THE EFFECTS OF TIME OF INSEMINATION ON FERTILITY IN BEEF HEIFERS SYNCHRONIZED WITH PROSTAGLANDIN $F_{2\alpha}$

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Two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) spaced 12 days apart were used to synchronize estrus in Hereford heifers. Animals were inseminated at 75 h (group 2), 80 h (group 3) or 85 h (group 4) after the second injection of $PGF_{2\alpha}$. Untreated control animals (group 1) were inseminated as detected in estrus over an observation period of approximately 35 days. Immediately before, and 24 h after each $PGF_{2\alpha}$ injection, blood was collected for progesterone assay. Fertility expressed as calving rates was as follows: group 1, 33/77 (43%); group 2, 30/79 (38%); group 3, 29/79 (37%); group 4, 20/73 (27%). Fertility was depressed at 85 h vs. control ($P < 0.05$) in $PGF_{2\alpha}$-treated animals but there were no other significant differences. Progesterone assays showed that 65% of animals had progesterone-secreting corpora lutea at the first injection of $PGF_{2\alpha}$. There was no relationship between fertility and either serum progesterone concentration or the day of the cycle at the second injection of $PGF_{2\alpha}$.

Deux injections de prostaglandine $F_{2\alpha}$ ($PGF_{2\alpha}$), à intervalles de 12 jours ont été pratiquées pour synchroniser l'oestrus de génisses Hereford. Les bêtes ont été inséminées 75 h (groupe 2), 80 h (gr. 3) ou 85 h (gr. 4) après la seconde injection. Les sujets témoins (gr. 1) ont été inséminés dès l'apparition des chaleurs, sur une période d'observation d'environ 35 jours. On a aussi mesuré la teneur en progestérone du sang immédiatement avant et 24 h après chaque injection. L'indice de fertilité, établi d'après le taux de vêlage a été de 43% pour le groupe 1, 38% pour le groupe 2, 37% pour le groupe 3 et 27% pour le groupe 4. La fertilité a été moins bonne ($P<0.05$) chez les génisses inséminées 85 h après injection que chez les génisses témoins mais à part cela, il n'y a pas eu de différences significatives. L'essai biologique a révélé que chez 65% des génisses, le corps jaune secrétait de la progestérone après la première injection. On n'a observé aucune relation entre la fertilité et la concentration en progestérone sérique ou le jour du cycle oestral atteint à la seconde injection.

Numerous studies have shown that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogues cause regression of functional corpora lutea in cattle (Rowson et al. 1972; Louis et al. 1974). This luteolytic property is the basis of the use of prostaglandin in regulating the estrous cycle of cattle so that they may be inseminated without estrus detection (Lauderdale et al. 1974; Hafs et al. 1975a,b). Previous studies (reviewed by Manns and Hafs 1976) have shown that a single insemination at 80 h or two inseminations at 70 and 88 h (Hafs et al. 1975b) or at 72 and 90 h (Lauderdale et al. 1974) can give fertility comparable to conventional artificial insemination (AI) procedures.

The present experiment was designed to determine the optimal time for single AI after the second of two prostaglandin injections.

MATERIALS AND METHODS

Animals

The study comprised two experiments. Experiment A used one hundred and thirty-one 2-yr-old grade Hereford heifers and was conducted in April and May. Experiment B used 177 grade Hereford heifers aged approximately 15 mo and was performed in June and July. All animals had been selected as reproductively normal by rectal palpation.

Experimental Procedure

Both experiments contained a control group (group 1) and three groups of $PGF_{2\alpha}$-treated animals (groups 2–4) (prostaglandin $F_{2\alpha}$ kindly supplied by the Upjohn Company, Kalamazoo, Michigan and given at a dose of 25 mg $PGF_{2\alpha}$ (33.4 mg of Tham salt)). Control heifers were inseminated after estrus detection over an interval of approximately 35 days beginning on the day of the first injection of $PGF_{2\alpha}$ to groups 2–4. Estrus detection was by observaton at least three times each day and was assisted by KaMaR (KaMaR Inc. Steamboat Springs, Colo.) heat mount patches.

$PGF_{2\alpha}$-treated heifers were injected intramuscularly with 25 mg $PGF_{2\alpha}$. There was an interval of 12 days between treatments. Jugular vein blood samples were collected by venipuncture immediately before, and 24 h after each injection. Blood was allowed to clot at room temperature and after centrifugation the serum was stored frozen until hormone assay. The intervals between the second injection of $PGF_{2\alpha}$ and single insemination were 75 h, 80 h, and 85 h for groups 2, 3 and 4, respectively. This was accomplished by timing the injections at 1000, 0800 and 0600 h and insemination 3 days later at 1300, 1600 and 1900 h in groups 2, 3 and 4, respectively.

In experiment A, heifers were bred to either a Simmental or an Angus bull. Two AI technicians were used and each inseminated the same number of heifers in each group. Inseminators alternated using Simmental and Angus semen. Total insemination time for each group was about 90 min; therefore, breeding started approximately 45 min before and extended 45 min beyond the designated time of 75, 80 or 85 h. Inseminator and breed of bull was recorded for each animal. Groups 2–4 were observed for estrus for a 25-day interval following the timed AI. If the timed AI was to a Simmental bull, the repeat was to an Angus bull and vice versa.

In experiment B, all animals were bred by two technicians to one polled Hereford bull. Technicians bred equal numbers in each group; the inseminator of each animal was recorded. Repeats within the first 10 days after timed AI were to an Angus bull. No repeat breedings were done until at least 24 h after the timed AI. Twenty-five days after the timed AI (35 days after beginning estrus detection in controls), heifers were exposed to bulls for 2 mo (experiment A) or 3 mo (Experiment B).

Pregnancy examinations were done by rectal palpation of the uterus 50 days after breeding in groups 2–4, and 40–60 days after breeding in group 1. Another pregnancy examination was done after removal of the bulls, and open animals were culled. Calving dates and breed of calf were recorded on all animals. Success of insemination was based on breed of calf and a gestation interval not more than 10 days longer than 283 days. The pregnancy diagnoses were used to supplement this information where necessary.

Statistical comparisons between treatments were made by chi-square analysis.

Progesterone Assay

Serum progesterone was assayed by a standard radioimmunological procedure using an antibody purchased from Dr. G. Abraham. The specificity of the antiserum has been published (Abraham et al. 1971). Samples were extracted with 85.6 ± 2% ($\bar{x}$ ± SEM, n = 10) efficiency with hexane, and all samples were corrected for recovery. Standards were assayed in triplicate at the beginning and each of each assay; unknowns were assayed in duplicate. Phosphate-buffered saline (Manns et al. 1975) was used for preparation of antibody, progesterone solutions and the charcoal suspension. The intra- and inter-assay coefficients of variation were 7.2% (n=10) and 17.8% (n=7) for samples with progesterone concentrations of 1.95 and 2.90 ng/ml, respectively.

RESULTS AND DISCUSSION

Progesterone assays revealed that of 228 cycling animals in groups 2–4 of both experiments, 149 (65.4%) responded to the first injection of $PGF_{2\alpha}$. This is very close to the theoretical value based on a knowledge of the days during the cycle when an animal should respond to $PGF_{2\alpha}$ (Rowson et al. 1972).

Table 1. Fertility in 2-yr-old beef heifers after timed insemination following $PGF_{2\alpha}$ treatment, experiment A

Group:	1	2	3	4
Interval, treatment-AI	Control	75 h	80 h	85 h
Animals in group	33‡	31	33	34
Calves to first AI	14	13	17	12
Calved (%)	42	42	52	35
Not cycling	4	1	1	0
Open end breeding season	0	0	0	0
Adjusted (%)†	48	43	53	35

†Animals excluded were those (a) not detected in estrus during the observation period (controls) (b) not cycling based on progesterone concentrations consistently below 1 ng/ml (c) open at the end of the breeding season.
‡27 control animals were bred during the first 21 days of observation; 13(44%) calved to that insemination.

Data on fertility are summarized in Tables 1–3. In experiment A (Table 1), there were no significant differences between any groups, although the 85-h insemination gave lowest fertility. This trend was more evident when fertility was adjusted for non-cycling animals. This adjustment was based on exclusion of animals which were not observed in estrus over a 35-day interval (controls) or had consistently low (< 1 ng/ml) progesterone at all four sampling times (groups 2–4). In experiment A, all animals were pregnant after exposure to bulls for a period of about 2 mo.

In experiment B (Table 2), heifers inseminated at 85 h had reduced fertility compared to controls (21 vs. 43%, $P < 0.05$); there was a strong tendency for the 80-h insemination to be reduced as well (26 vs. 43% $P < 0.10$). In experiment B, some animals were open after exposure to bulls for 3 mo, and in the adjusted data these animals have been excluded since they apparently had low fertility.

When the data were combined (Table 3), fertility in group 4 (85 h) was significantly lower ($P < 0.05$) than controls. There was a trend ($P < 0.10$) for groups 2 and 3 to have higher fertility than group 4. These data indicate that there is no difference between insemination with estrus detection or a timed insemination at 75 or 80 h. However, the 85-h insemination was too late to give optimal fertility.

In experiment A, 85% of animals were in standing estrus within 6 days of second $PGF_{2\alpha}$ injection; in experiment B, the comparable figure was 29%. The probable explanation is that in experiment B, the daily high temperature in the 5-day interval following $PGF_{2\alpha}$ injection averaged 30 C. The average for the 5 days preceeding

Table 2. Fertility in 15-mo-old beef heifers after timed inseminations following $PGF_{2\alpha}$, experiment B

Group:	1	2	3	4
Interval, treatment-AI	Control	75 h	80 h	85 h
Animals in group	44‡	48	46	39
Calved to first AI	19	17	12	8
Calved (%)	43	35	26	21
Not cycling	4	5	1	3
Open end breeding season	4	8	7	8
Adjusted (%)†	51	47	32	29

Control vs. 80 h, $P < 0.10$; control vs. 85, $P < 0.05$.
†See footnote to Table 1.
‡39 control animals were bred during the first 21 days of observation; 18(46%) calved to that insemination.

Table 3. Fertility in beef heifers after timed insemination following $PGF_{2\alpha}$ treatment. Results from experiments A and B are combined

Group:	1	2	3	4
Interval, treatment-AI	Control	75 h	80 h	85 h
Animals in group	77‡	79	79	73
Calved to first AI	33	30	29	20
Calved (%)	43	38	37	27
Not cycling	8	6	2	3
Open end breeding season	4	8	7	8
Adjusted (%)†	50	46	41	32

Control vs. 85, $P < 0.05$.
†See footnote to Table 1.
‡66 control animals were bred during the first 21 days of observation; 31 (47%) calved to that insemination.

injection was 23 C. The only shade available was a porosity fence 3 m in height; animals showed some signs of heat stress. However, progesterone assays revealed that a high percentage of animals responded to the second injection of $PGF_{2\alpha}$ with luteal regression, and fertility in both experiments was not markedly different when adjusted fertility was compared (Tables 1 and 2).

An analysis of fertility in relation to the progesterone concentration at the time of the second prostaglandin injection (Table 4) showed a broad range of progesterone concentration at the second $PGF_{2\alpha}$ injection. However, as long as the animal was cycling, the progesterone concentration did not appear to be related to the response to $PGF_{2\alpha}$ either in terms of luteal regression or fertility at AI. The apparent increase in fertility at a progesterone concentration of 3.0–3.9 ng/ml was not statistically significant.

Another factor considered in the present study was the effect of the day of cycle at second $PGF_{2\alpha}$ injection on fertility. Progesterone assay revealed which animals responded to the first injection of $PGF_{2\alpha}$. Those animals would be at approximately day 9 of the cycle when the second injection of $PGF_{2\alpha}$ was given, while the remainder would be between days 10 and 17. When all animals were pooled regardless of the insemination treatment, comparable fertility rates were 30.4% (42 of 138 animals) at day 9 and 41.6% (32 of 77 animals at days 10 to 17. This trend did not reach statistical significance.

Table 4. Progesterone concentration at second injection of $PGF_{2\alpha}$ and fertility to a single timed AI. Results from experiments A and B are pooled without respect to time of insemination

Progesterone at 2nd $PGF_{2\alpha}$ (ng/ml)	Fertility	
	N	%
1.0 – 1.9	14/36	39
2.0 – 2.9	14/40	35
3.0 – 3.9	23/49	47
4.0 – 4.9	11/33	33
> 5.0	21/54	39

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