THE EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ DURING VARIOUS STAGES OF THE OESTROUS CYCLE OF BEEF HEIFERS*

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Summary. The effects of prostaglandin $F_{2\alpha}$ on peripheral plasma progesterone and oestrogen levels, incidence of oestrous cycle alteration, and fertility were examined in thirty beef heifers. The $PGF_{2\alpha}$ was administered by insertion of an insemination pipette into the lumen of the uterus during three periods of the oestrous cycle: Days 3 to 5, Days 9 and 10, and Days 16 and 17. Oestrus occurred within 2 to 5 days in all animals treated except those treated on Days 3 and 4. Plasma progesterone levels had fallen significantly by the day following treatment and continued to fall in those heifers returning to oestrus. As progesterone fell, plasma oestrogen increased to a peak level on the day before or the day of oestrus. The hormone patterns were quite similar to those of the untreated animals as they approached oestrus and during the 16-day period after mating. The pregnancy rate was significantly lower in the treated heifers than in the untreated heifers. This was probably due to uterine infections rather than to the drug *per se*.

INTRODUCTION

Recent studies have shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may be an effective means of controlling the time of oestrus in the cow without impairing fertility. Rowson, Tervit & Brand (1972) reported precise synchronization of oestrus between Days 5 and 16 of the cycle by injecting 0.5 mg $PGF_{2\alpha}$ non-surgically twice into the uterine horn ipsilateral to the corpus luteum. Liehr, Marion & Olson (1972) reported similar results. Lauderdale (1972) reported that 30 mg $PGF_{2\alpha}$ administered subcutaneously after Day 5 of the oestrous cycle successfully synchronized the time of oestrus.

In 1966, Babcock queried whether prostaglandins might have a luteolytic effect in certain mammals and it has since been shown that administration of $PGF_{2\alpha}$ into the cow causes a marked decline in plasma progesterone within a few hours (Lewis, Hafs & Seguin, 1973). The present study was conducted to assess the possible luteolytic effects of $PGF_{2\alpha}$ by measurement of plasma

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progesterone and to determine (1) the effect of PGF_{2α} on follicular function by measuring plasma oestrogen, (2) whether normal gonadal hormone patterns occur after mating at the induced oestrus, and (3) the effect of intrauterine placement of PGF_{2α} on the incidence of oestrus and pregnancy rates.

MATERIALS AND METHODS

Thirty beef heifers, approximately 18 months of age, that had exhibited at least two oestrous cycles of normal length (17 to 23 days) were randomly assigned to four treatments. Group 1 consisted of twelve untreated heifers. Groups 2, 3 and 4 (six heifers each) were treated with 2 mg PGF_{2α} per heifer on Days 3 to 5, 9 or 10 or 16 or 17 of the oestrous cycle, respectively. The PGF_{2α} (free acid purchased from Ono Pharmaceutical Company, Osaka, Japan) was dissolved in 0.9% physiological saline and placed in the body of the uterus by means of a stainless steel insemination rod.

After treatment, the cows were checked for oestrus twice daily (07.00 and 18.00 hours Eastern Daylight Time). At oestrus, they were hand-mated to two bulls. One half of each group was randomly selected and slaughtered on Day 16 after mating, and the oviduct and uterine horn ipsilateral to the ovary bearing the corpus luteum were flushed to recover the embryo. The ovaries were examined, weighed, and the size and number of follicles and corpora lutea were recorded. The other half of each group was allowed to continue to term if they remained pregnant.

Blood samples were collected from the jugular vein into heparinized evacuated tubes every day from the day of treatment until the day of oestrus and mating, and then every other day following oestrus until Day 16. After centrifuging the blood, the plasma was removed and stored at -20°C until it was assayed for hormones.

Plasma was assayed for progesterone according to a method reported by Henricks, Hill, Dickey & Lamond (1973). It consisted of extracting 200 µl of the plasma sample with petroleum ether and submitting the ether extract to a radioimmunoassay using a progesterone-specific antiserum. The antiserum was used at a final dilution of 1:8000. Levels of authentic progesterone used in obtaining standard curves ranged from 25 to 1000 pg. The labelled form of the hormone used was [1,2,6,7-³H]progesterone (New England Nuclear Corp.). Of a suspension of 0.25% norite A charcoal and 0.5% dextran, 1 ml was pipetted into each assay tube to separate the antibody-bound progesterone from the unbound progesterone. The coefficient of variation of the progesterone concentration of a pool of plasma from a heifer that was included in all assays was 15.3%.

Plasma was assayed for total oestrogen according to a method reported by Henricks, Hill & Dickey (1971). It consisted of extracting 5 ml of a plasma sample with ethyl ether and submitting the extract to a radioimmunoassay using an antiserum that reacts equally well to oestradiol-17β and oestrone. There was a slight cross-reaction with oestriol and oestradiol-17α. The antiserum was used at a final dilution of 1:200,000. Levels of authentic oestradiol-17β in the standard curve ranged from 10 to 400 pg. The labelled form of the hormone

used was [2,4,6,7-³H]oestradiol-17β (New England Nuclear Corp.). Of a suspension of 0.25% norite A charcoal and 0.025% dextran, 1 ml was pipetted into each assay tube to separate the antibody-bound from the unbound oestrogen. A coefficient of variation of 12.7% was obtained for the oestrogen concentration of a pool of heifer plasma that was included in each assay. In both steroid assays, the buffer used to make the solutions of antiserum, radioactive steroid and suspension of dextran-charcoal was 0.01 M-sodium phosphate, 0.9% sodium chloride and 0.9% Knox gelatin (pH 7.9). Stripping of the steroids from their respective antibodies by the dextran-charcoal suspension was checked and did not occur in the oestrogen assay and was less than 2% between the first tube and the last tube in progesterone assays containing forty to fifty tubes.

RESULTS

The occurrence of oestrus in heifers given PGF_{2α} at various times of the oestrous cycle is summarized in Table 1. A synchronized oestrus is defined as one that

Table 1. The occurrence of oestrus in heifers treated by intrauterine injection of PGF_{2α} at various times of the oestrous cycle

	<i>Treatment with PGF_{2α} on:</i>		
	<i>Days 3 to 5</i>	<i>Day 9 or 10</i>	<i>Day 16 or 17</i>
No. of heifers treated	6	6	6
No. of heifers showing oestrus within 5 days of treatment	2	6	6
Days from treatment to oestrus	2	2 to 5	1 to 3

occurred within 5 days after the time of PGF_{2α} administration. When the prostaglandin was given on Day 9 or 10 or Day 16 or 17 of the oestrous cycle, oestrus occurred within 1 to 3 days following treatment in eleven of twelve treated heifers. The remaining heifer exhibited oestrus 5 days after treatment. By contrast, two of six heifers treated with PGF_{2α} on Days 3 to 5 had a synchronized oestrus. These two were treated on Day 5 of the cycle. The other four heifers were in oestrus 9 to 21 days after treatment. It is recognized that occurrence of oestrus soon after treatment with PGF_{2α} on Day 16 of the cycle does not represent an unequivocal response to the prostaglandin.

An examination of the plasma progesterone level can offer some insight into the response to PGF_{2α}. The progesterone levels in individual cows are shown in Text-fig. 1. As in the control heifers in which the progesterone levels declined steadily between Day 16 and oestrus, the levels also declined from the time of treatment in the heifers in which oestrus was synchronized. In the treated heifers that did not have a synchronized oestrus, the level of progesterone declined slightly or not at all, and then increased during the period of blood collection (see Text-fig. 2b). The control heifers exhibited a greater diversity in their hormone patterns than the treated heifers. The period of decline in progesterone levels after Day 16 ranged from 2 to 6 days in the controls and

TEXT-FIG. 1. Daily plasma progesterone concentrations in (a) untreated beef heifers, and after intrauterine administration of PGF_{2α}; (b) on Day 3 to 5; (c) on Day 9 or 10; (d) on Day 16 or 17. ↓ = Injection of PGF_{2α}; × = oestrus.

2 to 4 days in the treated animals. The fact that there was a sharper decline in progesterone levels is borne out by the mean levels on the day of and the day after treatment with PGF_{2α}. In the control heifers, the level declined from a mean of 3.6 ng/ml on Day 16 to 2.9 ng/ml on Day 17. In the three treated groups, the mean levels declined from 2.7 to 1.0 ng/ml in the two heifers treated on Day 5; 4.4 to 2.2 ng/ml in the six heifers treated on Day 9 or 10; and 4.4 to 1.3 ng/ml in the six heifers treated on Day 16 or 17. Levels appeared to decline more rapidly when treatment with PGF_{2α} was given early (Day 5) or late in the cycle (Day 16 or 17) rather than during the mid-luteal phase (Day 9 or 10).

In Text-fig. 2, the progesterone levels before oestrus and after mating are presented with normalization of the data using the day of oestrus as the reference point. In the fourteen heifers responding by showing early oestrus, the decline in plasma progesterone during pro-oestrus was similar to that in the control

TEXT-FIG. 2. Mean daily plasma progesterone concentrations in beef heifers after intrauterine administration of $\text{PGF}_{2\alpha}$. The data are normalized with respect to oestrus. ●, Controls; ×, $\text{PGF}_{2\alpha}$ on Day 5; ○, $\text{PGF}_{2\alpha}$ on Day 9 or 10; Δ, $\text{PGF}_{2\alpha}$ on Day 16 or 17.

group. After mating, the mean progesterone levels in all groups (treated and control) were also similar to one another indicating that $\text{PGF}_{2\alpha}$ treatment did not hamper the development or function of the ensuing corpus luteum.

Plasma oestrogen levels in individual cows in the control group and the three treated groups are shown in Text-fig. 3. In the control and treated heifers, oestrogen concentrations increased from less than 10 pg/ml on the day of treatment (or on Day 16 of the cycle in the control heifers) to a level usually greater than 12 pg/ml on the day of oestrus. In most of the heifers in all groups, the highest value was on the day of oestrus. The main difference between the treated and untreated heifers was the sharper increase in oestrogen level after treatment in all the treated heifers that returned to oestrus. The mean concentrations on the day following treatment were 9.8 pg/ml in the control group; 12.5 pg/ml in the two heifers treated on Day 5; 11.8 pg/ml in the six heifers treated on Day 9 or 10; and 11.5 pg/ml in the six heifers treated on Day 16 or 17. In the heifers that did not have a synchronized oestrus, the oestrogen level remained below 10 pg/ml.

In Text-fig. 4, the mean oestrogen levels before oestrus and after mating are represented with normalization of the data using oestrus as the reference point. On this basis, the oestrogen pattern was quite similar in the four groups. Oestrogen levels reached a peak the day before or on the day of oestrus. After oestrus and mating, they decreased to below 10 pg/ml and remained at a rather constant level in all groups.

Pregnancy rates, observed on Day 16 after mating and determined by the number of heifers calving, were affected by intrauterine administration of

TEXT-FIG. 3. Daily plasma oestrogen concentrations in (a) untreated beef heifers, and after intravaginal administration of PGF_{2α}; (b) on Days 3 to 5; (c) on Day 9 or 10; (d) on Day 16 or 17. ↓ = Injection of PGF_{2α}; x = oestrus.

TEXT-FIG. 4. Mean daily plasma oestrogen concentrations in beef heifers after intravaginal administration of PGF_{2α}. The data are normalized with respect to oestrus. ●, Controls; x, PGF_{2α} on Day 5; ○, PGF_{2α} on Days 9 or 10; Δ, PGF_{2α} on Days 16 or 17.

Table 2. Pregnancy in heifers on Day 16 after mating and at parturition after synchronization of oestrus with intrauterine administration of PGF_{2α}

	Control	Treatment with PGF _{2α} on:		
		Day 5*	Day 9 or 10	Day 16 or 17
Pregnancy rate on Day 16 after mating				
No. of heifers	6	2	3	3
No. pregnant†	4	1	2	1
Pregnancy rate at time of parturition				
No. of heifers	6	0	3	3
No. calving	4	0	0	1

* Initially, six heifers were in this group with an equal number being treated with PGF_{2α} on Days 3, 4 and 5 of the cycle. Only those treated on Day 5 exhibited a synchronized oestrus.

† Many of the heifers in the treatment groups that were killed on Day 16 after mating had uterine infections—see text.

PGF_{2α}. As shown in Table 2, an embryo was recovered on Day 16 from four of six control heifers and from four of eight heifers killed in the treated groups. Four of six heifers in the control group calved, but only one of six calved in the treated groups. Of the eight treated heifers killed on Day 16, two had large amounts of pus in the uterus. Several others showed some evidence of infection.

DISCUSSION

The intrauterine administration of PGF_{2α} after Day 4 of the oestrous cycle caused plasma progesterone to decrease within 1 day, while plasma oestrogens increased. As would be expected after a rapid decrease in progesterone and a rise in oestrogens, oestrus soon followed. The plasma progesterone and oestrogen concentrations measured in these heifers are similar to those reported previously during pro-oestrus and after mating for untreated heifers (Henricks *et al.*, 1971, 1973).

The plasma progesterone levels can be compared with those reported by Lewis *et al.* (1973). These investigators reported a 60% decrease within 12 hr when 30 mg PGF_{2α} was given intramuscularly. The decrease was slightly less when 30 mg PGF_{2α} was given intravaginally. The decrease was approximately 40% after 24 hr in the present experiment in which 2 mg PGF_{2α} were placed in the uterus. Lewis *et al.* (1973) reported no effect on progesterone levels in heifers treated before Day 5 of the cycle. Oestrogen levels were not reported. The effect of PGF_{2α} on plasma progesterone levels indicates that it is an effective luteolysin except during the initial stages of formation of the corpus luteum. These findings support the report by Rowson *et al.* (1972) regarding the effects of PGF_{2α} on the corpus luteum. One can speculate that oestrogen levels rose as a result of the regression of the corpus luteum and the absence of plasma progesterone. It is possible, however, that PGF_{2α} had a direct effect on oestrogen levels.

Pregnancy rates were much lower in heifers treated with PGF_{2α} in this experiment than have been reported by other workers (Lauderdale, Chinault,

Seguin & Thatcher, 1973) in a large experiment involving three experimental stations. In that report, pregnancy rates in cattle treated with $\text{PGF}_{2\alpha}$ were similar to those of control animals. In an experiment just completed at this laboratory involving eighty beef heifers, half of which were injected intramuscularly with $\text{PGF}_{2\alpha}$, pregnancy rates were approximately 55% in both treated and untreated groups. Thus, it would seem that the route of administration rather than $\text{PGF}_{2\alpha}$ *per se* was the cause of the poor fertility in the present experiment. In an abbreviated study involving seven post-partum cows, six became pregnant at the oestrus occurring after intrauterine placement of a solution containing $\text{PGF}_{2\alpha}$ and an antibiotic. If $\text{PGF}_{2\alpha}$ becomes available at low cost, intramuscular administration of $\text{PGF}_{2\alpha}$ will be more practical than intrauterine treatment.

After mating, the gonadal hormone concentrations in the treated group and untreated group changed in a parallel manner, suggesting that $\text{PGF}_{2\alpha}$ has no lasting effect on the reproductive system. There is also a normal temporal relationship between progesterone and oestrogen at the oestrus occurring soon after treatment. In preliminary studies on the effect of $\text{PGF}_{2\alpha}$ on hypophyseal hormone secretions and the time of ovulation, it appears that prostaglandin has no effect on LH secretion (C. U. Ellicott, unpublished results).

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