Theriogenology 42:917–929, 1994

FOLLICULAR DEVELOPMENT AND SUPEROVULATION RESPONSE IN COWS ADMINISTERED MULTIPLE FSH INJECTIONS EARLY IN THE ESTROUS CYCLE

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Received for publication: *April 20, 1993*
Accepted: *August 24, 1994*

ABSTRACT

To determine whether follicular development, superovulation and embryo production were affected by the absence or presence of a dominant follicle, cows were administered injections of FSH twice daily in the early (Days 2 to 6, estrus = Day 0) or middle stage (beginning on Day 10 or 11) of the estrous cycle. Treatment with FSH early in the cycle stimulated follicular development in 83 to 100% of all cows from 4 groups evaluated at different times after PGF2α treatment on Days 6 and 7. However, the proportion of cows with > 2 ovulations varied from 31 to 62.5%, indicating that induction of follicular development may occur in the absence of superovulation. When compared with cows treated in the middle of the cycle, no differences were observed in the proportion of cows with > 2 ovulations (31 vs 20%), ovulation rate (26.0 ± 6.3 vs 49.6 ± 25.8), production of ova/embryos (13.3 ± 3.2 vs 14.4 ± 3.4), or the number of transferable embryos (8.0 ± 3.6 vs 5.4 ± 1.5; early vs middle, respectively). The proportion of the total number of embryos collected that were suitable for transfer was greater ($P<0.01$) in cows treated early in the cycle (60%) than at midcycle (37.5%). The diameter of the largest follicle observed by ultrasound prior to initiation of FSH treatment in the early stage of the cycle (10.0 ± 2.0 mm) was smaller ($P<0.05$) than at midcyle (16.8 ± 1.3 mm). These results demonstrate that superinduction of follicular development is highly consistent after FSH treatment at Days 2 to 6 of the cycle and that superovulation and embryo production are not less variable than when FSH is administered during the middle of the cycle. However, superovulation in the early stage of the cycle may increase the proportion of embryos suitable for transfer.

Key words: FSH, superovulation, embryos, follicle dominance, cattle

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Acknowledgments

Mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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INTRODUCTION

The superovulation of cattle is used in conjunction with embryo transfer to expedite the propagation of animals with genetic merit for desirable traits. One major problem with superovulation has been the large variation in the number of ovulations and(or) embryos that result from this procedure. In standard superovulation protocols, injections of FSH are initiated during the mid luteal phase of the estrous cycle (i.e., 9 to 12 d after estrus). Evidence exists that the large variation in superovulatory response may be due in part to variation in the developmental stage of follicles present on the ovaries when treatment with FSH is initiated (15). The presence of a dominant follicle on the ovaries of a cow may decrease the number of follicles that mature and ovulate in response to FSH (8). One approach to overcoming this problem would be to begin treatment with FSH within a few days after estrus. The population of follicles on the ovaries at this time consists mainly of a cohort of small developing follicles, thought to be recruited by the postovulatory rise in FSH (19). Treatment of cows with a total of 56 mg FSH during Days 2 to 7 after estrus (Day 0) resulted in consistent induction of follicular development and superovulatory responses (18). In other studies utilizing lower dosages of FSH and fewer total days of treatment, superovulatory responses of cows treated with FSH in the early stage of the estrous cycle were reduced when compared with those of cows treated with FSH in the middle of the cycle (3,6,12). Alternatively, the absence of a dominant follicle on the ovaries of cows could be determined by ultrasound prior to the initiation of FSH treatment during the midluteal phase of the estrous cycle. Studies evaluating this approach have resulted in superovulatory responses that were enhanced (8,10) or not different (7,20) when compared with those of cows treated with FSH in the presence of a dominant ovarian follicle. Discrepancies in these studies may be attributed to differences in the criteria used to define the presence or absence of a dominant follicle. Additional research is needed to further evaluate the potential influence of a dominant follicle on ovarian response to exogenous treatment with FSH. In the present study, initiation of treatments with FSH during the early stages of the estrous cycle was selected over ultrasound examination of the ovaries of cows during the middle of the cycle for the following practical reasons: the high cost of an ultrasound machine, less handling of animals, and easier synchronization of donor cows with recipient cows.

The objectives of the present studies were to evaluate 1) follicular development after FSH treatment during the early stage of the estrous cycle; 2) different dosages of FSH for superovulating cows early in the estrous cycle; and 3) whether treatment with FSH early in the estrous cycle results in less variable superovulation responses than standard superovulation protocols in which FSH treatment is initiated in the middle of the cycle. These experiments were conducted to provide insight into the variation associated with follicular recruitment, ovulation and embryo yield.

MATERIALS AND METHODS

Animals and Materials

Ovaries and reproductive tracts of animals used in these experiments were palpated per rectum to assure no gross abnormalities were evident. All cows used in these 3 experiments exhibited estrus in response to 1 or 2 injections of prostaglandin (PGF2α; Lutalyse, Upjohn Co., Kalamazoo, MI). Administration of PGF2α was used to synchronize estrus so that injections of FSH (Schering-Plough Animal Health, Kenilworth, NJ) could be initiated simultaneously for all cows within an experiment. Dosages of FSH given below were based on the biopotency reported by Schering-Plough. Each experiment used a different lot of FSH and was conducted at different times of the year using mature, nonlactating beef cows of different genetic origin and managed under different conditions. Because of this, statistical comparisons were not made between experiments. Cows were stratified into treatments within experiments by age and breed.

Experiment 1

In Experiment 1, we evaluated follicular development in cows treated with FSH early in the cycle. In the first replication, twice daily injections (i.e., morning and evening) of 7, 6, 5 and 4 mg, i.m. of FSH were administered on Days 2, 3, 4 and 5 after estrus, respectively, for a total dose of 44 mg of bioactive FSH (biopotency = 37 mg/50 mg vial). Cows received 30 mg PGF2α on Day 5.5 after estrus and were slaughtered at 0, 12, 24, 36, or 48 h post PGF2α treatment (n = 5, 6, 6, 6 and 2 for each slaughter period, respectively). Radioimmunoassay of progesterone (14) concentrations in serum collected at the time of PGF2α administration and at slaughter indicated that only 12 of 20 FSH-treated cows slaughtered between 12 and 48 h after PGF2α treatment responded to PGF2α. Therefore, an additional replication was conducted in which the cows were injected twice daily with 6, 5, 4, 3 and 3 mg FSH/injection on Days 2, 3, 4, 5 and 6 after estrus, respectively, for a total of 42 mg FSH over a 5-d period. The cows were then injected with 25 mg PGF2α on Days 6.5 and 7 and slaughtered at 0, 24 or 48 h after the first injection of PGF2α (n = 3 per slaughter period). Cows not treated with FSH (controls) were given PGF2α between Day 6 and 8 of the cycle and were slaughtered 0, 24 or 48 h later (n = 6 or 7/slaughter group for Replicate 1 and n = 3/slaughter group in Replicate 2). Ovaries from animals in both replicates were dissected after slaughter, and the numbers of small (< 8 mm in diameter) and large (≥ 8 mm) follicles were recorded. Data on the number of small and large follicles collected from FSH-treated and control cows in Replicates 1 and 2 were analyzed on SAS by analysis of variance for a randomized block design, using the replicate as the blocking factor and time after PGF2α as the covariate (5). Chi-square analysis was used to compare the proportion of cows responding to PGF2α as determined by circulating levels of progesterone. An analysis of variance for a completely random design was

used to determine if follicle populations differed between the cows that did and did not respond to PGF2α treatment.

Experiment 2

In Experiment 2, we evaluated the superovulatory response of 5- to 11-yr-old crossbred cows treated with different dosages of FSH beginning at 1 to 4 d after estrus (pooled mean ± SEM = 2.5 ± 0.2 d after estrus). Injections of FSH were given twice daily for 5 d. The amount of FSH administered in each injection on each of the 5 d of treatment was 5, 5, 4, 3 and 3 mg, respectively (total = 40 mg of bioactive FSH; biopotency = 38.9 mg/50 mg vial), for cows (n = 12) in the low dosage treatment group, and it was 7, 6, 5, 4 and 3 mg, respectively (total = 50 mg), for cows (n = 12) in the high dosage group. An injection of 25 mg PGF2α was given at the last FSH injection and again the next morning to induce luteolysis. Cows were bred by bulls and were slaughtered 6 to 8 d later. Reproductive tracts were collected and flushed to recover embryos and unfertilized ova. All embryos were classified by stage of development and evaluated for suitability for transfer based on morphological appearance (14).

During the second or third day of FSH treatments, 3 cows from the low FSH dosage group were mistakenly injected with one or more doses of FSH scheduled for the high dosage group; therefore, these 3 cows received a higher dosage than scheduled. Results from these 3 cows were analyzed as a separate treatment group and are designated as the Mix group. Chi-square analysis was used to determine if the proportion of cows that exhibited estrus or the proportion cows that had multiple (i.e., > 2) ovulations differed among the 3 different FSH dosage groups. General linear model analysis (SAS) of variance procedure for a completely randomized design was used to evaluate treatment differences in the number of ovulations (number of CL); unfertilized ova; degenerated embryos; 2- to 16-cell embryos; morulae; early blastocysts; blastocysts; late blastocysts; expanded blastocysts; total number of ova and embryos collected per cow; the recovery rate of embryos (the ratio of embryos and ova collected/CL); and the number of transferable embryos. Because treatment with FSH was initiated 1 to 4 d after estrus, the initial analysis included the day after estrus at which FSH was initiated as the covariate (5). However, this did not significantly ($P \geq 0.2$) affect any of the parameters evaluated and was thus excluded from the model. Multiple range comparisons were performed with a SAS least squares means procedure when significant ($P < 0.05$) differences were observed in the analysis of variance (5).

Experiment 3

In Experiment 3, we evaluated superovulation and embryo production in 19 cows treated with FSH beginning at 1 or 2 d after estrus (designated as the early cycle group) and 25 cows treated with FSH beginning at 10 or 11 d after estrus (designated as mid-cycle group). Cows were stratified by breed and age (3 to 10 yr) within the 2 groups. On the day before FSH injections were initi-

ated, the ovaries of each cow were visualized by ultrasonography to characterize follicle populations. The dose of FSH given during each injection on each of the 5 d of treatment was 5, 6, 4, 3 and 3 mg, respectively, for a total of 42 mg bioactive FSH (biopotency = 44.2 mg/50 mg vial) over the 5-d period. In addition, cows in the early cycle group were given Norgestomet implants (Syncro-Mate-B, Sanofi Animal Health, Overland Park, KS) on the first day of FSH treatment to suppress estrous behavior. The rationale for the dosage of FSH and the use of Norgestomet implants was based on observations made in Experiment 2 (see Results and Discussion). An injection of 1 mg fenprostalene (Bovilene, Syntex, West Des Moines, IA) was given at the last FSH injection and again the next morning to induce luteolysis. Fenprostalene was used in this experiment instead of PGF2α because the longer half-life of fenprostalene was expected to be more efficacious in the early stage of the cycle. Norgestomet implants were removed from the early group at the time of the second fenprostalene injection. Cows were artificially inseminated at 12 and 24 h after the first detection of standing estrus. Cows not observed in estrus were artificially inseminated at 72 and 84 h after the first fenprostalene injection. All cows were slaughtered 6 to 8 d after breeding, and their reproductive tracts were flushed to recover ova or embryos. Embryos were characterized and evaluated as described in Experiment 2. Serum samples were collected at ultrasound, at breeding, at 1 to 2 d prior to slaughter and at slaughter to evaluate circulating progesterone concentrations. Analysis of data was also performed as described in Experiment 2. In addition, the proportion of cows exhibiting estrus, the proportion of cows with more than 3 large follicles and(or) more than 2 CL and the proportion of embryos suitable for transfer were evaluated by Chi-square analyses.

RESULTS

Experiment 1

Treatment of cows with FSH during Days 2 to 5 (Replicate 1) or Days 2 to 6 (Replicate 2) after estrus increased ($P<0.01$) the number of large follicles (≥ 8 mm in diameter) on ovaries collected at slaughter (LS mean ± SEM = 24.9 ± 1.3 vs 1.7 ± 1.6 for FSH-treated vs control cows, respectively). The number of large follicles was not affected ($P>0.1$) by replicate, replicate-by-treatment interaction, or hour after PGF2α treatment. Induction of follicular development by FSH was highly consistent since 32 of the 34 FSH-treated cows (94%) had 14 or more large follicles on their ovaries when slaughtered, while the remaining 2 cows had 5 and 9 large follicles, respectively, at slaughter.

The number of small follicles decreased ($P<0.001$) after treatment with FSH (14.0 ± 2.0) when compared with that of the control cows (29.3 ± 2.3). Time of slaughter and the interaction of replicate and treatment did not influence ($P>0.1$) the number of small follicles, indicating that continuing treatment for an additional day did not affect follicle numbers.

Changes in levels of progesterone in serum samples collected at the time of PGF2α administration and at slaughter 12 to 48 h after PGF2α demonstrated that 8 of 20 FSH-treated cows from Replicate 1 and 1 of 6 FSH-treated cows from Replicate 2 did not respond to PGF2α ($P>0.1$). All control cows responded to PGF2α ($P<0.01$; control vs FSH). However, the control cows were generally given PGF2α 1 d later in the cycle than FSH-treated cows. The number of small and large follicles present on the ovaries of FSH-treated cows did not differ ($P>0.1$) between cows that did or did not respond to PGF2α treatment.

Experiment 2

In Experiment 2, we evaluated ovulation and embryo production in cows given 3 different dosages of FSH (Table 1). The proportions of cows that had more than 2 CL were similar ($P>0.1$) among the 3 treatment groups. Recovery rates for embryos and(or) ova

Table 1. Ovarian response and embryo production in cows treated with different dosages of FSH during the early stage of the estrous cycle (Experiment 2)

	FSH dose		
	Low[a]	High[b]	Mix[c]
No. of cows treated	9	12	3
No. of cows exhibiting estrus	8	10	3
No. of cows with a CL	5	9	3
No. of cows with >2 CL	5	7	3
No. of CL[d]	19 ± 3.7	25 ± 9.2	44 ± 2.8
Range in no. of CL[d]	15-34	4-34	38-47
No. of unfertilized ova[d]	7.6 ± 4.6	5.8 ± 2.6	2.3 ± 1.9
No. of degenerated embryos[d]	2.4 ± 0.6	1.1 ± 0.5	6.0 ± 1.5[e]
No. of 2- to 16-cell embryos[d]	0.4 ± 0.2	0.9 ± 0.7	3.7 ± 1.8
No. of morulae[d]	4.8 ± 1.7	2.9 ± 1.6	17.0 ± 7.1[e]
No. of blastocysts[d,f]	1.4 ± 1.4	0.9 ± 0.6	5.0 ± 1.0[e]
No. of transferable embryos[d]	6.6 ± 2.4	4.4 ± 2.7	25.6 ± 5.2[e]
Total no. of ova and embryos[d]	16.6 ± 2.4	11.6 ± 2.8	34 ± 5.1[e]

[a] Cows were injected twice daily for 5 d with 5, 5, 4, 3 and 3 mg FSH/injection/day.
[b] Cows injected twice daily for 5 days with 7, 6, 5, 4 and 3 mg FSH/injection/day.
[c] Cows from the low dosage treatment that were mistakenly injected with doses of FSH scheduled for the high dosage group on the second or third day of treatment.
[d] Values represent the mean ± SEM or range from animals with > 2 CL.
[e] Value differs ($P<0.05$) from the low and high dosage groups.
[f] Includes all stages of blastocyst development.
CL = corpus luteum.

from cows with more than 2 CL (i.e., number of ova and(or) embryos recovered divided by the number of CL for each cow) were 87, 64 and 78% for the low, high, and mixed FSH dosage groups, respectively ($P > 0.05$). No differences were found between the low and high dosage groups for any of the parameters measured. Ovulation response was highly consistent in the mixed dosage group; and, although not significantly different, it was almost twice that obtained in the low or high group. The number of degenerated embryos, morulae, blastocysts and transferable embryos obtained from the mixed dosage group was greater ($P<0.05$) than from either the low or high FSH dosage treatment groups.

Timing of estrus did not vary between treatments (pooled mean ± SEM = 35.5 ± 3.1 h after the first injection of PGF2α), and all but 3 cows exhibited estrus (Table 1). One cow from the low dosage group and 2 cows from the high group did not exhibit estrus when exposed to bulls. At slaughter, the cow from the low dosage group had 2 regressing CL, while the 2 cows from the high group had luteinized follicles on their ovaries in addition to 0 or 4 CL. Two of these cows, 1 from each group, accounted for a portion of the 7 cows that did not ovulate during the breeding period (see number of cows with CL in Table 1). The remaining cows that did not ovulate were characterized as follows: 2 cows had luteinized follicles (low dosage group), 1 cow had a 4-cm cystic follicle (high group), and 2 cows (one from each group) had 5 or more large follicles. Thus, 4 of 7 nonovulators had luteinized follicles and 1 cow had a cystic follicle; the remaining 2 cows had no apparent abnormalities.

Evaluation of ovaries from the 9 cows (4 from the low-dosage and 5 from the high-dosage cows) that did not have more than 2 CL at slaughter indicated that 5 cows (2 from the low and 3 from the high dosage groups) had 5 or more large follicles at slaughter. Thus, stimulation of follicular development occurred in 83% (20 of 24) of the FSH-treated animals, and superovulation occurred in 62.5% (15 of 24) of the cows.

An additional observation made in Experiment 2 was that 5 cows from the low dosage group and 7 cows from the high group exhibited estrus after the second or third day of FSH treatment. Visual appraisal of the ovaries at slaughter indicated that ovulation did not result from the premature episodes of estrous behavior observed in these cows. Because of the increased production of transferable embryos in the mixed dosage group and the expression of estrus during treatment with FSH, a biphasic dosage of FSH and the use of Norgestomet implants were adopted for Experiment 3.

Experiment 3

Reproductive parameters for cows treated with FSH in the early or middle stage of the estrous cycle were evaluated in Experiment 3. Only 32% of early-treated and 20% of midcycle-treated cows had more than 2 ovulations ($P>0.1$). The large standard error associated with the number of ovulations in the midcycle group (Table 2) was due to 1 cow having more than 150 CL; the next highest number of

Table 2. Ovulation response and embryo production in cows treated with FSH in the early or middle stage of the cycle (Experiment 3)

	Treatment group	
	Early cycle	Midcycle
No. of cows treated	19	25
No. of cows observed in estrus	7	11
No. of cows with 1 or more CL	10	8
No. of cows with > 2 CL	6	5
No. of CL[a]	26.0 ± 6.3	49.6 ± 25.8
No. of Cl[a] (range)	15 to 55	12 to 150
No. of unfertilized ova[a]	2.0 ± 0.4	5.8 ± 4.57
No. of degenerated embryos[a]	n = 1	0
No. of 2- to 16-cell embryos[a]	2.3 ± 1.4	2.6 ± 1.2
No. of morulae[a]	4.3 ± 1.3	4.2 ± 2.1
No. of blastocysts[a,b]	4.5 ± 3.4	1.8 ± 0.9
No. of transferable embryos[a]	8.0 ± 3.6	5.4 ± 1.5
Total no. of ova and embryos[a]	13.3 ± 3.2	14.4 ± 3.4

[a] Values represent the mean ± SEM or range from animals with greater than two ovulations.
[b] Includes all stages of blastocyst development.

ovulations was 47, and the mean number of ovulations was 24.5 when the cow with more than 150 CL was excluded. Results of analyses were similar, regardless of whether or not this cow was included. Recovery rates of ova and(or) embryos per CL were 59 and 51% for cows with multiple ovulations in the early and midcycle groups, respectively ($P>0.1$). Although the average number of transferable embryos was higher for cows treated with FSH early in the cycle, the difference was not significant ($P>0.1$) for the 2 groups when evaluated by analysis of variance. However, treatment with FSH early in the cycle resulted in an increase ($P<0.01$) in the proportion of the total number of ova and embryos that were characterized as transferable embryos (60 vs 37.5%). The only other difference observed was that the diameter (mm) of the largest follicle on the ovaries of cows before FSH treatment was smaller ($P<0.05$) in the early cycle group (mean ± SEM = 10.0 ± 2.0) than in the midcycle group (16.8 ± 1.3).

The proportion of cows exhibiting estrus and ovulating after fenprostalene was low for both treatment groups, with 37 and 44% of the cows exhibiting estrus and 53 and 32% ovulating when treated in the early and middle stage of the cycle, respectively. Onset of estrus with respect to time from the first fenprostalene injection was similar for the 2 treatments (pooled mean ± SEM = 49.3 ± 3.6 h).

To gain insight into the possible reasons why cows did not superovulate, cows that had ≤ 2 CL were grouped into 1 of 4 categories based on ovarian structures and circulating concentrations of progesterone (Table 3): Category 1 consisted of cows in which superinduction of follicular development occurred in the absence of superovulation. Ovaries from these cows contained numerous large

Table 3. Classification of cows that did not superovulate as assessed by ovarian structures and circulating concentrations of progesterone (Experiment 3)

Category	Number of large follicles	Progesterone (ng/ml) at: Breeding	Progesterone (ng/ml) at: Slaughter	Number of cows: Early group	Number of cows: Midcycle group
1	≥ 4	< 1	< 1	5[ab]	11[ab]
2	≥ 4	> 1	< 1	4	7[b]
3	≥ 4	< 1	> 1[c]	1	
		> 1	> 1[c]		1
4	< 4	< 1	< 1	1	1
		< 1	> 1[c]	1	
		> 1	> 1	1[d]	

[a] Ovaries from 2 early and 4 midcycle group cows contained blood-filled follicles at slaughter. In addition, 1 or more luteinized follicles were observed on the ovaries of 2 early and 3 midcycle group cows at slaughter.

[b] Two cows from the early group (Category 1) and 2 from the midcycle group (1 Category-1 cow and 1 Category-2 cow) had 1 or 2 new CL at slaughter (< 1 ng P_4/ml in serum).

[c] A functional CL (i.e., > 1 ng P_4/ml serum) was present at slaughter.

[d] One cow in the early group had a 2.8-cm luteinized follicular cyst at the time of slaughter and progesterone concentrations greater than 1 ng/ml in all the samples.

follicles, and circulating levels of progesterone at artificial insemination were less than 1 ng/ml. One cow from the early group and 6 cows from the midcycle group were observed in estrus, while the remaining cows were time-bred. Category 2 consisted of cows that responded to FSH with respect to induction of follicular development but did not respond to fenprostalene treatment. Cows in this category had numerous large follicles on their ovaries, but progesterone concentrations at breeding were greater than 1 ng/ml. None of these cows demonstrated estrous behavior. Category-3 cows had 4 or more large follicles and 1 or 2 functional CL at slaughter (progesterone concentrations > 1 ng/ml). Category-4 cows had fewer than 4 large follicles at slaughter. The mean number of large follicles observed at slaughter for cows grouped into Categories 1 through 3 did not differ ($P>0.1$) between the early (10.3 ± 1.3) and midcycle (13.5 ± 3.0) treatments. Based on the total number of cows in Categories 1, 2 and 3 and on the number of cows that had more than 2 CL, stimulation of follicular development occurred in 84% of the early cycle-treated (16 of 19) and in 96% of the midcycle-treated (24 of 25) cows ($P>0.1$). Chi-square analyses showed that the incidence of induced follicular development occurred more frequently ($P<0.01$) than the incidence of superovulation in both the early and midcycle treatment groups.

DISCUSSION

The results from this study do not demonstrate that the superovulation response of cows treated during the early stage of the estrous cycle is less variable than the superovulation response during the middle of the cycle. The incidence in which superinduction of follicular development occurred and the number of follicles stimulated by FSH (as determined by ovulation rate or by the number of large follicles present in animals with 2 or fewer ovulations) did not differ between cows treated with FSH in the early or middle stage of the estrous cycle. These findings therefore demonstrate that induction of follicular development by exogenous treatment with FSH is not greatly enhanced when FSH is given in the absence of a dominant follicle on Days 2 to 6 of the estrous cycle.

The results of this study further show that induction of follicular development by treatment with FSH was more consistent than induction of multiple ovulations. In Experiment 1, we demonstrated that the incidence of superinduction of follicular development achieved by administering injections of FSH on Days 2 to 6 of the estrous cycle was highly consistent, with all the cows having 5 or more large follicles after FSH treatment. Unfortunately, this same consistency was not realized in the superovulation response when similar treatments were used on 2 additional groups of cows in Experiments 2 and 3. The incidence of superovulation was 62.5%, while stimulation of follicular development occurred in 83% of cows in Experiment 2. The incidence of superovulation in Experiment 3 was also lower (31.5 or 20%) than the induction of follicular development (84 or 96%), regardless of whether or not the cows were treated in the early or middle stage of the cycle. Therefore, one source of variation in the superovulation response achieved after treatment with FSH may involve the mechanisms responsible for luteal regression and induction of ovulation.

Data in Table 2 illustrate that only 37 and 44% of early and midcycle cows were observed in estrus during the 5-d period after fenprostalene injections in Experiment 3. Apparently, luteal regression did not occur (see Category 2 in Table 3) in 33 and 50% of the early and midcycle group cows that did not exhibit estrus. Lindsell et al. (12) reported that 10 to 37% of FSH-treated cows were excluded from their study for failure to superovulate; the proportion of cows excluded was similar whether FSH treatment was initiated on Day 3, 6, 9 or 12 of the cycle. These researchers reported that luteolysis did not occur in 57% of the cows excluded from their study and that luteolysis had occurred in the remaining 43% of the excluded cows but these cows failed to ovulate. Recently, Calder and Rajamahendran (3) reported that the incidence of estrus after treatment with FSH was much lower when injections were initiated on Day 2 rather than Day 9 of the cycle. As in our present study, these researchers found that induction of follicular development was highly consistent and did not differ between early- and midcycle-treated cows. Redmer et al. (18) also observed that while a highly consistent superovulation response (range in ovulation = 19 to 34) occurred in 11 of 15 cows treated with FSH during

Days 2 to 7 of the cycle, 4 cows failed to exhibit estrus and ovulate following fenprostalene injection. Collectively, these studies provide evidence that mechanisms involved in luteal regression and (or) induction of ovulation contribute to the lack of superovulation response after FSH treatment.

The results from Experiment 3 indicate that superovulation response did not differ between cows treated with FSH either in the early or middle stage of the estrous cycle. In contrast, other studies (3,6,12) have shown that superovulation responses were lower after treatment with FSH early in the cycle than after treatment during the middle of the cycle. The reasons for the discrepancy between these studies and our present study are not clear but may involve differences in day of initiation, duration and dosage of FSH and(or) the timing and compound used to induce luteolysis.

Experiments 2 and 3 provide evidence that losses also occur after ovulation, which may be due to problems with oocytes not being collected into the oviduct, fertilization, and(or) subsequent embryo development (1,9,11,16). While ovulation rates in Experiments 2 and 3 were respectable in animals that responded to FSH treatment, the proportion of embryos or ova recovered per ovulation ranged from 64 to 87% in Experiment 2 and was 59 and 51% for the early and midcycle groups in Experiment 3. Lindsell et al. (12) found 66 and 52% recovery rates for early- and midcycle-treatment cows. In addition to this 40 to 50% loss, morphological appearance of embryos collected in Experiment 3 and in previous studies (6,12) indicated that only approximately 50% of the embryos were suitable for transfer. The reason that the total numbers of embryos and ova recovered were generally high in comparison to the number of transferable embryos remains to be determined. However, results from Experiment 3 indicate that treatment with FSH early in the cycle may result in an increase in the proportion of the total number of embryos collected that are suitable for transfer. In an unpublished study by Redmer et al., the percentage of CL resulting in transferable embryos after FSH treatment on Days 2 to 7 of the estrous cycle was improved when compared with that of treatment on Days 10 to 15. Therefore, while it appears that treating cows with FSH early in the cycle does not improve the incidence of superovulation, it may, however, contribute to the successful development of the embryos. Additional research is needed to further clarify this finding.

The results of Experiment 2 demonstrated that high circulating levels of estrogen, resulting from the superinduction of follicular development, stimulated estrous behavior after 2 or 3 d of treatment with FSH in the absence of luteal progesterone. Although estrous activity in these cows occurred in the absence of ovulation prior to treatment with PGF2α, there was concern that oviduct and uterine function may have been adversely affected by exposure to high levels of estrogen during treatment with FSH in the absence of luteal phase levels of progesterone (14). A Norgestomet implant was administered to cows in the early group during the FSH treatment in Experiment 3 to overcome any possible adverse effects that sustained high levels of estrogen might have during the early stage of the cycle.

Although estrous behavior was not observed in cows during treatment with FSH in Experiment 3, the proportion of transferable embryos collected from cows with Norgestomet implants was not improved over that observed in the mixed group in Experiment 2. Previous research (17) demonstrated that the number of degenerated embryos and unfertilized ova was decreased in cows that had been treated with Norgestomet implants during FSH administration in the middle of the estrous cycle. Improved embryo yields were also observed in subfertile cows (open 1 or more years) when FSH treatment was begun 4 d after Norgestomet implants were inserted, regardless of stage of cycle (4). In contrast, embryo quality was decreased in FSH-treated cows synchronized with progesterone releasing intrauterine devices (PRID) when compared to cows synchronized with PGF2α (2). Administration of PRIDs to cows treated with FSH during Days 2 to 6 of the cycle did not improve the number of cows expressing estrus after PGF2α on Day 7 or embryo production (3). Therefore, any beneficial effects that progestagens have on embryo development remain to be determined.

It is concluded from these experiments that while recruitment and induction of follicular growth appears to be very consistent after treatment with FSH, the subsequent processes involved in the production of transferable embryos are variable. The consistency of the follicular response to treatment was evident in the 3 experiments using genetically different animals managed at different locations and treated at different times of the year. Therefore, the large variation associated with the production of multiple embryos may have been due to problems associated with luteal regression, ovulation, collection of ova into the oviduct and fertilization as well as subsequent embryo development (1,11,14,16). Moreover, at least part of the variation may have been due to differences in developmental status and in the number of follicles present at the time when treatment with FSH was initiated, as described by Monniaux et al. (15). Thus, the processes involved in recruitment and growth of follicles may be only a partial series of the events required for the development of a mature follicle that is fully competent with respect to the biochemical processes and secretions needed for the production of a viable oocyte, for ovulation, ova transport, fertilization and embryonic development (1,9,11,13,14,16).

REFERENCES

1. Armstrong DT. Recent advances in the superovulated cow. Theriogenology 1993;39:7-24.
2. Boland MP, Goulding D, Roche JF. Alternative gonadotrophins for superovulation of cattle. Theriogenology 1991;35:5-17.
3. Calder M, Rajamahendran R. Follicular growth, ovulation and embryo recovery in dairy cows give FSH at the beginning or middle of the estrous cycle. Theriogenology 1992;38:1163-1174.
4. Ellington JE, Elefson EE, McCall RM. Use of a Norgestomet implant as an aid when superovulating low-fertility dairy cows. Theriogenology 1987;27: 227 abstr.
5. Freund RS, Littell RC. SAS for Linear Models, SAS Institute, Cary, NC, 1981.

6. Goulding D, Williams DH, Duffy P, Boland MP, Roche JF. Superovulation in heifers given FSH initiated either at day 2 or day 10 of the estrous cycle. Theriogenology 1990;34:767-778.
7. Gray BW, Cartee RE, Stringfellow DA, Riddell MG, Riddell KP, Wright JC. The effects of FSH-priming and dominant follicular regression on the superovulatory response of cattle. Theriogenology 1992;37:631-639.
8. Guilbault LA, Grasso F, Lussier JG, Rouillier P, Matton P. Decreased superovulatory responses in heifers superovulated in the presence of a dominant follicle. J Reprod Fertil 1991;91:81-89.
9. Hawk HW. Gamete transport in the superovulated cow. Theriogenology 1988; 29:125-142.
10. Huhtinen M, Rainio V, Aalto J, Bredbacka P, Maki-Tanila A. Increased ovarian responses in the absence of a dominant follicle in superovulated cows. Theriogenology 1992;37:457-463.
11. Hyttel P, Callesen H, Greve T, Schmidt M. Oocyte maturation and sperm transport in superovulated cattle. Theriogenology 1991;35:91-108.
12. Lindsell CE, Murphy BD, Mapletoft RJ. Superovulatory and endocrine responses in heifers treated with FSH-P at different stages of the estrous cycle. Theriogenology 1986;26:209-219.
13. Loos FAM de, Bevers MM, Dieleman SJ, Kruip ThAM. Follicular and oocyte maturation in cows treated for superovulation. Theriogenology 1991;95: 537-546.
14. Maurer RR, Echternkamp SE. Hormonal asynchrony and embryonic development. Theriogenology 1982;17:11-22.
15. Monniaux D, Chupin D, Saumande J. Superovulation response of cattle. Theriogenology 1983;19:55-81.
16. Moor RM, Kruip ThAM, Green D. Intraovarian control of folliculogenesis: Limits to superovulation? Theriogenology 1984;21:103-116.
17. Prather RS, Spire MF, Schalles RR. Norgestomet incorporation into a superovulation regime. Theriogenology 1984;21:256 abstr.
18. Redmer DA, Kirsch JD, Fricke PM, Reynolds LP. Superinduction of follicular growth and superovulation in cows treated with FSH beginning on day 2 after estrous. J Anim Sci 1991;69(Suppl 1):129 abstr.
19. Turzillo AM, Fortune JE. Suppression of the secondary FSH surge with bovine follicular fluid is associated with delayed ovarian follicular development in heifers. J Reprod Fertil 1990;89:643-653.
20. Wehrman ME, Wilson M, Bergfeld E, Cupp A, Kojima N, Mariscal V, Peters K, Scholz S, Sanchez T, Kittok R, Kinder J. Modification of follicular development prior to superovulation with two doses of exogenous progesterone does not alter response to superovulation. J Anim Sci 1992;70(Suppl 1):253 abstr.