Effects of the Persistent Dominant Follicle on the Ability of Follicle Stimulating Hormone to Induce Follicle Development and Ovulatory Responses

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ABSTRACT

Three experiments were conducted to evaluate the effect of an induced first wave persistent dominant follicle on folliculogenesis and ovulatory responses induced by FSH. On d 6 of a synchronized estrous cycle (d 0 = estrus), cows were treated with a Syncromate-B implant and two injections of PGF$_2$α (25 mg, 0700 h; 15 mg, 1900 h, i.m.). Cows in the control group retained a first-wave persistent dominant follicle, but in the aspirated group, the first-wave dominant follicle was removed via transvaginal aspiration on d 10 (d 0 = estrus). Beginning on d 12, cows received 32 mg of FSH-P i.m. in decreasing doses at 12-h intervals over a 4-d period. On d 15, the Syncromate-B implant was removed, and cows were ovariotomized (experiment 1, n = 8) or inseminated (experiment 2, n = 11) at 10 and 22 h after the onset of estrus. Cows in experiment 1 received a used controlled intravaginal drug releasing (CIDR) device and two injections of PGF$_2$α (25 mg, 0700 h; 15 mg, 1900 h; i.m.) on d 6. On d 8, the first-wave dominant follicle was aspirated (n = 6) or left intact (n = 5), and FSH treatment was initiated (20 mg of Folltropin in decreasing doses at 12-h intervals over a 4-d period), and on d 10 the used CIDR device was removed from all cows. Ovarian follicle size and number were examined daily by ultrasonography from d 5 of the estrous cycle. The persistent dominant follicle increased in size from 10.7 mm on d 5 to 15.4 mm on d 10 (experiments 1 and 2), and from 9 mm on d 5 to 20.4 mm on d 11 (experiment 3). From d 11 to 14, the number of class 1 (2 to 5 mm) follicles was lower in the aspirated group than in the control group; the number of class 2 (6 to 9 mm) follicles was higher on d 12 and 13 for the aspirated group (experiments 1 and 2). The number of class 3 ($\geq$10 mm) follicles was higher in the aspirated group on d 14 to 16, but the same on d 17. Ovarian and embryo responses to superovulation did not differ between groups. In experiment 3, the numbers of class 1, 2, and 3 follicles, as well as ovarian and embryo responses following ovulation did not differ between groups. Initiation of exogenous FSH treatment appears to override any systemic inhibitory effect that a persistent dominant follicle may be exerting at the pituitary and possibly the ovary. (Key words: follicle-stimulating hormone-P, dominant follicle, follicular dynamics, superovulation)

Abbreviation key: CIDR = controlled intravaginal drug releasing, CL = corpus luteum, corpora lutea, DF = dominant follicle, $E_2$ = estradiol-17$\beta$, $P_4$ = progesterone, PRID = $P_4$-releasing intravaginal device, UFO = unfertilized oocytes.

INTRODUCTION

Investigations of endocrinological processes by ultrasonography have demonstrated that the growth of dominant follicles (DF) during the bovine estrous cycle occurs primarily as two or three waves. A DF is defined as a large ovarian follicle that is recruited and selected during a follicular wave, has an initial linear growth rate, and is at least 2 mm greater than the largest subordinate follicle (57). During each wave of follicular growth, the DF develops and suppresses the growth of subordinate follicles and inhibits the recruitment of a new follicular wave (23, 28, 48, 49, 56).

One goal of superovulation is to suppress atresia in a greater number of follicles, thereby allowing them to attain preovulatory status (30). There is a high degree of unpredictability in superovulatory responses that de-
creases the efficiency and profitability of embryo transfer programs. Monniaux et al. (33) reported that ovarian status at the time of the superovulatory regimen can account for a large portion of the variability in superovulatory response. For example, recent reports (8, 17, 21, 63) suggest that the presence or absence of a DF can affect superovulatory responses of cows. Superoxovulatory responses are reduced in cows treated in the presence of a DF compared with responses of cows without a DF at the onset of the superovulatory treatment.

The ovarian response in cows with a DF at the time of superovulation induction can be improved by removing the DF by transvaginal aspiration (5). This procedure is also used to collect immature bovine oocytes repeatedly from the same donor (39). Immediately after the elimination of the DF, FSH concentrations rise and induce the recruitment of a new follicular wave (3, 7). However, the presence of a DF may possibly alter ovarian responsiveness to exogenous gonadotropins. Follicles <6 mm in diameter are recruited during gonadotropin treatment (33), and the presence of a DF may inhibit the recruitment process (63).

Progesterone (P4) has an important effect on the growth and turnover of the DF (2, 9, 51, 52, 59). A negative feedback of P4 on LH pulse frequency is a mechanism by which P4 affects follicular growth and the demise of the first-wave DF (1, 51, 59). Induction of a low P4 environment leads to the development of an estrogen active, persistent DF and has been used as a model for the study of follicular dominance in cattle (51). Injection of PGF2α, and insertion of a Syncromate-B implant (51) or a used controlled intravaginal drug release (CIDR) device on d 8 of the estrous cycle (52), or injection of PGF2α, and insertion of a P4-releasing intravaginal device (PRID) on d 5 of the estrous cycle (62) reduces concentrations of plasma P4 and leads to the development of a persistent DF. Wehman et al. (62) reported that FSH treatment initiated 5 d after insertion of a 0.5 PRID reduces the number of corpora lutea (CL), total ova, and transferable embryos compared with a group with FSH treatment initiated 5 d after the insertion of 2 PRID. However, this differential response between 0.5 versus 2 PRID was not evident when FSH treatment was initiated at either 2 or 8 d after PRID insertion. The presence of a persistent DF was associated with a large number of small (≤5 mm) and very few medium size (6 to 9 mm) follicles, which is indicative of a block in follicle recruitment and selection.

The primary objective of the present studies was to evaluate whether the presence or absence of a persistent DF would alter FSH-P induction of follicle development, subsequent ovulatory responses, and embryo yields.

**MATERIALS AND METHODS**

**Experiment 1**

An initial experiment was conducted to evaluate the effect of removal, via aspiration, of an active and persistent first-wave DF on folliculogenesis in response to an FSH superstimulation protocol. Eight cycling dairy cows were synchronized with an injection of GnRH (8 µg i.m. of Receptal; Hoechst-Roussel AgriVet, Somerville, NJ) followed 7 d later by an injection of PGF2α, (25 mg i.m.; Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI). Brightly colored enamel-based paint (Impervo, Benjamin Moore, Montvale, NJ) was applied to the tailhead of each cow 1 d before injection of PGF2α. On the day of PGF2α, injection, the paint strip was covered with a contrasting color of chalk (All-Weather Paintstick; Lake Chem. Co., Chicago, IL) (61). The extent of tail paint and chalk removal was scored based on a modification of the method of Macmillan et al. (29) using a five-point scale (5 = full presence of paint and chalk, not in estrus, to 0 = absence of paint and chalk, standing estrus) (61). The scoring of tail paint and chalk and the visual detection of estrus were performed twice daily (0600 to 0700 h and 1800 to 1900 h) for 3 d after injection of PGF2α. On d 6 of the synchronized estrous cycle (estrus = d 0), all cows received a 6-mg Norgestomet ear implant without injection of the solution of Norgestomet and estradiol valerate (Syncromate-B; Sanofi Animal Health, Inc., Overland Park, KS) and two injections of PGF2α (25 mg a.m. and 15 mg p.m., i.m.) to induce luteolysis and create an environment low in progesterone for development of an active persistent DF (52). The implant was left in place for 9 d. Cows were assigned randomly to either the nonaspiration group (control group; n = 4) or the aspirated group in which the induced active persistent DF was removed by transvaginal aspiration (aspirated group; n = 4). The first-wave DF was removed via transvaginal ultrasound guided aspiration on d 10 (e.g., 4 d after insertion of Norgestomet implant), utilizing an Aloka Echo-Camera SSD-500 unit (Aloka Co., LTD, Japan) equipped with a 5.0-MHz transvaginal convex array transducer and a needle guide. Epidural anesthesia was induced with 5 ml of 2% lidocaine, and the perineal region was scrubbed and disinfected. The lubricated transducer with needle guide was inserted deep into the vagina. A 17-gauge, 60-cm, single channel sterile needle, fitted with a 19-gauge, 2.5-cm disposable tip was used in the needle guide to puncture the vaginal wall and peritoneum. By manipulation of the reproductive tract per rectum, ovaries were positioned and held firmly in front of the
transducer in close apposition to the vaginal wall. After the on-screen needle travel indicator line was aligned to appear perpendicular to the follicle, the needle was advanced through the wall of the vagina, into ovarian stroma, and through the wall of the follicle. As soon as the tip of the needle entered the follicle, follicular contents were aspirated into a sterile plastic tube using a suction unit (Pioneer Medical, Inc., Madison, CT) preset to a flow rate of 20 ml/min at a preset pressure of 100 mm Hg. Collection of follicular fluid into the tube and collapse of the DF on screen were regarded as a successful aspiration of the follicle.

Because an immediate rise in concentrations of FSH occurs after couterization of the DF (3) or after removal of the ovary bearing the DF and precedes recruitment of a new follicular wave by 2 d (7), follicular superinduction was initiated 2 d after aspiration of the active persistent DF. For follicular superinduction, a total of 32-mg Armour units of FSH-P (FSH-P; Schering-Plough, Animal Health Kenilworth, NJ) were given per cow. Injections were initiated on d 12 of the experimental estrous cycle (i.e., 2 d after follicular aspiration) at 0700 h and continued at 12-h intervals in a decreasing regimen over a 4-d period (a.m./p.m. = 6/6, 5/5, 3/3 and 2/2 mg/injection). A real-time ultrasound scanner (Aloka Echo-Camera SSD-500), equipped with a 7.5-MHz transrectal linear array transducer, was used to monitor the size and number of ovarian follicles ≥2 mm in diameter from d 5 until ovariectomy on d 15 of the experimental estrous cycle. Ovarian maps that included the relative position of follicles and CL were drawn during each examination. According to the diameter, follicles were grouped into the following size classification: class 1, 2 to 5 mm; class 2, 6 to 9 mm; and class 3, ≥10 mm.

**Experiment 2**

The objective of the second experiment was to evaluate folliculogenesis, ovulatory responses, and embryo yields to a superovulatory protocol using FSH-P administered in the presence or absence of an active persistent DF. Eleven cycling dairy cows were used in the experiment (control group, n = 6; aspirated group, n = 5). The synchronization of estrus, induction of an active persistent DF, follicle aspiration, and induction of superovulation procedures were the same as in experiment 1. However, ultrasonography was extended until estrus was detected on d 17. Ultrasonography was used to confirm ovulation 2 d and again 7 d after estrus to count the number of CL on the day of embryo recovery.

Cows were inseminated twice at 12-h intervals starting 10 h after estrus was detected, as described for experiment 1. Embryos were recovered nonsurgically on d 6 after insemination, following a procedure described by Putney et al. (41). At the time of embryo collection, the number of embryos retrieved per cow was recorded, and the embryos were classified by stage of development and quality according to the criteria of the International Embryo Transfer Society (22). Embryos were grouped into six stages: unfertilized oocytes (UFO), 2 to 12 cells, early morula, morula, early blastocyst, and blastocyst. Embryos were given a quality classification score (1 = excellent, perfect embryo; 2 = good, trivial imperfections; 3 = fair, definite but not severe problems such as extruded cells or a small amount of degeneration; and 4 = poor, partly degenerated or vesiculated cells) (41). Potential embryos were classified as UFO plus all embryos. Freezable and transferable embryos included only embryos with a quality score of 1 or 2 that were in the early morula or blastocyst stages of development.

Blood samples (10 ml) were collected daily from a coccygeal vessel into heparinized tubes from d 0 (first estrus) until the day of ovariectomy (experiment 1) or until the day of embryo recovery (d 6 of next cycle, experiment 2). Blood samples were stored in an ice bath, and plasma was separated by centrifugation (1800 × g for 15 min) and stored at −20°C until assayed for P4 and estradiol-17β (E2). Concentrations of P4 were measured by radioimmunoassay (25) in all samples. Sensitivity of the P4 assay was 0.28 ng ml⁻¹. Intraassay and interassay coefficients were 11.0 and 14.2%, respectively. Concentrations of E2 in plasma from d 0 to 15 (day of ovariectomy, experiment 1) or until day of estrus (experiment 2) were measured by radioimmunoassay as described by Tortone et al. (60) and modified by Badinga et al. (7). Sensitivity of the assay was 0.8 pg ml⁻¹, and the intraassay and interassay coefficients of variation were 8.5 and 22.8%, respectively.

**Experiment 3**

The objective of the third experiment was to evaluate folliculogenesis, ovulatory responses, and embryo yields to a superovulatory protocol using Folltropin-V (Vetrepham Canada Inc., London, Ontario, Canada) administered in the presence or absence of an active persistent DF, in which superstimulation with Folltropin-V was initiated immediately on the day the persistent DF was aspirated. Synchronization of estrus was induced by a single injection of PGF2α (25 mg i.m.; Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI) in 20 dairy cows. Eleven cycling dairy cows were observed in estrus and used in the experiment. On d 6, an active persistent DF was induced by insertion of a used CIDR device to induce subluteal concentrations of P4 (2.9 ± 0.3 ng/ml; 52) and two injections of PGF₂α (25 mg a.m.
and 15 mg p.m., i.m.) to cause luteolysis and create a low P₄ environment. The used CIDR device was left in place for 4 d. In contrast to experiment 2, aspiration of the DF was done on d 8 for the treatment group, and both control and treatment groups received sequential Folltropin-V injections beginning on d 8 immediately after the time of follicular aspiration. For follicular superinduction, a total of 20-mg of Folltropin-V was given per cow. Injections were initiated on d 8 (e.g., the same day as follicular aspiration) at 0 h and continued at 12-h intervals in a decreasing regimen over a 4-d period (a.m./p.m. = 4/4, 3/3, 2/2 and 1/1 ml per injection). Ultrasonography was performed daily from d 3 until d 11. Estrus occurred on d 12 following removal of the used CIDR on d 10. Cows were inseminated twice at 12-h intervals starting 10 h after the detection of estrus, which was performed as described for experiment 1. Additional ultrasonography was performed 2 d after estrus to confirm ovulation and to count the number of CL on the day of embryo recovery (d 7). Embryo recovery and evaluation were performed as described in experiment 2.

**Statistical Analysis**

Data were analyzed by least squares analysis of variance using the general linear model procedure of the SAS (46). Data from experiments 1 and 2 were analyzed together because the inclusion of a replicate in the mathematical model was not significant. The mathematical model used to analyze the number of follicles within discrete size classes, size of the DF, and plasma concentrations of P₄ and E₂ included effects of treatment, cow nested within treatment, day of the cycle, and interaction of treatment and day. The main effect of treatment was tested using the mean square of cow nested within treatment as the error term. When the interaction of treatment and day was significant, data were analyzed using orthogonal contrasts, comparing hormonal concentration between groups. Plasma concentrations of E₂ were analyzed in two periods from d 0 to 10 and from d 10 to 16. Plasma concentrations of P₄ were analyzed from d 0 to day of embryo recovery. To correct for heterogeneity of variance, plasma concentrations of P₄ were analyzed after logarithmic transformation (natural log) of P₄ concentration + 5, from d 16 to day of embryo recovery.

A one-way ANOVA was used to analyze discrete responses to superovulation treatment, such as follicles >9 mm on the day of estrus, number of ovulatory follicles (difference between the number of follicles >9 mm present in the ovary on the day of estrus and the number of nonovulatory follicles 2 d after estrus), number of ovulatory follicles adjusted (covariate) for number of follicles >9 mm in the ovaries on the day of estrus, number of nonovulatory follicles (number of follicles >9 mm remaining in the ovary 2 d after ovulation), number of CL (number of CL present in the ovary the day of embryo recovery as determined by ultrasonography), number of potential embryos (sum of UFO plus all embryos), the number of UFO plus embryos adjusted for number of ovulatory follicles, the number of UFO plus embryos adjusted for CL number, number of embryos, number of embryos adjusted for total number of UFO and embryos, number of transferable embryos, number of transferable embryos adjusted for number of UFO and embryos, number of transferable embryos adjusted for number of embryos, number of embryos adjusted for number of CL, and number of transferable embryos adjusted for number of CL.

Because experiment 3 involved a different experimental protocol, with superstimulation of follicle development beginning on the day of follicle aspiration and use of a different FSH source (Folltropin®-V vs. FSH-P), a separate statistical analysis was done following the same analytical approaches described above for follicular and embryonic responses.

**RESULTS**

**Experiments 1 and 2: Follicular Dynamics**

The first-wave DF was identified in all cows during the ultrasound examination on d 5 of the cycle. Under a low P₄ environment, created by insertion of the Norgestomet ear implant and PGF₂α, injections on d 6, the persistent DF increased in size from 11.0 ± 0.6 mm on d 5 to 15.8 ± 0.6 mm on d 10 in the control group and from 10.3 ± 0.6 mm on d 5 to 15.1 ± 0.6 mm on d 10 for the aspirated group (Figure 1A). For the control group, the persistent DF continued growing and reached 19.9 ± 0.7 mm on d 16 of the estrous cycle. Follicular aspiration in the aspirated group was associated with the disappearance of the active persistent DF.

The number of class 1 follicles (Figure 1B) did not differ between groups from d 5 to 10 or from d 15 to 17. The number (Figure 1B) of class 1 (2 to 5 mm) follicles was lower (P < 0.05) in the aspirated group from d 11 to 14. The number of class 1 follicles on d 10 (34.1 ± 3.1; Figure 1B) decreased to 3.2 ± 3.9 on d 16. In contrast, for the control group, the number of class 1 follicles increased from d 10 (30.7 ± 2.9) until d 12 (41.4 ± 3.1). The immediate decrease in number of class 1 follicles occurred before the injection of FSH-P on d 12.

The reduction in class 1 follicles in the aspirated group was associated with increased recruitment (3.3 > 0.6 ± 1.2; P < 0.1) of class 2 (6 to 9 mm) follicles (Figure 2A) on d 12, just before FSH-P injections and on d 13 (1 d after FSH-P injections began) to 8.2 > 1.4 ± 1.2.
Figure 1. Pattern of growth of the first wave dominant follicle (A) from d 5 to 15 of the estrous cycle in cows of control (■; n = 10 from d 5 to 15; n = 6 from d 16 to 17) and aspirated (●; n = 9 from d 5 to 15; n = 5 from d 16 to 17) groups and number (B) of class 1 follicles (2 to 5 mm) from d 5 to day of estrus in cows of control (■; n = 10 from d 5 to 15; n = 6 from d 16 to 17) and aspirated (●; n = 9 from d 5 to 15; n = 5 from d 16 to 17) groups (experiments 1 and 2). Asterisk indicates differences (P < 0.01) between mean concentrations within day. SMB = Synchromate-B implant.

Figure 2. Number of class 2 follicles (6 to 9 mm; A) and class 3 follicles (≥ 10 mm; B) from d 5 to day of estrus in cows of control (■; n = 10 from d 5 to 15; n = 6 from d 16 to 17) and aspirated (●; n = 9 from d 5 to 15; n = 5 from d 16 to 17) groups (experiments 1 and 2). Asterisk indicates differences (P < 0.01) between means within day. SMB = Synchromate-B implant.

class 2 follicles (P < 0.001). The decline in the number of class 1 follicles of the control group (Figure 1B) did not occur until d 14 (30.9 ± 3.1) at which time the number of class 2 follicles (9.6 ± 1.3; Figure 2A) increased. This increase continued until d 15 for the control group (18.9 ± 1.3) when the number of class 2 follicles was significantly fewer and declining in the aspirated group (9.3 ± 1.4; P < 0.001; Figure 2A). The decline in the number of class 2 follicles of the aspirated group was associated with an earlier increase and greater number of class 3 (≥10 mm) follicles on d 14 (5.9 ± 1.2 vs. 1.0 ± 1.1; P < 0.001; Figure 2B), d 15 (15.9 ± 1.2 vs. 5.6 ± 1.1; P < 0.001; Figure 2B), and d 16 (15.6 ± 1.5 vs. 9.7 ± 1.3; P < 0.001) compared with values for the control group. The presence of an active persistent DF delayed the FSH-induced increase in class 3 follicles by 1 to 2 d. The number of class 3 follicles was not different between groups at d 17 (23.7 ± 2.3 vs. 19.6 ± 2.3; P > 0.1).

Experiments 1 and 2: Plasma $P_4$ and $E_2$ Concentrations

Concentrations of $P_4$ in plasma did not differ between the two groups (Figure 3A). Concentrations of plasma $P_4$ increased from d 1 (0.6 ± 0.6 ng ml$^{-1}$) until d 6 (5.6 ± 1.9 ng ml$^{-1}$), at which time two injections of PGF$_{2\alpha}$ were administered. After an injection of PGF$_{2\alpha}$, plasma concentrations of $P_4$ decreased to basal levels and remained low (<1 ng ml$^{-1}$) until d 19 (2 d postovulation) when concentrations of $P_4$ in plasma were 3.0 ± 3.5 ng ml$^{-1}$ for the control group and 4.7 ± 3.8 ng ml$^{-1}$ for the
OVARIAN AND EMBRYO RESPONSES TO FSH-P IN COWS

Figure 3. Concentrations of progesterone (A) from day of estrus (d 0) to day of embryo recovery and estradiol-17β (B) from day of estrus until d 16 of the estrous cycle in cows of control (■; n = 10 from d 5 to 15; n = 6 after d 15) and aspirated (●; n = 9 from d 5 to 15; n = 5 after d 15) groups (experiments 1 and 2). Asterisk indicates differences (P < 0.01) between means within day. SMB = Synchromate-B implant.

aspirated group. On the day of embryo recovery, plasma concentrations of P4 were the same for both groups (P > 0.10). The tendency of higher P4 concentrations in the control group is due to one cow that had 108.4 ng ml⁻¹ of P4 and 19 CL.

The concentrations of plasma E₂ were higher (P < 0.001) in the control group than in the aspirated group on d 8, 9, and 10 and after follicular aspiration on d 11, 12, and 13 of the estrous cycle (Figure 3B).

Experiments 1 and 2: Ovarian Responses to Superovulation Treatment

On the day of detected estrus, the number of follicles >9 mm, the number of ovulatory follicles, the number of ovulatory follicles adjusted for number of follicles >9 mm on day of estrus, and the number of nonovulatory follicles did not differ between treatments (Table 1). The array of other ovarian and embryo responses (Table 2) measured on the day of embryo recovery did not differ between groups.

Experiment 3: Follicular Dynamics

The first-wave DF was identified in all cows during the ultrasound examination on d 5 of the cycle. Insertion of the used CIDR device and PGF₂α injections on d 6 induced a low P₄ environment (Figure 4) such that the persistent DF increased in size from 11.0 ± 0.6 mm on day 5 to 15.0 ± 0.6 mm on d 8 for the control group and from 10.8 ± 0.6 mm on d 5 to 14.8 ± 0.6 mm on d 8 for the aspirated group (Figure 5A). The persistent DF continued to grow for the control group, reaching a size of 20.4 ± 0.6 mm on d 11 of the estrous cycle. After aspiration, the persistent DF disappeared.

The number of class 1 follicles (Figure 6B) did not differ between groups from d 5 to 11. The number of class 1 follicles decreased from d 9 (52.2 ± 3.7) to 11 (21.8 ± 3.7) in the aspirated group and from d 9 (49 ± 4.0) to 11 (22.0 ± 4.0) in the control group. The reductions in number of class 1 follicles for the aspirated (FSH and aspiration) and control (FSH) groups were associated concurrently with increased recruitment of class 2 follicles (Figure 6A) on d 10 for both groups (control group: 15.8 ± 4.1; aspirated group: 19.2 ± 3.8). There was a subsequent increase in number of class 3 follicles (Figure 6B) on d 11 for control (7.8 ± 1.0) and aspirated (10.7 ± 0.9) groups which did not differ between groups.

Experiment 3: Ovulatory Responses to Superovulation Treatment

On the day of detected estrus, the number of follicles >9 mm, the number of ovulatory follicles, the number of ovulatory follicles adjusted for number of follicles >9 mm on day of estrus, and the number of nonovulatory follicles estimated as number of follicles >9 on day of estrus minus number of nonovulatory follicles (>9 mm) at d 2 after estrus.

| Table 1. Ovulatory responses (means ± SEM) to a superovulatory treatment measured the day of detected estrus (experiments 1 and 2). |
|---------------------------------|-----------------|-----------------|
| **Follicular aspiration**       | **Aspirated**   | **Control**     |
| (n = 5 cows)                    | (n = 6 cows)    |                 |
| Total follicles >9 mm, no.      | 18.4 ± 4.4      | 13.0 ± 3.9      |
| Ovulatory follicles, no.¹       | 12.0 ± 5.4      | 7.2 ± 4.9       |
| Ovulatory follicles (no.) adjusted for follicles >9 mm (no.) on day of estrus | 9.1 ± 3.5 | 9.6 ± 3.1     |
| Nonovulatory follicles, no.     | 6.4 ± 3.2       | 5.8 ± 2.9       |

¹Ovulatory follicles estimated as number of follicles >9 on day of estrus minus number of nonovulatory follicles (>9 mm) at d 2 after estrus.
Table 2. Ovulatory response (means ± SEM) to superovulatory treatment measured the day of embryo recovery (experiments 1 and 2).

<table>
<thead>
<tr>
<th>Follicular aspiration</th>
<th>Aspirated (n = 5 cows)</th>
<th>Control (n = 6 cows)</th>
</tr>
</thead>
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<tr>
<td>Corpora lutea (ultrasound), no.</td>
<td>10.2 ± 4.6</td>
<td>7.5 ± 4.2</td>
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<td>Ova and embryos, no.</td>
<td>5.0 ± 3.8</td>
<td>6.2 ± 3.5</td>
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<td>Ova and embryos (no.) adjusted for corpora lutea, no.</td>
<td>3.9 ± 1.7</td>
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<td>Ova and embryos (no.) adjusted for ovulatory follicles, no.</td>
<td>3.4 ± 2.0</td>
<td>7.5 ± 1.8</td>
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<tr>
<td>Embryos, no.</td>
<td>2.6 ± 1.9</td>
<td>2.7 ± 1.7</td>
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<tr>
<td>Embryos (no.) adjusted for ova and embryos, no.</td>
<td>2.8 ± 1.6</td>
<td>2.5 ± 1.5</td>
</tr>
<tr>
<td>Transferable embryos, no.</td>
<td>1.0 ± 1.6</td>
<td>2.3 ± 1.5</td>
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<tr>
<td>Transferable embryos (no.) adjusted for ova and embryos, no.</td>
<td>1.1 ± 1.6</td>
<td>2.2 ± 1.5</td>
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<tr>
<td>Transferable embryos (no.) adjusted for embryos, no.</td>
<td>1.0 ± 0.7</td>
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<tr>
<td>Embryos (no.) adjusted for corpora lutea, no.</td>
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<tr>
<td>Transferable embryos (no.) adjusted for corpora lutea, no.</td>
<td>0.8 ± 1.5</td>
<td>2.5 ± 1.4</td>
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</table>

Follicles did not differ between treatments (Table 3). The array of other ovarian and embryo responses (Table 4) measured on the day of embryo recovery did not differ between groups. Throughout the blood sampling period (d 1 to 19) plasma concentrations of progesterone did not differ other than d 17 and 18 (P < 0.05; Figure 4). The differences on these days were due to elevated P4 concentrations in two of five cows (19 ng ml−1) of the control group, whereas none of six cows had this elevated P4 concentration in the aspiration group. The overall number of CL at embryo recovery (d 7) did not differ between groups (Table 4).

**DISCUSSION**

The growth of the first-wave DF is one of the most predictable events in ovarian follicular dynamics during the estrous cycle of cattle (7, 13). The demise of...
low amplitude during proestrus and metestrus and low frequency, high amplitude pulses during diestrus (43). Progesterone and E2 are important in the regulation of pituitary LH secretion. Under a low progestin environment, in the absence of a CL, the pattern of LH secretion that causes persistent follicles is intermediate between a high follicular phase pulse frequency and the low pulse frequency of the midluteal phase (24). In such a low progestin environment and an increased LH pulse frequency, the first-wave DF continues to grow and suppresses the growth of other follicles (51, 52). In the present studies, the induction of a low progestin environment resulted in persistence and increased size of the DF from d 6 to 16 (experiments 1 and 2) or to d 11 (experiment 3), which is in agreement with results of others (52, 58). Prolonging the period of follicular dominance is an alternative model to study whether a DF can alter induced follicular development in response to exogenous FSH.

The phenomenon of dominance is central to understanding folliculogenesis because it suggests that some follicles survive in a milieu that suppresses the growth of other follicles. In addition, dominance is related to the success of superovulation, both in humans and in domestic animals (14). There are numerous reports (11, 27, 31, 35, 38) of superovulation programs in cattle with variable results. The ovarian response to a superovulation program is determined by many factors, such as ovarian responsiveness of donors, fertilization rate, embryo viability, factors related to physiological status of the animal such as pregnancy, and management (convenience and cost effectiveness of superovulation protocols) (5).

Ovarian status at the time of superovulatory treatment has been postulated to be a major factor determining ovarian response (33). The increase in number of class 2 follicles at d 2 after aspiration of the active persistent DF of experiments 1 and 2 and prior to the first injection of FSH indicates that the persistent DF suppressed the growth of follicles. This suppressive influence may have been through secretion of some factors, such as estradiol or inhibin, that deprived follicles of the gonadotropin support that is critical for their further development (12, 13). Recruitment was delayed in the presence of the active persistent DF as shown by a lower number of class 2 follicles in the control group on d 12 just before the beginning of FSH injections, which was sustained until 2 d after initiation of FSH treatment (d 14). Similar results were reported by Guilbault et al. (17) when they superovulated heifers in the presence of a DF. At d 14, recruitment was evident in the control group, as the number of class 2 follicles increased in association with a decrease in number of class 1 follicles. This process occurred 2 d after estrus.

### Table 3. Ovulatory responses (means ± SEM) to a superovulatory treatment measured the day of detected estrus (experiment 3).

<table>
<thead>
<tr>
<th>Follicular aspiration</th>
<th>Aspirated (n = 6 cows)</th>
<th>Control (n = 5 cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total follicles &gt;9 mm, no.</td>
<td>12.0 ± 1.7</td>
<td>7.8 ± 1.9</td>
</tr>
<tr>
<td>Ovulatory follicles(^1), no.</td>
<td>4.5 ± 1.7</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>Ovulatory follicles (no.) adjusted for follicles &gt;9 (no.) on day of estrus</td>
<td>4.2 ± 1.8</td>
<td>4.8 ± 2.0</td>
</tr>
<tr>
<td>Nonovulatory follicles, no.</td>
<td>9.0 ± 2.8</td>
<td>3.6 ± 3.1</td>
</tr>
</tbody>
</table>

\(^1\)Ovulatory follicles estimated as number of follicles >9 mm on day of estrus minus number of nonovulatory follicles (>9 mm) at d 2 after estrus.
earlier in the aspirated group (d 12) in which the active persistent DF was aspirated on d 10. Similarly, the number of class 3 follicles increased earlier in the aspirated group without an active persistent DF. However, by d 17, the number of class 3 follicles was the same for the two groups, indicating that exogenous FSH eventually overrode the inhibitory influence of the active persistent DF. This is further supported by the same number of ovulatory and embryo responses.

It is evident that the increase in number of class 3 follicles in the group that had the DF aspirated did not occur until d 14, or 2 d after the beginning of FSH injections in experiments 1 and 2. The number of class 2 follicles had increased slightly but significantly on d 12, which is the day FSH injections were initiated (2 d after aspiration of the DF), and the number of class 1 follicles declined on d 11 (1 d after follicle aspiration and 1 d before FSH injection). Thus, aspiration of the DF led to a recruitment of follicles before the injection of exogenous FSH. This recruitment earlier than that of the control group is likely due to an increase in endogenous FSH following aspiration of the DF. The increase in FSH probably did not occur in the control group in which the active persistent DF was maintained. It is possible that this earlier recruitment, due to endogenous FSH, sensitized the responsive follicle pools to respond earlier to follicular superinduction to exogenous FSH compared with the control group. Based on these observed responses, an alternative experimental approach was used in experiment 3 to determine whether presence of an active DF can alter follicular dynamics in response to exogenous FSH. Exogenous FSH injections in experiment 3 were initiated immediately upon aspiration of the DF on d 8 in both groups. Under this scenario, any increase in endogenous FSH in the group with the aspirated DF will occur concurrently with the exogenous FSH and represent a small amount of total FSH. When FSH injections were initiated on the day of follicle aspiration in experiment 3, there were no major differences in dynamics of class 1, 2, and 3 follicles between cows bearing or not bearing a persistent DF. The sequential decrease in number of class 1 follicles and subsequent increase in class 2 and 3 follicles was characteristic of follicle superinduction, as observed in experiments 1 and 2. In experiments 1, 2 and 3, the FSH injections overrode the effect of the DF that was present in cows of the control groups.

The initiation of the superovulatory regimen in the absence of a DF has been shown to improve the ovarian response to FSH (8, 17, 21, 50, 63). However, Gray et al. (16) found no difference in yield of transferable or total embryos when FSH treatment began during the period of morphological regression of the DF versus a control group in which superovulatory treatment began on d 10 when DF was morphologically dominant. The ovulation rate decreased when FSH was given in the presence of a DF, resulting in fewer recovered embryos than from initiation of treatment when a DF was not present (17, 21). The beneficial effect of no DF was not shown in the present experiment, in which none of the variables measured on the day of estrus or the day of embryo recovery was different. Similarly, follicular development, ovulation rate, and embryo recovery rate did not differ when the first-wave DF was ovulated, with human chorionic gonadotropin and superovulation treatment started in the absence of a DF (44). In a crossover design, Stock et al. (58) reported no differences in the number of ovulatory size follicles, ovulation rates, or yields of total and transferable embryos between heifers superovulated in the absence of a DF on d 1 versus the presence of a DF on d 6 of the cycle. This comparison was made in seven Holstein heifers that exhibited estrus following FSH treatment in both the absence of a DF on d 1 versus presence of a DF on d 6. Eight of the 17 heifers in the group with the DF present on d 6 ovulated during FSH treatment. Authors sug-

### Table 4. Ovulatory response (means ± SEM) to superovulatory treatment measured the day of embryo recovery (experiment 3).

<table>
<thead>
<tr>
<th>Follicular aspiration</th>
<th>Aspirated (n = 6 cows)</th>
<th>Control (n = 5 cows)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpora lutea (ultrasound), no.</td>
<td>5.2 ± 1.6</td>
<td>6.2 ± 1.8</td>
</tr>
<tr>
<td>Ova and embryos, no.</td>
<td>1.5 ± 0.9</td>
<td>1.0 ± 1.0</td>
</tr>
<tr>
<td>Ova and embryos (no.) adjusted for corpora lutea, no.</td>
<td>1.7 ± 0.6</td>
<td>0.8 ± 0.7</td>
</tr>
<tr>
<td>Ova and embryos (no.) adjusted for ovulatory follicles, no.</td>
<td>1.4 ± 0.8</td>
<td>1.1 ± 0.9</td>
</tr>
<tr>
<td>Embryos, no.</td>
<td>1.0 ± 0.7</td>
<td>0.6 ± 0.8</td>
</tr>
<tr>
<td>Embryos (no.) adjusted for ova and embryos, no.</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Transferable embryos, no.</td>
<td>0.7 ± 0.5</td>
<td>0.2 ± 0.6</td>
</tr>
<tr>
<td>Transferable embryos (no.) adjusted for ova and embryos, no.</td>
<td>0.5 ± 0.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Transferable embryos (no.) adjusted for embryos, no.</td>
<td>0.4 ± 0.02</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>Embryos (no.) adjusted for corpora lutea, no.</td>
<td>1.2 ± 0.5</td>
<td>0.4 ± 0.5</td>
</tr>
<tr>
<td>Transferable embryos (no.) adjusted for corpora lutea, no.</td>
<td>0.8 ± 0.4</td>
<td>0.1 ± 0.4</td>
</tr>
</tbody>
</table>
suggested that ovulation of the DF during superovulation may explain poor ovulatory responses when superovulation is implemented in the presence of a DF. In the present experiment, the active persistent DF was present in the ovary of all control cows during FSH-P treatment for experiments 1, 2, and 3, and all persistent DF ovulated after estrus. Wehrman et al. (62) reported a reduced number of CL, total ova, and transferable embryos when FSH treatment was initiated 5 d after insertion of a 0.5 PRID compared with a group with FSH treatment initiated 5 d after insertion of 2 PRID. However, this differential response between 0.5 versus 2 PRID was not evident when FSH treatment was initiated at either 2 or 8 d after PRID insertion.

Passive immunization of ewes against steroid-free follicular fluid or against synthetic inhibin peptides increased ovulation rates two- to fourfold (36, 54). The ovulation rates of the cow and prolificacy are more difficult to manipulate by inhibin vaccination (37). However, immunization of heifers or cows against steroid-free follicular fluid can increase the ovulation rate (15, 34, 40, 53) and the number of transferable embryos (4). Thus, immunoneutralization of inhibin increases ovarian follicular development.

Follicular dominance appears to be controlled by a number of mechanisms acting in concert, including alterations in peripheral FSH concentrations in response to E2 and inhibin secreted by the DF as well as the possible production of local ovarian factors that inhibit development of subordinate follicles (6, 10). Two hypotheses have been advanced to explain how the DF exerts dominance. The DF could cause regression of subordinate follicles indirectly, via a negative feedback on FSH secretion (14). Alternatively, the DF may secrete a factor that directly impairs further growth and development of subordinate follicles (2, 14, 26). In monotocous species, such a factor would clearly have to be endocrine in nature, because it would need to inhibit recruitment and induce regression of subordinate follicles on both ovaries. In addition to the peripheral action of inhibin on FSH secretion, strong evidence supports the regulatory actions of inhibin-like molecules within the ovary (18, 19, 20, 55, 64). Sato et al. (47) suggested that an inhibin-like substance inhibits FSH action at the ovarian level through binding to the FSH receptor on the granulosa cell. More recently, a report by Schneyer et al. (55) indicated that inhibin α-subunits bind to FSH receptor sites and inhibit FSH bioactivity in granulosa cell cultures. Results from experiment 3 do not support the hypothesis that factors from the DF exert inhibitory effects on follicle growth at the ovarian level. Follicular dynamics after injections of FSH were the same in either the presence or absence of a DF.

Neither the number of CL nor plasma P4 on the day of embryo recovery differed between groups (experiments 1, 2, and 3). Higher concentrations of E2 in plasma during d 8, 9, and 10 of the estrous cycle before aspiration of the persistent DF were due to greater variability among cows of the control group than that among cows of the aspirated group before aspiration of the persistent DF on those days (P < 0.01). This result was due to the increase >5 pg ml\(^{-1}\), in plasma E2 for eight of 10 cows in the control group. Only five of nine cows in the aspirated group had this increase. On d 11, 12, and 13, the difference between treatments in plasma E2 was due to presence of an estrogenic active persistent DF in the control group and initiation of a new follicular wave in the aspirated group, before exogenous FSH treatment was initiated. A larger number of class 3 follicles on d 15 and d 16 in the aspirated group was not associated with higher concentrations of E2 in plasma. For experiments 2 and 3, yields of transferable embryos were low compared with what is achieved in the commercial embryo transfer industry (42). To test the potential effect of a persistent DF on responses to FSH in these experiments, a low progesterone environment was induced that may have had an adverse effect on physical recovery of oocytes and embryos, as well as alterations in oocyte and embryonic development from FSH induced follicles. Prolonged dominance of follicles induced by a high LH pulse frequency, as a consequence of a low progesterone environment, initiated premature maturation of the bovine oocyte (32) and reduced oocyte viability (45).

**IMPLICATIONS**

Increased follicle recruitment following the removal of the persistent DF indicates that an active persistent DF inhibits ovarian follicular development. However, exogenous FSH treatment appears to override any inhibitory effect that a persistent DF may be exerting systemically at either the ovary or pituitary. The presence of a low progesterone environment throughout the period of superovulation appeared to reduce the yield of embryos.

**REFERENCES**


