breeds indicating a breed difference in time to estrus after  $\text{PGF}_{2\alpha}$ .

The dose of  $\text{PGF}_{2\alpha}$  did not influence the interval to estrus. Hafs et al. (1975b) found no difference in the distribution of estrus among heifers treated with 20, 30 and 40 mg  $\text{PGF}_{2\alpha}$  or in cows treated with 30 or 60 mg  $\text{PGF}_{2\alpha}$ . Louis et al. (1975) treated diestrous heifers with either 30 mg  $\text{PGF}_{2\alpha}$  (im), two injections (im) of 15 mg  $\text{PGF}_{2\alpha}$  at 6-hr intervals or 60 mg  $\text{PGF}_{2\alpha}$ .



The interval from  $\text{PGF}_{2\alpha}$  to the onset of estrus and ovulation was not different between treatments.

Generally, the time interval to estrus following prostaglandin treatment is near 72 hr (Louis et al., 1973; Louis et al., 1974; Britt et al., 1978), but values from 48 to 92 hr have been reported (Britt et al., 1978; Thimonier et al., 1975; Johnson, 1978, Burfening et al., 1978). Many factors such as day of the cycle (Ellicott et al., 1974; Dobson et al., 1975; Jackson et al., 1979a), number of prostaglandin treatments (Johnson, 1978; Hardin et al., 1980; Thimonier et al., 1975), season of the year (Britt et al., 1978), age of animal (Burfening et al., 1978) and breed of animal (Moore, 1975) appear to influence this interval. However, in most of these trials these factors were confounded. Projects need to be designed where each of these factors can be studied individually. Day of the estrous cycle appears to be the major factor affecting time to estrus and may account for the variation attributed to other factors. Time of day of prostaglandin treatment (Britt et al., 1978) and dose of prostaglandin (Hafs et al., 1975b; Louis et al., 1975) appear to have no effect on interval to estrus.

#### Fertility After Prostaglandins

The fertility of the induced estrus is an important consideration of any method of synchronization. When cattle were inseminated by estrus following one or more prostaglandin treatments, the fertility was equal to untreated animals. Roche (1974) gave beef heifers a single  $\text{PGF}_{2\alpha}$  injection if they had not been

in estrus during the last 5 days. Treated heifers were inseminated 12 to 18 hr after estrous onset. Untreated controls were inseminated as they showed spontaneous estrus. The fertility was similar in treated and untreated animals. Cooper (1974) found no reduction in fertility in Friesian heifers that were inseminated at the end of estrus following the second of two cloprostenol injections given 11 days apart.

In lactating dairy cows treated with cloprostenol on Days 7 to 16 of the cycle, the fertility of treated animals inseminated by estrus was significantly higher than untreated herd-mates inseminated by estrus (Macmillan et al., 1980). Seguin et al. (1978), however, showed no difference in fertility in dairy cattle treated with cloprostenol and inseminated by estrus.

Lauderdale et al. (1981) gave seven consecutive injections of  $\text{PGF}_{2\alpha}$  at 10 to 12 day intervals to beef heifers. After the seventh treatment, the heifers were inseminated as they showed estrus. The fertility of the treated animals was equal to untreated controls. Thus, repeated treatment with  $\text{PGF}_{2\alpha}$  did not reduce fertility.

Estrous detection during artificial insemination programs requires much labor. One advantage of estrous synchronization is that time inseminations may be possible and estrous detection eliminated. However, time insemination must not lower conception rates. Lauderdale et al. (1974) gave a single  $\text{PGF}_{2\alpha}$  injection to cows and heifers which had palpable corpora lutea. Following

treatment the animals were either inseminated 12 hr after the onset of estrus or time inseminated at 72 and 90 hr after  $\text{PGF}_{2\alpha}$ . Untreated controls were inseminated 12 hr after estrous onset. The fertility was similar to controls when cattle were inseminated at the synchronized estrus or at predetermined times after  $\text{PGF}_{2\alpha}$ . Leaver et al. (1975) gave dairy heifers a single injection of cloprostenol on Days 5 to 16. The conception rate of heifers inseminated at 72 and 96 hr was equal to that of heifers inseminated by estrus. In those time inseminated, a greater proportion conceived to the 72-hr insemination suggesting that the optimum time for a single insemination is nearer to 72 hr.

Since estrus is synchronized more closely after two injections of prostaglandins given 10 to 12 days apart, a single time insemination may be possible. Hafs et al. (1978) gave beef cows and beef and dairy heifers two injections of  $\text{PGF}_{2\alpha}$  10 to 12 days apart. Treated animals were inseminated at 80 hr or at 70 and 88 hr after the second injection. Control animals were inseminated as they showed natural estrus. The conception rates of the three groups were similar. They concluded that fertility of prostaglandin treated cattle inseminated without estrous detection was equal to that in untreated animals, and that fertility from one insemination at 80 hr was equivalent to that from two inseminations. Others have reported similar results (Hafs et al., 1975c; Louis et al., 1975; Manns et al., 1976).

Burfening et al. (1978) inseminated cycling beef heifers at either 80 or 72 and 96 hr after the second  $\text{PGF}_{2\alpha}$  injection.

The conception rate to the 80-hr insemination was significantly lower than that to the double insemination. Of those inseminated at 72 and 96 hr, all but one calf resulted from the 72-hr insemination. In this trial 80 hr was too late to inseminate beef heifers. Hardin et al. (1980) found that conception rates were significantly lower in Brahman crossbred heifers and cows after a single insemination at 80 hr than in those inseminated by estrus after the second cloprostenol injection.

Prostaglandins do not lower fertility when cattle are inseminated by estrus (Lauderdale et al., 1974; Roche, 1974; Cooper, 1974; Lauderdale et al., 1981). Two time inseminations at approximately 70 and 90 hr following one or two prostaglandin treatments give conception rates equal to untreated animals (Leaver et al., 1975; Hafs et al., 1978). A single time insemination at 80 hr following the second of two prostaglandin injections given 10 to 12 days apart has given conception rates equal to control animals (Hafs et al., 1975c; Louis et al., 1975; Manns et al., 1976). However, in other trials conception rates have been significantly lower to the 80-hr insemination (Burfening et al., 1978; Hardin et al., 1980). Eighty hours appears to be too late to inseminate heifers (Burfening et al., 1978; Leaver et al., 1975). The optimum time for a single time insemination may be affected by breed of animal, type of prostaglandin, stage of the cycle at treatment, etc. These factors have not been investigated.

THE EFFECT OF STAGE OF THE ESTROUS CYCLE ON INTERVAL  
TO ESTRUS AND FERTILITY AFTER  $\text{PGF}_{2\alpha}$  INJECTION  
IN BEEF FEMALES

SUMMARY

Beef heifers and cows were given two injections of  $\text{PGF}_{2\alpha}$  and the day of the estrous cycle at the time of the second  $\text{PGF}_{2\alpha}$  injection was determined from the last observed estrus. Animals were either artificially inseminated 12 to 18 hr after the onset of estrus or inseminated at approximately 80 hr after the second injection. Blood samples were collected at 4-hr intervals after the second  $\text{PGF}_{2\alpha}$  injection from Day 7 and Day 14 heifers and assayed for progesterone.

The interval from the second  $\text{PGF}_{2\alpha}$  injection to the onset of estrus was shorter in heifers than in cows (53.6 vs 61.9 hr). The day of the estrous cycle at the second  $\text{PGF}_{2\alpha}$  injection significantly affected the interval to estrus, and based on those results both cows and heifers were divided into early cycle (Days 5 to 9) and late cycle (Days 10 to 15) groups. Both heifers and cows receiving their second injection of  $\text{PGF}_{2\alpha}$  early in the cycle showed estrus significantly earlier than those late in the cycle.

The conception rate of early cycle heifers inseminated at 80 hr after the second  $\text{PGF}_{2\alpha}$  injection was lower ( $P < .05$ ) than the late cycle heifers inseminated at 80 hr. When heifers were inseminated by estrus, there was no difference in fertility between early and late cycle animals. It appears that the 80 hr

insemination is too late to inseminate early cycle heifers. There were no differences in conception rates between early and late cycle cows inseminated at 80 hr ( $P=.28$ ) or inseminated by estrus ( $P>.50$ ). The 80-hr insemination appears to be an appropriate time to inseminate both early and late cycle beef cows.

In both Day 7 and Day 14 heifers, serum progesterone declined significantly by 4 hr post-injection and continued to decline linearly through 36 hr. The day of the estrous cycle at  $\text{PGF}_{2\alpha}$  injection had no effect on the rate of progesterone decline ( $P>.40$ ). However, Day 14 heifers had a longer interval to estrus after the  $\text{PGF}_{2\alpha}$  injection than Day 7 heifers ( $P<.05$ ). This suggests that the rate of progesterone decline is not a factor in the shorter interval to estrus in cattle injected with  $\text{PGF}_{2\alpha}$  early in the cycle.

#### INTRODUCTION

Prostaglandin  $\text{F}_{2\alpha}$  causes luteolysis and return to estrus in cattle when given on Days 5 to 16 of the estrous cycle (Lauderdale, 1972; Rowson et al., 1972). Since  $\text{PGF}_{2\alpha}$  is only luteolytic on Days 5 to 16 of the estrous cycle, a single treatment will not synchronize estrus in an entire herd of randomly cycling cattle. However, two  $\text{PGF}_{2\alpha}$  injections given 10 to 12 days apart should theoretically synchronize all cycling animals after the second injection (King and Robertson, 1974; Lauderdale et al., 1974).

Generally, the mean interval to estrus following prostaglandin treatment is near 72 hr (Louis et al., 1973; Louis et al., 1974; Britt et al., 1978), but values have ranged from 48 to 92 hr (Britt et al., 1978; Thimonier et al., 1975;

Johnson, 1978; Burfening et al., 1978). The day of the estrous cycle when prostaglandin is given appears to affect the interval to estrus (Ellicott et al., 1974; Dobson et al., 1975; Jackson et al., 1979a). Other factors such as season of the year (Britt et al., 1978), age of animal (Burfening et al., 1978) and breed of animal (Moore, 1975) appear to affect this interval, but day of the cycle at treatment was not known in those trials.

Fertility of cattle inseminated by estrus after  $\text{PGF}_{2\alpha}$  treatment is normal (Inskeep, 1973; Lauderdale et al., 1974; Roche, 1974). A single insemination at 80 hr following the second of two injections of  $\text{PGF}_{2\alpha}$  given 10 to 12 days apart has resulted in conception rates equal to untreated control animals inseminated by estrus (Louis et al., 1975; Manns et al., 1976; Hafs et al., 1978). However, in other trials conception rates have been lower at an 80-hr insemination (Burfening et al., 1978; Hardin et al., 1980).

Chenault et al. (1976) found that animals with a higher concentration of plasma progesterone at  $\text{PGF}_{2\alpha}$  treatment had a longer interval to estrus. Refsal and Seguin (1980) reported that serum progesterone at the time of cloprostenol administration was positively correlated to the interval to estrus. This indicates that the pattern of progesterone decline after  $\text{PGF}_{2\alpha}$  injection is a factor controlling interval to estrus.

The objectives of this study were to determine: (1) the effect of stage of the estrous cycle at the time of a second  $\text{PGF}_{2\alpha}$  injection on the interval to estrus in beef cows and

heifers; (2) if an 80-hr insemination is an appropriate time to inseminate both cows and heifers; (3) if the rate of serum progesterone decline after  $\text{PGF}_{2\alpha}$  injection affects the interval to estrus in animals treated early and late in the cycle.

#### MATERIALS AND METHODS

The effect of stage of the estrous cycle at the time of  $\text{PGF}_{2\alpha}$  injection on subsequent reproductive events in beef females was studied in four trials conducted in 1980 and 1981.

All injections consisted of 25 mg  $\text{PGF}_{2\alpha}$  (THAM salt)<sup>1</sup> given intramuscularly. The onset of estrus (Day 0) was defined as the time when an animal would stand to be mounted by another animal. Pregnancy was determined by rectal palpation 45 to 65 days after insemination.

Trial 1. Observations for estrous behavior were made twice daily for 6 days in 87 Angus, Hereford and Simmental yearling heifers confined in dry lot at Kansas State University. All 87 heifers were then injected with  $\text{PGF}_{2\alpha}$  and observed for estrus for another 6 days. Heifers observed in estrus during those 12 days were given a second  $\text{PGF}_{2\alpha}$  injection 11 days after the first. Day of the estrous cycle at the second injection was determined from the last observed estrus.

Following the second injection, heifers were checked for estrus at 4-hr intervals (0200, 0600, 1000, 1400, 1800 and 2200 hr) for 6 days by visual observation and time to estrus was

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<sup>1</sup>Prostaglandin  $\text{F}_{2\alpha}$  (dinoprost tromethamine), the Upjohn Company, Kalamazoo, Michigan 49001.



recorded. Heifers were artificially inseminated approximately 80 hr after the second  $\text{PGF}_{2\alpha}$  injection.

Heifers were fed a ration meeting the NRC requirements for growing beef heifers.

Trial 2. Lactating Angus and Hereford cows 32 to 72 days postpartum were injected with  $\text{PGF}_{2\alpha}$ . Cows were on native range pasture at Kansas State University. Estrus was checked three times daily for 2 to 5 days after treatment. All cows showing estrus received a second  $\text{PGF}_{2\alpha}$  injection on either Days 5 to 8 or Days 12 to 15 after estrus. The groups were equalized for the day animals showed estrus, breed, days postpartum and age. After the second injection, estrus was observed twice daily until 80 hr. All cows were artificially inseminated approximately 80 hr after the second injection.

Trial 3. Estrus was observed twice daily beginning 5 days before and continuing through 6 days after the first  $\text{PGF}_{2\alpha}$  injection in lactating Hereford cows. Estrus was detected by visual observation and aided by androgenized cows equipped with chin ball markers. Cows were kept on native pasture near Winfield, Kansas. All cows observed in estrus during the 11-day observation period received a second  $\text{PGF}_{2\alpha}$  injection 11 days after the first. Day of the cycle at the second injection was determined from the last observed estrus.

Following the second injection, estrus was checked daily at 0700, 1000, 1400, 1700 and 2200 hr for 6 days. Half of the cows injected early in the cycle (Days 5 to 9) and half of the

cows injected late in the cycle (Days 10 to 15) were artificially inseminated 12 to 18 hr after the onset of estrus; the other half of each group was inseminated approximately 80 hr after the second injection.

Trial 4. Estrus was checked twice daily in 104 Angus, Hereford, Polled Hereford and Simmental yearling heifers beginning 5 days before and continuing until 6 days after a  $\text{PGF}_{2\alpha}$  injection. Heifers were maintained in dry lot at Kansas State University and were fed a ration meeting NRC requirements for growing beef heifers.

Approximately half of the heifers received a second  $\text{PGF}_{2\alpha}$  injection on Days 6 to 8 of their estrous cycle while the other heifers were reinjected on Days 13 to 15. Following the second injection, estrus was observed at 4-hr intervals as in Trial 1.

Heifers in estrus at 1000, 1400 and 1800 hr were artificially inseminated at approximately the next 0700 hr. Those in estrus at 2200, 0200 and 0600 hr were inseminated at approximately the next 1900 hr.

Blood samples were obtained from all heifers by jugular venipuncture immediately before the second  $\text{PGF}_{2\alpha}$  injection. In addition, blood was taken from 10 heifers receiving their second injection on Day 7 and from 10 heifers on Day 14 (each group contained 5 Polled Hereford and 5 Simmental heifers) at 4-hr intervals from 4 hr before to 36 hr after the second injection. Blood was collected in evacuated tubes, immediately refrigerated, allowed to clot for approximately 24 hr and centrifuged. Serum

was stored at  $-20^{\circ}\text{C}$  until analyzed. Serum progesterone was determined by radioimmunoassay as previously described (Stevenson et al., 1981).

Only animals on Days 5 to 15 of their estrous cycle at the time of the second  $\text{PGF}_{2\alpha}$  injection were used in the data analysis. Data on interval to estrus and serum progesterone were analyzed by the SAS General Linear Model procedure (Barr et al., 1979); data on conception was analyzed either by Chi-square tests (Snedecor and Cochran, 1967) or by Fisher exact tests (Zar, 1974).

## RESULTS AND DISCUSSION

Interval to Estrus. Data on interval to estrus after the second  $\text{PGF}_{2\alpha}$  injection was pooled for heifer trials and for cow trials since there were no differences in interval to estrus between trials using the same type of animal ( $P > .10$ ).

The interval from the second  $\text{PGF}_{2\alpha}$  injection to the onset of estrus was shorter ( $P < .01$ ) in heifers than in cows (table 1). This agrees with the results of Burfening et al. (1978). They found that the interval to estrus after  $\text{PGF}_{2\alpha}$  was 51.8 hr in heifers but was 74.6 hr in cows. The longer interval in cows may be due to the suckling stimulus, but further work is needed to determine the exact mechanism. The mean interval to estrus was shorter in both heifers and cows than previously reported (Britt et al., 1978; Adeyemo et al., 1979; Hardin et al., 1980; Chenault et al., 1976; Louis et al., 1974). However, Dobson et al. (1975) and Burfening et al. (1978) have reported similar

TABLE 1. INTERVAL FROM SECOND PGF<sub>2α</sub> INJECTION TO ESTRUS  
IN HEIFERS AND COWS

Type of animal	No. of animals	Hours from injection to estrus <sup>a</sup>
Heifers <sup>b</sup>	88	53.6 ± 1.0 <sup>c</sup>
Cows <sup>b</sup>	76	61.9 ± 1.1 <sup>d</sup>

<sup>a</sup>Least-squares means ± standard error of least-squares means.

<sup>b</sup>Data pooled from two heifer trials and two cow trials since there was no significant difference in interval to estrus between trials (P>.10).

<sup>c,d</sup>Values significantly different (P<.01).

intervals in heifers and Welch et al. (1975) similar intervals in cows.

The day of the estrous cycle at the time of the second  $\text{PGF}_{2\alpha}$  injection significantly affected the interval to estrus (table 2). In both heifers and cows, animals treated on Days 6 to 9 had similar intervals to estrus; there was a large increase in time to estrus between Days 9 and 10 in heifers and between Days 9 and 11 in cows. Intervals to estrus were similar in heifers treated on Days 10 to 14 and in cows treated on Days 11 to 15. When using the two injection system (two  $\text{PGF}_{2\alpha}$  injections 11 days apart), few animals would be on Days 10 and 11 at the second injection. Thus, early and late cycle groups in both heifers and cows were divided between Days 9 and 10.

Early cycle heifers showed estrus an average of 12.1 hr earlier than late cycle heifers after the second  $\text{PGF}_{2\alpha}$  injection (table 3). Early cycle cows came into estrus 9.6 hr earlier than late cycle cows. Similar results on the effect of stage of the estrous cycle on interval to estrus have been reported (Dobson et al., 1975; Thimonier et al., 1975; Johnson, 1978; Jackson et al., 1979a). Early cycle heifers had a shorter ( $P < .01$ ) interval to estrus than early cycle cows. A similar relationship was found between late cycle heifers and cows.

Mid-cycle follicular growth occurs on Days 6 to 8 of the bovine estrous cycle with the number of large follicles (diameter  $> 8\text{mm}$ ) reaching a peak on Day 8. The number of large follicles is low on Days 9 to 14 (Schams et al., 1977). This period

TABLE 2. EFFECT OF DAY OF CYCLE ON INTERVAL TO ESTRUS IN HEIFERS AND COWS AFTER PGF<sub>2α</sub> INJECTION

Day of cycle	Heifers		Cows	
	No. of animals	Hours to estrus <sup>b</sup>	No. of animals	Hours to estrus <sup>b</sup>
5	--	--	1	68.0 + 8.7 <sup>de</sup>
6	7	46.9 + 3.3 <sup>c</sup>	5	51.2 + 3.9 <sup>cd</sup>
7	24	46.4 + 1.8 <sup>c</sup>	18	58.1 + 2.0 <sup>cd</sup>
8	5	47.0 + 3.9 <sup>c</sup>	18	57.3 + 2.0 <sup>cd</sup>
9	8	52.1 + 3.1 <sup>cd</sup>	2	55.5 + 6.1
10	2	61.0 + 6.1 <sup>cde</sup>	--	--
11	1	62.5 + 8.7 <sup>cde</sup>	1	69.5 + 8.7 <sup>de</sup>
12	6	59.6 + 3.5 <sup>cd</sup>	4	71.0 + 4.3 <sup>e</sup>
13	6	54.0 + 3.5 <sup>cd</sup>	4	67.9 + 4.3 <sup>de</sup>
14	28	59.4 + 1.6 <sup>f</sup>	13	65.8 + 2.4 <sup>de</sup>
15	1	96.0 + 8.7	10	65.4 + 2.9 <sup>de</sup>

<sup>a</sup>Day of the estrous cycle at the second PGF<sub>2α</sub> injection. Day of the cycle had a significant effect on interval to estrus ( $P < .01$ ).

<sup>b</sup>Hours from the second PGF<sub>2α</sub> injection to the onset of estrous (least-squares means + standard error of least-squares means).

<sup>c,d,e,f</sup>Means with different superscripts were significantly different ( $P < .05$ ).

TABLE 3. EFFECT OF STAGE OF THE ESTROUS CYCLE ON INTERVAL TO ESTRUS AFTER PGF<sub>2α</sub> INJECTION

Type of animal	No. of animals		Hours to estrus <sup>a</sup>	
	Early cycle <sup>b</sup>	Late cycle <sup>b</sup>	Early cycle <sup>b</sup>	Late cycle <sup>b</sup>
Heifers	44	44	47.6 ± 1.4 <sup>c</sup>	59.7 ± 1.4 <sup>d</sup>
Cows	44	32	57.1 ± 1.4 <sup>d</sup>	66.7 ± 1.6 <sup>e</sup>

<sup>a</sup>Hours from the second PGF<sub>2α</sub> injection to the onset of estrus (least-squares means + standard error of least-squares means).

<sup>b</sup>Early cycle animals were on Days 5 to 9 and late cycle animals on Days 10 to 15 of the estrous cycle at the second PGF<sub>2α</sub> injection.

<sup>c,d,e</sup>Means with different superscripts were significantly different (P<.01).

of follicular growth corresponds to the stage of the estrous cycle when the interval to estrus is shorter following  $\text{PGF}_{2\alpha}$  treatment. The shorter interval to estrus in early cycle animals may be due to the presence of large follicles which continue to mature and ovulate after the CL is regressed by  $\text{PGF}_{2\alpha}$ . In late cycle animals, follicular growth appears to occur after luteolysis which results in a longer interval to estrus. This thesis is supported by previous work (Edqvist et al., 1975; Thimonier et al., 1975; Jackson et al., 1979a).

Results on the interval to estrus indicate that the optimum time for a single time insemination after the second  $\text{PGF}_{2\alpha}$  injection is dependent on the type of animal (heifer vs cow) and the stage of the estrous cycle at treatment.

Previous work has shown season of the year (Britt et al., 1978) and breed of animal (Moore, 1975) to affect the interval to estrus after  $\text{PGF}_{2\alpha}$ . However, in the present data, when the effects of the animal being a cow or heifer and stage of the cycle were removed, season of year and breed of animal had no effect on interval to estrus (table 4).

Conception. The data on conception rates is summarized in table 5. The conception rates of animals inseminated at 80 hr was calculated by dividing the number of animals pregnant by the number of animals given the second  $\text{PGF}_{2\alpha}$  injection. The conception rates of animals inseminated by estrus was calculated by dividing the number of animals pregnant by the number of animals showing estrus and inseminated after the second  $\text{PGF}_{2\alpha}$  injection.



TABLE 4. INFLUENCE OF VARIOUS FACTORS ON INTERVAL TO  
ESTRUS AFTER PGF<sub>2α</sub> INJECTION

Factor	Probability of having no effect
Heifer or cow	P = .0001
Day of cycle	P = .0001
Early cycle or late cycle	P = .0001
Progesterone concentration at time of injection	P = .0313
Breed of heifer	P = .9742
Breed of cow	P = .1515
Season of year	P = .2750
Year of trial	P = .5985
Age of cow	P = .7107
Age <sup>2</sup> of cow	P = .8244

TABLE 5. CONCEPTION RATES OF HEIFERS AND COWS INSEMINATED AT 80 HOURS OR BY ESTRUS AFTER PGF<sub>2α</sub> INJECTION

Type of animal	Insemination time <sup>b</sup>	Conception rate <sup>a</sup> (no. of animals)		
		Early cycle <sup>c</sup>	Late cycle <sup>c</sup>	Total
Heifers	80 hr	28.6 (14) <sup>d</sup>	62.5 (16) <sup>e</sup>	46.7 (30)
Heifers	By estrus	68.8 (32)	72.4 (29)	70.5 (61)
Cows	80 hr	40.0 (35)	53.3 (30)	46.2 (65)
Cows	By estrus	47.1 (17)	62.5 (8)	52.0 (25)

<sup>a</sup>The conception rates of animals inseminated at 80 hr was calculated by dividing the number of animals pregnant by the number of animals given the second PGF<sub>2α</sub> injection. The conception rates of animals inseminated by estrus was calculated by dividing the number of animals pregnant by the number of animals showing estrus and inseminated after the second PGF<sub>2α</sub> injection.

<sup>b</sup>In animals inseminated at 80 hr, the inseminations were centered around 80 hr after the second PGF<sub>2α</sub> injection. Those inseminated by estrus were inseminated 12 to 18 hr after the onset of estrus.

<sup>c</sup>Animals on Days 5 to 9 at the time of the second PGF<sub>2α</sub> injection were classified as early cycle, those on Days 10 to 15 were classified as late cycle.

<sup>d,e</sup>Conception rates of early and late cycle heifers inseminated at 80 hr were significantly different ( $P < .05$ ).

The conception rate of early cycle heifers inseminated at 80 hr after the second  $\text{PGF}_{2\alpha}$  injection was lower ( $P < .05$ ) than late cycle heifers inseminated at 80 hr. When heifers were inseminated by estrus, there was no difference in fertility between early and late cycle groups. Thus, the lower conception rate of early cycle heifers inseminated at 80 hr appears to be due to the time of insemination and not to reduced fertility. Early cycle heifers that were time inseminated were inseminated an average of 32.4 hr after the onset of estrus. Some were inseminated as much as 44 hr after the onset of estrus. The 80-hr insemination appears to be too late for early cycle heifers but results in acceptable conception rates in late cycle heifers. Since the two injection system tends to group the majority of animals on Days 7 to 9 at the second injection (Johnson, 1978), higher conception rates may be possible in heifers by time inseminating near 70 hr after the second  $\text{PGF}_{2\alpha}$  injection (Leaver et al., 1975; Burfening et al., 1978). Heersche et al. (1979) reported that conception rates were not lowered when beef females were inseminated up to 30 hr after the onset of estrus. Another alternative to improve conception rates in heifers following two  $\text{PGF}_{2\alpha}$  injections may be to observe estrus from 36 to 50 hr after the second  $\text{PGF}_{2\alpha}$  injection and inseminate those heifers by estrus. The remaining heifers could be inseminated at 80 hr.

There was no difference in fertility between early and late cycle cows inseminated by estrus ( $P > .50$ ). The conception rates of early and late cycle cows inseminated at 80 hr were not

different ( $P=.28$ ). These results indicate that 80 hr after the second  $\text{PGF}_{2\alpha}$  injection is an appropriate time to inseminate cows and agrees with data of Hafs et al. (1978), Hafs et al. (1975c) and Louis et al. (1975).

Synchronization Failure. Only animals on Days 5 to 15 of the estrous cycle at the second  $\text{PGF}_{2\alpha}$  injection were used in the data analysis, and theoretically all could be expected to show a synchronized estrus. However, 10.0, 27.5, 13.2 and 11.3% of the animals did not show synchronized estrus after the second  $\text{PGF}_{2\alpha}$  injection in Trials 1 to 4 respectively. The higher failure rate in Trial 2 may have been due to estrus only being observed twice daily through 80 hr after the second injection. In the other trials estrus was checked 5 to 6 times a day for 6 days.

Causes for synchronization failure are summarized in table 6. In eight animals, apparently the CL did not regress after  $\text{PGF}_{2\alpha}$  injection since the animals returned to estrus 18 to 23 days after the estrus before the second  $\text{PGF}_{2\alpha}$  injection. Those eight animals were all 5 to 8 days after estrus when injected indicating that  $\text{PGF}_{2\alpha}$  was not as effective in regressing the CL in early cycle animals as it is in late cycle animals. Others have also reported incomplete luteolysis after  $\text{PGF}_{2\alpha}$  (King and Robertson, 1974; Refsal and Seguin, 1980; Bachlaus et al., 1980).

The CL appeared to regress in seven animals, but either estrus was not observed or these animals ovulated without expressing estrus since they showed estrus 21 to 27 days after  $\text{PGF}_{2\alpha}$  injection. This group included both early and late cycle

TABLE 6. ESTROUS SYNCHRONIZATION FAILURE AND DISTRIBUTION OF ANIMALS BY THE DAY OF THE CYCLE AT SECOND PGF<sub>2α</sub> INJECTION ACCORDING TO TYPE OF SYNCHRONIZATION FAILURE

Trial	Percent failure	CL did not regress <sup>a</sup>		CL did regress but <sup>b</sup> estrus not observed		Responded late <sup>c</sup> after treatment	
		Day of PGF <sub>2α</sub> injection	No. of animals	Day of PGF <sub>2α</sub> injection	No. of animals	Day of PGF <sub>2α</sub> injection	No. of animals
1	10.0 (3/30)	5	1	6	1	6	2
2	27.5 (11/40)	6	4	7	1	9	2
3 <sup>d</sup>	13.2 (7/54)	7	1	12	2	10	1
4	11.3 (8/71)	8	2	13	1		
Total	14.9 (29/194)			14	2		

<sup>a</sup>Animals returned to estrus 18 to 23 days after the estrus prior to the second PGF<sub>2α</sub> injection.

<sup>b</sup>Animals returned to estrus 21 to 27 days after the second PGF<sub>2α</sub> injection.

<sup>c</sup>Animals exhibited estrus 7 to 10 days after the second PGF<sub>2α</sub> injection.

<sup>d</sup>Information was not available to determine cause of synchronization failure.

animals. Five additional animals exhibited estrus 7 to 10 days after the second  $\text{PGF}_{2\alpha}$  injection. This may have been due to slow corpus luteum regression.

Degree of Synchronization. The degree of estrous synchronization in heifers after the second  $\text{PGF}_{2\alpha}$  injection is presented in table 7. Both early and late cycle heifers had a high degree of synchronization (93.2 and 86.5%, respectively) during a 24-hr period around the mean time to estrus. When the two groups were combined, only 70.5% were in estrus during a 24-hr period. The greater degree of synchronization among animals at similar stages of the estrous cycle agrees with previous work (Johnson, 1978; Refsal and Seguin, 1980). Results in the present data indicate that if animals were at a similar stage of the cycle at  $\text{PGF}_{2\alpha}$  treatment, a single time insemination would give acceptable conception rates.

Progesterone. The decline in serum progesterone in Day 7 and Day 14 heifers after the second  $\text{PGF}_{2\alpha}$  injection is presented in figure 2. Day 7 heifers had a significant increase in serum progesterone during the 4 hr before the  $\text{PGF}_{2\alpha}$  injection indicating that the CL was growing and secreting increasing amounts of progesterone. Day 14 heifers had similar concentrations of progesterone at the time of the injection as 4 hr earlier.

Day 14 heifers had a higher serum progesterone concentration ( $P < .01$ ) at the time of the second  $\text{PGF}_{2\alpha}$  injection than did Day 7 heifers (3.9 vs 3.2 ng/ml). By 4 hr post-injection progesterone levels were similar between the groups. In both groups progesterone fell significantly by 4 hr post-injection and continued to

TABLE 7. THE DEGREE OF ESTROUS SYNCHRONIZATION  
IN HEIFERS FOLLOWING TWO PGF<sub>2α</sub>  
TREATMENTS<sup>a</sup>

Stage of the cycle <sup>b</sup>	Percent in estrus in a 24-hour period <sup>c</sup>
Early cycle	93.2
Late cycle	86.5
Overall	70.5

<sup>a</sup>Estrus was checked every 4 hr for 6 days following the second PGF<sub>2α</sub> injection.

<sup>b</sup>Early cycle animals received the second PGF<sub>2α</sub> injection on Days 5 to 9, late cycle animals on Days 10 to 15.

<sup>c</sup>The percent of animals that showed estrus after the second PGF<sub>2α</sub> injection that were in estrus during a 24-hr period around the mean time to estrus (mean time to estrus + 12 hr).

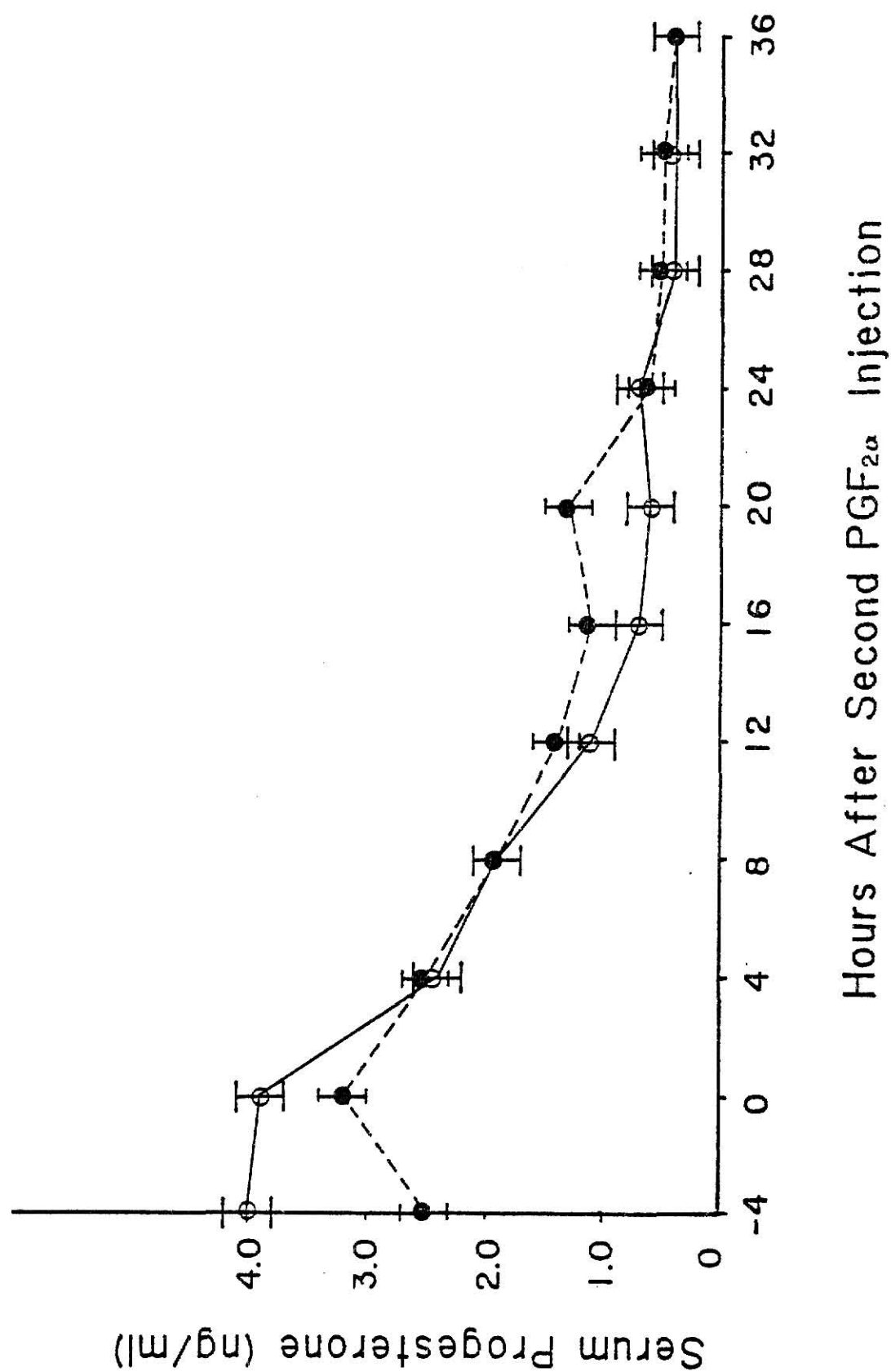


Figure 2. Least-squares means of serum progesterone concentrations in Day 7 (● - - - ●) and Day 14 (○ - - - ○) heifers following PGF<sub>2α</sub>.



decline linearly through 36 hr. Others have reported similar results (Louis et al., 1973; Louis et al., 1974; Dobson et al., 1975). The day of the cycle at injection had no effect on rate of progesterone decline ( $P>.40$ ). Day 14 animal fell below 1.0 ng progesterone/ml by 16 hr while Day 7 animals fell below this level by 24 hr.

The rate of progesterone decline does not explain the difference in interval to estrus between early and late cycle animals after  $\text{PGF}_{2\alpha}$  treatment. Although there was no significant difference in rate of progesterone decline between Day 7 and Day 14 heifers, the Day 14 heifers tended to have a more rapid progesterone decline and reached basal levels earlier. However, Day 14 heifers had a longer interval to estrus ( $P<.05$ ) than Day 7 heifers. This indicates that the rate of progesterone decline after  $\text{PGF}_{2\alpha}$  is not a factor in the shorter interval to estrus in cattle injected with  $\text{PGF}_{2\alpha}$  early in the cycle.

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THE EFFECT OF STAGE OF THE ESTROUS CYCLE ON INTERVAL  
TO ESTRUS AND FERTILITY AFTER PGF<sub>2α</sub> INJECTION  
IN BEEF FEMALES

by

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The effect of stage of the estrous cycle at the time of  $\text{PGF}_{2\alpha}$  injection on subsequent reproductive events in beef females was studied in four trials involving 194 animals. All animals were given two injections of 25 mg  $\text{PGF}_{2\alpha}$  intramuscularly. Observations for estrus were made before and after the first  $\text{PGF}_{2\alpha}$  injection, and day of the cycle at the second injection was determined from the last observed estrus (day of estrus=Day 0). Following the second  $\text{PGF}_{2\alpha}$  injection, observations for estrus were made two to five times daily in the cow trials and six times daily in the heifer trials. Animals were artificially inseminated either 12 to 18 hr after the onset of estrus or approximately 80 hr after the second  $\text{PGF}_{2\alpha}$  injection. Pregnancy was determined by rectal palpation 45 to 65 days after insemination.

Blood samples were collected at 4-hr intervals beginning 4 hr before until 36 hr after the second  $\text{PGF}_{2\alpha}$  injection from 10 heifers on Day 7 and 10 heifers on Day 14. Serum progesterone was determined by radioimmunoassay.

The interval from the second  $\text{PGF}_{2\alpha}$  injection to the onset of estrus was shorter ( $P<.01$ ) in heifers than in cows (53.6 vs 61.9 hr). The day of the estrous cycle at the second  $\text{PGF}_{2\alpha}$  injection significantly affected interval to estrus. Based on those results both cows and heifers were divided into early cycle (Days 5 to 9) and late cycle (Days 10 to 15) groups. Both heifers and cows receiving their second  $\text{PGF}_{2\alpha}$  injection early in the cycle showed estrus significantly earlier than those late in cycle (47.6 vs 59.7 hr in heifers; 57.1 vs 66.7 hr in cows).

The conception rate of early cycle heifers inseminated at 80 hr after the second  $\text{PGF}_{2\alpha}$  injection was lower ( $P < .05$ ) than late cycle heifers inseminated at 80 hr (28.6 vs 62.5%). When heifers were inseminated by estrus, there was no difference in fertility ( $P = .75$ ) between early and late cycle animals (68.8 vs 72.4%). It appears that 80 hr is too late to inseminate early cycle beef heifers. There were no differences in conception rates between early and late cycle cows inseminated at 80 hr ( $P = .28$ ) or inseminated by estrus ( $P > .50$ ). The 80-hr insemination appears to be an appropriate time to inseminate both early and late cycle beef cows.

Only animals on Days 5 to 15 of the estrous cycle at the second  $\text{PGF}_{2\alpha}$  injection were used in the data analysis, and theoretically all could be expected to show a synchronized estrus. However, of the 141 animals that subsequent estrus data after the synchronization period was available, 14.2% of the animals did not have synchronized estrus. In eight animals, apparently the CL did not regress after  $\text{PGF}_{2\alpha}$  since those animals returned to estrus 18 to 23 days after the estrus before the second  $\text{PGF}_{2\alpha}$  injection. Those animals were all 5 to 8 days after estrus when injected indicating that  $\text{PGF}_{2\alpha}$  was not as effective in regressing the CL in early cycle animals as it is in late cycle animals. The CL appeared to regress in seven animals, but either estrus was not observed or those animals ovulated without expressing estrus. Five additional animals exhibited estrus 7 to 10 days after the second  $\text{PGF}_{2\alpha}$  injection.

In both Day 7 and Day 14 heifers, serum progesterone declined significantly by 4 hr post-injection and continued to decline linearly through 36 hr. The day of the estrous cycle at  $\text{PGF}_{2\alpha}$  injection had no effect on rate of progesterone decline ( $P>.40$ ). However, Day 14 heifers had a longer interval to estrus after  $\text{PGF}_{2\alpha}$  than did Day 7 heifers ( $P<.05$ ). This suggests that the rate of progesterone decline is not a factor in the shorter interval to estrus in cattle injected with  $\text{PGF}_{2\alpha}$  early in the cycle.