

THE USE OF PROSTAGLANDIN F_{2α} GIVEN
BY EITHER INTRAUTERINE INFUSION
OR BY INTRAMUSCULAR INJECTION
FOR THE CONTROL OF OESTRUS
AND OVULATION IN CATTLE

N. W. MOORE

*Department of Animal Husbandry, University of Sydney Farms,
Camden, 2570 (Australia)*

SUMMARY

In a series of experiments, several breeds and ages of cattle (*Jersey, Freisian, Hereford, Santa Gertrudis* and *Brahman*) were treated with prostaglandin F_{2α} (PG) administered by either intrauterine infusion or intramuscular injection.

A single infusion of 4 mg PG was found to be the most effective intra-uterine treatment. There appeared to be no advantage to be gained in the use of two infusions administered either 8 or 24 hours apart.

Intramuscular injection was as effective as intrauterine infusion, but the effective dose was some 4-5 times that given by infusion. However, irrespective of method of administration and dose of PG, rarely did more than 65 p. 100 of animals exhibit oestrus within 6 days after treatment. The peak of incidence of oestrus occurred 2-4 days after treatment. Generally, there was little difference between breeds or ages in either the proportion of cows that exhibited oestrus or in the time of oestrus after treatment. However, in *Brahmans* the time of oestrus was less precise and the peak incidence of oestrus occurred some 24 hours later than in other breeds.

Fertility at the controlled oestrus appeared to be depressed. Of 36 *Santa Gertrudis* and 42 *Brahmans* artificially inseminated at oestrus after intrauterine PG, only 14 (33 p. 100) and 18 (50 p. 100) were diagnosed as pregnant 2-3 months after insemination.

Pregnant mare serum gonadotrophin (PMSG, 1,000 IU) given 2 days before intramuscular PG (10 or 20 mg) had no beneficial effects.

It is concluded that the variable, and generally low, proportion of cows that exhibited oestrus, and the lack of marked precision in time of oestrus could place limitations on the wide scale practical use of PG for synchronisation of oestrus in cattle.

INTRODUCTION

In cattle, as in other farm animals, numerous benefits can be gained from control of the time of oestrus and ovulation, but to be of practical value techniques for the control of oestrus should satisfy a number of prerequisites :

1. Simplicity.
2. Control achieved in a large proportion of animals.
3. Marked precision in the time of oestrus and ovulation after treatment.
4. Fertility at the controlled oestrus must not be adversely affected.
5. Preparations used must be cheap, readily available and without unacceptable side effects.

Early reports on the use prostaglandin $F_{2\alpha}$ (PG) in cattle suggested that some, or even all, of these pre-requisites might be satisfied (LAUDERDALE, 1972; LOUIS *et al.*, 1972; ROWSON *et al.*, 1972).

In an initial experiment (MOORE, 1974) involving over 140 cows, in which an attempt was made to induce superovulation at pre-determined times, pregnant mare serum gonadotrophin (PMSG) or an equine anterior pituitary extract (HAP) was given in conjunction with either daily injections of progesterone (40 mg/day) or PG administered by intrauterine infusion on two consecutive days (0.5 mg/infusion). Both progesterone and PG controlled the time of ovulation and PMSG and HAP induced superovulation, but only some 60 p. 100 of animals exhibited oestrus after treatment.

In a series of experiments which followed the initial investigation, PG was administered by intrauterine infusion or intramuscular injection, in a range of breeds of cattle. Prostaglandin $F_{2\alpha}$ was chosen because of its relative ease of availability and because of an assumed absence of unacceptable side effects.

EXPERIMENTAL PROCEDURES AND RESULTS

PG (Fuji Chemicals, Japan) was supplied in solution in ethanol (25 mg/ml) and was stored at 5°C. Immediately prior to use the ethanol solution was diluted with cold normal saline containing 200 IU/ml penicillin and 100 IU/ml streptomycin sulphate to give final concentrations of 1 mg/ml for intrauterine and 5 mg/ml for intramuscular treatment.

Intrauterine infusions were carried out using a « Neilson » catheter inserted via the cervix into the lumen of the uterine horn ipsilateral to the ovary containing the corpus luteum, while intramuscular injections were given in the neck area. At the time of treatment both ovaries were palpated *per rectum* and animals which did not show a well developed corpus luteum were discarded.

During the course of the experiments the animals were inspected at least twice daily for oestrous behaviour. Only those cows which stood to be mounted by other cows or by vasectomised bulls were considered to have exhibited oestrus.

Experiment 1.

The effect of PG administered once or twice by intrauterine infusion

Two tests were involved and in Test 1 eleven mature *Santa Gertrudis* cows and 40 heifers were used. The heifers were from 1½ to 2½ years of age and the cows had calved at least 4 months prior to the experiment.

The cows and heifers were divided as equally as possible between the different treatment groups and each animal received a total dose of 2 or 4 mg PG given as a single infusion or as two equal infusions 24 hours apart (table 1). PG was administered between the 6th and 16 days after oestrus.

Animals that exhibited oestrus after treatment were inseminated with frozen semen collected from *Santa Gertrudis* bulls and fertility was assessed by rectal palpation 8 weeks after insemination.

TABLE I

The incidence of oestrus and fertility following PG administered once or twice by intrauterine infusion

Expt. 1, Test 1

Total dose PG (mg)	Number of infusions (1)	Number of cows in oestrus				Total cows		
		Days after PG (2)				Treated	In oestrus (%)	Pregnant (3)
		2	3	4	5			
2	1	1	6	1	1	13	9-69	6-67
2	2	0	2	4	3	12	9-75	2-22
4	1	1	9	1	2	15	13-87	3-27
4	2	2	2	7	0	11	11-100	3-23
Total		4	19	13	6	51	42-82	14-33

Santa Gertrudis heifers and cows

(1) PG administered either as one infusion or as two infusions 24 hours apart.

(2) Days after first PG infusion.

(3) Results of rectal palpations.

Of the 51 cows treated 42 were observed in oestrus during the first 5 days after treatment. The administration of PG on two consecutive days was no more effective than a single treatment but there was a suggestion that the oestrous response following 4 mg was more effective than after 2 mg (24 of 26 *v* 18 of 25 cows in oestrus; $\chi^2 = 3.62$; $P \approx 0.05$). Fertility to insemination at the controlled oestrus was low.

In Test 2 *Freisian* heifers of 1½ of 2½ years of age and non-lactating cows were used and each animal received a standard dose of 4 mg PG administered at one infusion (table 2). The stage of the oestrous cycle at which the PG was administered was unknown, but all animals had well developed corpora lutea at the time of treatment.

Of 118 animals treated, 71 (60 p. 100) were observed in oestrus during the first 6 days after treatment, 46 of the 71 (65 p. 100) were in oestrus on the second or third day after treatment. It appeared that treatment might have been more effective in heifers than in the dry cows, but the difference failed to achieve significance (58 of 90 *v* 13 of 28; $\chi^2 = 2.91$, $P > 0.05$).

The cows were not mated and hence no data are available on fertility at the controlled oestrus.

TABLE 2

The incidence of oestrus in the Freisian following a single intrauterine infusion of 4 mg PG
Expt. 1, Test 2

Type of cow	Number of cows in oestrus										Total cows	
	Days after treatment										Treated	In oestrus (%)
	1½	2	2½	3	3½	4	4½	5	5½	6		
Heifer	2	3	7	20	9	6	3	6	0	2	90	58.64
Dry cow	0	2	1	2	2	0	0	6	0	0	28	13.44
Total	2	5	8	22	11	6	3	12	0	2	118	71.60

Experiment 2.

The effect of time interval between successive infusions of PG

Two tests were involved and as in Expt. 1, Test 1 was a small scale investigatory trial involving several treatments, while Test 2 was a larger scale trial carried out to assess the efficiency of the most promising treatment used in Test 1. In both tests the stage of oestrous cycle at which the animals were treated was unknown, but all animals had well formed corpora lutea at the time of treatment.

Test 1.

Thirteen mature non-lactating Jersey cows and 17 beef breed heifers (*Hereford* and *Shorthorn*) of 1½ to 2 years of age were treated with a single intrauterine infusion of 2 mg PG or with two infusions each of 2 mg administered at intervals of 8 or 24 hours (table 3). The cows and heifers were divided as equally as possible between the three treatments but there was no difference between them in their response to treatment.

TABLE 3

The incidence of oestrus following PG administered by intrauterine infusion once, or twice at intervals of 8 or 24 hours
Expt. 2, Test 1

Number of infusions and time interval	Number of cows in oestrus								Total cows	
	Days after treatment (1)								Treated	In oestrus
	1½	2	2½	3	3½	4	4½	5		
One	0	1	0	1	0	0	0	0	10	2
Two (24 hrs)	0	2	1	1	0	0	1	1	10	6
Two (8 hrs)	1	2	4	2	0	0	0	0	10	9
Total	1	5	5	4	0	0	1	1	30	17

(1) Days after first infusion.
2 mg PG given at each infusion.

Seventeen of the 30 cows were in oestrus within 5 days of treatment, and 14 of the 17 were first observed in oestrus over a period of 24 hours from 2 to 3 days after the first PG infusion. Overall there was a significant effect ($P < 0.01$) of number and time elapsing between PG infusions and it appeared that two infusions spaced 8 hours apart might be the most effective treatment.

The cows were not mated, and hence no data are available on fertility at the controlled oestrus.

Test 2.

Brahman cows and heifers, *Jersey* cows and *Hereford* heifers were treated with two infusions each of 2 mg PG spaced 8 hours apart (table 4). The *Brahman* cows were either non-lactating (dry) or had calves of at least 4 months of age at foot (wet); the *Jerseys* were not lactating and all heifers were of at least 14 months of age. *Brahmans* that exhibited oestrus after treatment were inseminated with frozen semen of *Brahman* bulls of known high fertility, whilst the *Jerseys* and *Herefords* were not mated. Fertility at the controlled oestrus was assessed by palpation *per rectum* 10-12 weeks after insemination.

TABLE 4

The incidence of oestrus and fertility following two intrauterine infusions each of 2 mg PG given 8 hours apart
Expt. 2, Trial 2

Type of cow	Number of cows in oestrus										Total cows		
	Days after treatment (1)										Treated	In oestrus	Pregnant (2)
	1½	2	2½	3	3½	4	4½	5	5½	6			
<i>Brah.</i> Heifer	1	0	1	1	0	3	1	0	1	1	34	9	3
<i>Brah.</i> Cow-wet	0	0	1	0	1	1	1	0	1	0	14	5	4
<i>Brah.</i> Cow-dry	2	1	3	0	6	4	2	2	0	2	25	22	11
<i>Jer.</i> Cow-dry	1	4	24	19	12	7	1	0	0	1	122	69	—
<i>Her.</i> Heifer	0	2	0	6	1	1	0	0	0	0	17	10	—
Total <i>Brah.</i>	3	1	5	1	7	8	4	2	2	3	73	36.49 %	18.50 %
Total <i>Jer.</i> + <i>Her.</i>	1	6	24	25	13	8	1	0	0	1	139	79.57 %	—

(1) Days after first infusion.

(2) Results of rectal palpation.

Brah. : *Brahman*.

Jer. : *Jersey*.

Her. : *Hereford*.

Overall, results of Test 2 when compared with those of Test 1 were disappointing. Of 212 cows treated only 115 were observed in oestrus. Whilst there was little difference between breeds in the proportion of treated cows that exhibited oestrus, there was a marked difference between breeds in the precision of time of oestrus. Of the 79 *Jersey* and *Herefords* which showed oestrus 62 (78 p. 100) were observed in oestrus between 2½ and 3½ days after treatment, whilst in the *Brahmans* there was less

precision in the time of oestrus and the peak of oestrus occurred between 3½ and 4½ days after the first PG infusion (19 of 36 in oestrus, 53 p. 100).

All 36 *Brahmans* in oestrus after treatment were inseminated, but only 18 were diagnosed as pregnant 10-12 weeks after insemination.

Experiment 3.

The effect of PG administered by intramuscular injection

Mature, non-lactating *Jersey* cows were given 15, 20 or 27 mg PG by intramuscular injection (table 5). The stage of the oestrous cycle at which animals were treated was unknown, and the cows were not mated after treatment.

TABLE 5

The incidence of oestrus following PG administered by intramuscular injection
Expt. 3

Dose PG (mg)	Number of cows in oestrus			Total cows	
	Days after treatment			Treated	In oestrus (%)
	2	3	4		
15	3	4	2	17	9.53
20	3	7	4	17	14.82
27	5	4	5	17	14.82
Total	11	15	11	51	37.73

Non-lactating *Jersey* cows

Thirty seven of the 51 cows treated exhibited oestrus, all over the period of 2 to 4 days after the injection of PG. Dose PG had no effect upon the time of oestrus, but the proportion of cows which exhibited oestrus following 15 mg PG was less than that following the higher doses (9 of 17 *v* 28 of 34 ; $P < 0.05$).

Experiment 4.

The effect of pregnant mare serum gonadotrophin (PMSG) on the response to intramuscular treatment with PG

Sixteen beef breed heifers (*Hereford* and *Shorthorn*) were used in the experiment (table 6). All received a single intramuscular injection of 10 or 20 mg PG given between Days 10 and 12 after oestrus and half received a single subcutaneous injection of 1,000 IU PMSG two days before PG treatment.

The effectiveness of treatment was assessed by ovarian palpation *per rectum*, by peripheral plasma levels of progesterone and by the incidence of oestrus after treatment. Progesterone levels were measured by a competitive protein binding assay (NEILL *et al.*, 1967) carried out on jugular vein plasma collected daily for 4 days prior to PG treatment, twice daily on the day of PG treatment and for the following 4 days, and thereafter daily or every second day for a further 20 days.

TABLE 6

The incidence of oestrus in heifers following PMSG and PG administered by intramuscular injection

Expt. 4

Treatment		Number of cows	
PMSG (IU)	PG (mg)	Treated	In oestrus
1,000	10	4	3
1,000	20	4	3
Nil	10	4	3
Nil	20	4	3
Total		16	12

Beef breed heifers

PMSG given 2 days before PG.

Twelve heifers were in oestrus following treatment, all of the 6 that received PG alone were in oestrus on the 3rd day after PG, while of the 6 that received PMSG and PG, 2 were in oestrus on the 5th day and one on each of the 6th, 7th, 9th and 11th days after PG. Dose of PG had no effect upon number of heifers in oestrus, time of oestrus, or plasma progesterone levels. PMSG had little effect upon number of ovulations. Of the 8 heifers treated with PMSG, all but one had only one corpus luteum, the remaining animal had 2, while all heifers given PG alone had one corpus luteum.

Irrespective of the treatment employed, progesterone levels declined within 24 hours after PG injection, but there were marked differences in the patterns of progesterone levels between heifers that received PG alone and those given PMSG and PG. In all 8 animals treated with PG alone, progesterone levels declined to 1 ng/ml or less, within 24 hours of treatment. These low levels were maintained for 5-6 days after which they rose and then declined between 20 and 22 days after PG treatment. In animals treated with PMSG and PG a uniform pattern of progesterone levels was not observed. Levels declined after PG treatment, but in only 2 heifers were concentrations of 1 ng/ml or less maintained for more than 3 consecutive days. In the remaining heifers given PMSG and PG levels in individual animals varied between 2 ng/ml and undetectable for up to 10 days following treatment, after which they rose (5 heifers), or levels returned, within 48 hours, to those observed before PG treatment (1 heifer).

Ovarian palpations supported the information gained from data on the incidence of oestrus and on progesterone levels. In all heifers, corpora lutea present at the time of PG treatment regressed, and regression was followed by ovulation and the formation of new corpora lutea with a subsequent rise in peripheral progesterone levels. However, progesterone levels during the oestrous cycle following treatment

appeared to be somewhat higher than those observed during the cycle preceding treatment. In the 12 heifers that exhibited oestrus following treatment, the mean progesterone levels on the 9th day of the oestrous cycle before and after treatment were 4.6 ± 0.6 and 6.9 ± 0.8 ng/ml plasma ($P < 0.001$).

DISCUSSION

The series of experiments clearly demonstrated the luteolytic activity of PG when administered by either intrauterine infusion or by intramuscular injection. The administration of PG, particularly by intramuscular injection, is relatively simple and rapid, but the variable and frequently low proportion of cows which exhibited oestrus following treatment and the lack of any marked precision in time of oestrus after treatment might well place some limitations on the wide scale use of PG for practical purposes. Even when the best available treatments were used on appreciable numbers of animals the proportion which exhibited oestrus rarely exceeded 65 p. 100, and oestrus was spread over some 4-5 days.

Observations on fertility were limited to two trials in which 33 p. 100 of *Santa Gertrudis* and 50 p. 100 of *Brahman* cows inseminated at the controlled oestrus were diagnosed as pregnant 2-3 months after insemination. Fertility rate in the *Santa Gertrudis* was low, but that for *Brahmans* might be acceptable.

When given by intrauterine infusion there was no advantage gained from giving more than a single treatment. A single infusion of 4 mg appeared to be the optimum treatment. However, had somewhat lower doses of PG been used then some advantage may have been gained by the use of two infusions as indicated by the study of ROWSON *et al.* (1972).

In breeds other than the *Brahmans* there was no major difference between the responses of heifers and cows to intrauterine treatment. In *Brahmans* (Expt. 1, Trial 2, table 4) the majority of non-lactating cows were in oestrus after treatment (22 of 25) while only 9 of 34 heifers and 5 of 14 lactating cows exhibited oestrus. The trial illustrated a further difference between breeds. The majority of *Jersey* cows and *Hereford* heifers were in oestrus between the 2nd and 4th days after treatment, whereas within *Brahmans* there was less precision in the time of oestrus and peak incidence of oestrus occurred 24 hours after that in the *Jerseys* and *Herefords*.

Intramuscular injections of PG were as effective as intrauterine infusions but the effective dose seems to be some 4-5 times that required for intra-uterine treatment. PMSG given one or two days before either intramuscular or intrauterine treatment with an analogue of prostaglandin $F_{2\alpha}$ (ICI-79939) has been shown to reduce the time elapsing between treatment and oestrus and to enhance the precision in time of oestrus (TERVIT *et al.*, 1973). However, in Expt. 4 (table 6) PMSG increased the time elapsing between treatment and oestrus and decreased the precision. In the study of TERVIT *et al.*, a dose of 2,000 IU PMSG, sufficient to result in a marked degree of superovulation was used, whereas in Expt. 4 animals received only 1,000 IU and only one animal showed any evidence of superovulation. TERVIT *et al.*, claimed that the effect of PMSG was due to the increased number of ovarian follicles present at the time of PG administration, a situation which did not occur in the present study.

If PMSG is to be used in conjunction with PG it would seem desirable to restrict the dose of PMSG to avoid the problems of abortion, foetal death and calving difficulties associated with multiple pregnancies in cattle (GORDON *et al.*, 1970 ; TESTART *et al.*, 1970).

*Colloque : Control of sexual cycles in domestic animals
October 27-30, 1974, Nouzilly.*

ACKNOWLEDGEMENTS

Some of the data presented in tables 2 and 4 were kindly made available by Dr. J. N. SHELTON of Australian Transplant Breeders, Mittagong, N. S. W.

RÉSUMÉ

UTILISATION DES PROSTAGLANDINES F_{2α} DONNÉES
SOIT PAR INFUSION INTRAUTÉRINE,
SOIT PAR INJECTION INTRAMUSCULAIRE POUR LE CONTRÔLE
DE L'ŒSTRUS ET DE L'OVULATION CHEZ LES BOVINS

Au cours d'une série d'expériences, les prostaglandines ont été administrées par infusion intrautérine ou injections intramusculaires chez diverses races de vaches. La prostaglandine PFG_{2α} a été choisie à cause de sa relative facilité d'obtention et à cause d'une absence présumée d'effets secondaires inacceptables. Les infusions intrautérines (2 à 4 mg de PGF_{2α}) étaient effectuées à travers le cervix dans la lumière de la corne utérine ipsilatérale à l'ovaire portant le corps jaune tandis que les injections intramusculaires étaient faites dans le cou. Les animaux qui ne montraient pas un corps jaune bien développé étaient rejetés. Les animaux qui manifestaient l'œstrus après traitement étaient inséminés et la fertilité étaient évaluée par palper rectal. Cette série d'expériences a été réalisée sur 478 animaux.

Une proportion variable et souvent faible de vaches montrèrent l'œstrus après traitement. Même lorsque les meilleurs traitements furent utilisés sur un nombre suffisant d'animaux, la proportion des œstrus observés excéda rarement 65 p. 100 et s'évala sur 4-5 jours.

Le taux de fertilité des vaches *Santa Gertrudis* a été faible (33 p. 100) mais celui des vaches *Brahmas* acceptable (50 p. 100).

Les injections intramusculaires de PG ont été aussi efficaces que les infusions intrautérines mais la dose efficace 4-5 fois plus élevée que celle exigée par le traitement intrautérin.

REFERENCES

- GORDON I., WILLIAMS G., EDWARDS J., 1962. The use of serum gonadotrophin (PMS) in the induction of twin-pregnancy in the cow. *J. Agric. Sci.*, **59**, 143-198.
- LAUDERDALE J. W., 1972. Effects of PGF_{2α} on pregnancy and estrous cycle of cattle. *J. Anim. Sci.* **35**, 246.
- LOUIS T. M., HAFS H. D., MORROW D. A., 1972. Estrus and ovulation after uterine PGF_{2α} in cows. *J. Anim. Sci.*, **35**, 247-248.
- MOORE N. W., 1974. The control of time of oestrus and ovulation and the induction of superovulation in cattle. *Aust. J. Agric. Res.* (In Press).
- NEILL J. D., JOHANSSON E. D. B., DATTA J. K., KNOBIL E., 1967. Relationship between the plasma levels of luteinising hormone and progesterone during the normal menstrual cycle. *J. Clin. Endocr. Metab.*, **27**, 1167-1173.

- ROWSON L. E. A., TERVIT R., BRAND A., 1972. The use of prostaglandins for synchronisation of oestrus in cattle. *J. Reprod. Fert.*, **29**, 145.
- TERVIT H. R., ROWSON L. E. A., BRAND A., 1973. Synchronisation of oestrus in cattle using a prostaglandin $F_{2\alpha}$ analogue (ICI-79939). *J. Reprod. Fert.*, **34**, 179-181.
- TESTART J., BOSC M. J., du MESNIL du BUISSON F., 1970. Étude de la survie embryonnaire après induction de superovulation chez la Vache. *Ann. Biol. anim. Bioch. Biophys.*, **10**, 99-111.
-