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A Retrospective Study on the Effects of FSH and Prostaglandin on Superovulation Responses in Dairy Cattle

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ABSTRACT

Two hundred-eleven uterine flushings following superovulation conducted in 80 Holstein cows were examined in retrospect to assess the effects of different doses of FSH (28 to 50 mg) and prostaglandin $F_2 \alpha$ on superovulatory responses. Prostaglandin $F_{2\alpha}$ (25 mg) was administered in either one or two injections. Split Prostaglandin $F_{2\alpha}$ injections after FSH injection resulted in the best estrous responses (87 vs 79% estrous expression) compared to single Prostaglandin $F_{2\alpha}$ injection. Split Prostaglandin $F_{2\alpha}$ injections resulted in less time to estrus (55.7 h) than single Prostaglandin $F_{2\alpha}$ injection (57.4 h). Estrus occurred earlier with 35 mg FSH (48h) than with 40 mg FSH (59h), 37 mg FSH (62h) or 30 mg FSH (56h). The 35 mg FSH dose resulted in more unfertilized ova than other FSH doses. Number of embryos per flush was not affected by FSH or Prostaglandin $F_{2\alpha}$ injection schemes. Number of excellent and good quality embryos was not affected by dose of FSH, Prostaglandin $F_{2\alpha}$ and number of times the animal was superovulated. It was concluded that estrous response and time to estrus are influenced by FSH and Prostaglandin $F_{2\alpha}$ but they did not affect total number of embryos recovered or number of excellent and good quality embryos collected.

INTRODUCTION

The use of exogenous gonadotrophins to induce superovulation in dairy cattle has been investigated by several workers. Casida et al. (1943) induced superovulation in 4 out of 7 cows by giving an FSH extract followed by an LH extract beginning on d 2 or 3 of the estrous cycle. Bellows et al. (1969) induced a high percentage of double ovulations and pregnancies in dairy cattle by using 6.25 mg of FSH over a 5 d period. In addition, Elsden et al. (1978) compared the effectiveness of FSH and pregnant mares serum gonadotropin (PMSG) to induce superovulation. They reported that FSH treated cows had more corpora lutea (CL) and resulting pregnancies than those tested with PMSG. Chupin and Procureur (1982) reported that when a total of 32 mg of FSH was administered in decreasing doses over a 4 d period, it was more effective in inducing superovulation than the same dose administered over a 5 d period. The relationship between dose of FSH and timing of administration and embryo production has been studied (Donaldson, 1984; Donaldson and Ward, 1986). The conclusion from these studies was that FSH is an important source of variation and that there was no distinct advantage of

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administering FSH over a 4 d period compared to a 5 d period or of using a decreasing dose schedule rather than an equivalent dosage schedule.

The use of prostaglandin (PG) to control reproduction in the cow has been described (Betterage, 1977). Seidel et al. (1978) reported that when PG was administered to cows which were superovulated, estrus followed sooner than when PG was administered to untreated animals. Estrus occurred at 48 h following PG in superovulated cows compared to 72 or 96 h in control cows. Also, Nelson et al. (1979) compared two time schedules (48 and 72 h after FSH administration) for administration of PG to superovulated cows. When the PG was administered at 48 h the cows had more palpable CL, but when given at 72 h after FSH administration, the cows produced more transferable embryos. Donaldson (1983) reported that when a total PG dose of 30 mg was given in 3 doses of 10 mg each, more cows showed estrus than when the same dose was given in only 2 injections of 15 mg. The objective of this retrospective study was to evaluate different doses and schedules of FSH and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) on superovulation response in dairy cattle.

MATERIALS AND METHODS

Following superovulation, a total of 211 uterine flushes were conducted on 80 Holstein cows. Before superovulation treatment rectal palpations were carried out by a veterinarian to identify reproductive unsoundness. Cows were observed for estrus twice daily and those that showed two estrous cycles of normal duration were then superovulated between d 9 and 13 of the cycle according to the FSH regimes shown in Table 1. The total FSH dose ranged from 28 to 50 mg and was administered over a 3 or 4 d period in 6 to 8 injections at 12 h intervals. Two treatments were similar except that one treatment consisted of two injections of 2.5 mg each on d 3 and 4 of the estrous cycle (Rajamahendran et al., 1987). Because of limited animal numbers in some treatments, treatments of 28, 30 and 32 mg (designated 30 mg FSH), and 40 and 50 mg (designated 40 mg) FSH were combined into pooled treatments for statistical analyses. To induce luteolysis, 25 mg of $PGF_{2\alpha}$ in either 1 (control) or 2 injections was administered starting at various times after the first FSH injection (Table 2). The first three treatments consisted of one injection given at 60, 72 or 84 h after the first FSH injection, respectively. Due to low numbers in the 84 h treatment group, all single injection groups were pooled. In the next three treatments, the total dose (25 mg) was divided into two equal injections administered 12 h apart, starting at 48, 60 and 72 h after FSH initiation. Again, because of low numbers all treatments were pooled into a single double injection group for statistical analyses. Cows in estrus were artificially inseminated at onset of estrus, 12 and 24 h later. Cows which were not seen in estrus were inseminated at 72 and 84 h after the first FSH injection. Finally, 6 d after the first insemination all the cows were subjected to nonsurgical uterine flushing to recover embryos (Rajamahendran et al., 1987), which were then graded into viable and non-viable categories (Greeve et al., 1979).

Regression analysis was used to evaluate estrus response to PG, timing to estrus, and number of ova and embryos per flush.

RESULTS AND DISCUSSION

Estrous response to PG was affected only by the PG regimen ($p<0.07$). Eighty-three percent of all cows exhibited estrus in response to PG. There was a

TABLE 1. FSH injection regimes.

Treatment Number	(n)[1]	Total Dose (mg)	Day of Superovulation[2,3] 1	2	3	4
1	4	28	5:5	4:4	3:3	2:2
2	8	30	5:5	5:5	5:5	--
3	101	32	6:6	5:5	3:3	2:2
4	44	35	5:5	5:5	5:5	5:0
5[4]	31	37	6:6	5:5	3:3	2:2
6	19	40	5:5	5:5	5:5	5:5
7	4	50	7.5:7.5	7.5:7.5	5:5	5:5

[1] (n) number of cows superovulated.
[2] FSH started either AM or PM.
[3] 1,2,3 or 4 refer to day 9 to 13 of the estrous cycle on which FSH injection began.
[4] 2.5 mg FSH also were given on day 3 and 4 of the estrous cycle.

TABLE 2. Prostaglandin F2α (PG) injection regimens.

Treatment Number	(n)[1]	Total[2] Dose	Number of injections	Number of Hours after FSH[3]
1[4]	57	25	1	60
2	34	25	1	72
3	4	25	1	84
4	2	25	2	48-60
5	107	25	2	60-72
6	7	25	2	72-84

[1](n) number of cows superovulated.
[2]PG started AM or PM.
[3]Time of administration after initial FSH injection.
[4]Treatments 1, 2 and 3 and 4, 5 and 6 were combined for statistical analysis to assess one vs two injections.

79.2 ± 4.5% ($\bar{x}$ ± SEM) response in terms of observed estrus for cows which received a single PG injection compared to 86.9 ± 4.7% response for cattle injected twice with 12.5 mg PG at 12 h intervals. Our data are consistent with those of Donaldson (1984) who obtained an 86.6% response in cows given two PG injections. Under physiological conditions, a relatively short pulse of PG (every 6 h) over a period of 25 to 30 h was reported as the minimal requirement for CL regression in sheep (McCracken et al., 1984). Time to observation of estrus was influenced by PG regimen ($p<0.01$). Time to estrus following one PG injection averaged 57.4 ± 1.5 h compared to 55.7 ± 1.6 h for cattle injected twice with PG.

FSH regime influenced ($p<0.05$) the time to observation of estrus. Cows injected with 35 mg FSH averaged 48.4 h to onset of estrus (Table 3). This was a shorter time interval ($p < 0.01$) than all other treatments. Cows which received 2.5 mg FSH on days 3 and 4 of the estrous cycle and 32 mg FSH (37 mg) for superovulation had 5.8 h more time ($p < 0.05$) before mean onset of estrus than cows given the 30 mg FSH only for superovulation. It appears that the response to the 35 mg dose of FSH may be related to a reduction in time to the onset of estrus following luteal regression with PG. Greeve et al. (1980) and Donaldson (1983) recorded intervals of 42 to 48 h to onset of estrus in synchronized cows. Seidel et al. (1978) found that time to estrus following PG was about 48 h in superovulated cows but this was increased to 60 to 72 h in single ovulation cows.

There was a significant interaction ($p < 0.01$) between the FSH and PG regimens for time to onset of estrus. A single injection of PG with 37 and 40 mg FSH and a double PG injection with 30 mg resulted in a greater amount of time to the onset of estrus than the other treatments (Table 4). The double PG injection with 35 mg FSH resulted in a shorter interval to estrus than double PG injections with 37 and 30 mg FSH and all single PG injections.

The total number of embryos per flush was not affected by day of FSH initiation, FSH injection regime, PG injection scheme or number of superovulations to which the cow had been subjected ($p > 0.10$). The average number of embryos per flush was 7.9 for all cows. Table 5 shows the number of embryos per flush for each FSH regimen. Our data are in contrast to others who have found differences in superovulatory response to the injection regime (Garcia et al., 1982; Hill et al., 1985; Looney et al., 1981) and day of the cycle on which FSH treatment was initiated (Donaldson, 1984; Lerner et al., 1986; Lindsell et al., 1986). The total embryos per flush agrees with Rajamahendran et al. (1987) and is higher than that reported by Looney et al. (1981).

The mean number of unfertilized ova (1.4 ± 0.3) per flush was not affected ($p > 0.10$) by day of FSH initiation, PG injections or number of superovulations, but was affected by FSH dose (Table 5). The 35 mg FSH dose resulted in the highest number of unfertilized ova. The overall fertilization rate of 83% is in agreement with reported fertilization rates of 70 to 85% (Elsden et al., 1978; Sreenan and Diskin, 1982).

The number of embryos classified as excellent or good was not affected by FSH regimen ($p > 0.10$), PG regimen ($p > 0.10$) and number of times cows were superovulated ($p > 0.10$). The number of excellent and good embryos (4.5 ± 4.5) was lower than that reported by Lindsall et al., (1986), but greater than that of Garcia et al. (1982). Lerner et al.,(1986) have reported decreased embryo quality and reduced numbers of transferable embryos with increasing donor age and increased number of transferable embryos with higher doses of FSH. They speculate that age related changes in embryo numbers and quality could be due to reduced numbers of growing follicles or stage of development of follicles at the time of FSH stimulation not being compatible with endocrine and gonadotrophin profiles of younger animals. Pregnancy rates of excellent and good quality embryos increase with initial embryo quality score (Coleman et al., 1987).

In conclusion, a greater percentage of cattle receiving PG in two injections were observed in estrus than those receiving one PG injection. While time to observe estrus was less in cattle receiving two PG injections than with one injection, total number of embryos recovered per flush, number of unfertilized ova and number of

TABLE 3. Hours to estrus by FSH treatment.

FSH Dose (mg)	Time (h)[1]		(n)[2]
	$\bar{x}$	SE	
30	56.3	1.2[ad]	97
35	48.4	1.9[b]	37
37	62.1	2.6[ac]	26
40	59.4	3.1[a]	16

[1] Least squares means and SE.
[2] Number of cows observed in estrus.
[a-d] means with different superscripts differ ($p < 0.05$).

TABLE 4. Hours to estrus in cows injected with different doses of FSH and one or two injections of Prostaglandin F2α(PG).

FSH Dose (mg)	PG injection regimen[1]	
	1 Injection	2 Injections
30	54.7 ± 1.7[bd]	57.6 ± 1.6[a]
35	57.8 ± 3.6[bd]	45.3 ± 2.1[bc]
37	67.5 ± 5.3[a]	55.5 ± 4.1[bd]
40	67.5 ± 3.5[a]	– –

[a-d] means with different superscripts differ ($p < 0.05$).
[1]Least squares means and SE.

TABLE 5. Least squares mean ± SE number of embryos and unfertilized ova (UFO) per flush by FSH dosage.

FSH Dose (mg)	Number of Embryos		Number of UFO	
	$\bar{x}$	SE	$\bar{x}$	SE
30	8.4	0.8	1.1	0.4[a]
35	9.6	1.3	3.7	0.6[b]
37	7.8	1.8	0.7	0.9[a]
40	5.9	2.0	0.3	1.0[a]

[a,b] Means with different superscripts differ ($p < 0.01$).

embryos classified as excellent or good were not affected by PG. The 35 mg FSH resulted in earlier estrous manifestation and more unfertilized ova than other treatments. But number of embryos recovered, and number of excellent and good embryos were not affected by FSH treatment. These data suggest that a wide variety of FSH doses and PG treatments can be used for successful superovulation and estrous synchronization in dairy cattle.

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