

Decreased superovulatory responses in heifers superovulated in the presence of a dominant follicle

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Summary. Dairy heifers were superovulated in the presence (dominant group, N = 8) or absence (non-dominant group, N = 6) of a dominant follicle at the start of a superovulatory treatment on Days 7–12 of the oestrous cycle (Day 0 = oestrus). Daily ultrasonographic observations of ovaries (recorded on videotape) starting on Day 3 were used to assess the presence or absence of a dominant follicle (diameter >9 mm, in a growing phase or at a stable diameter for <4 days) and to monitor follicular development before and during treatment. The number of CL estimated by ultrasonography (7.1 ± 1.8 vs 13.5 ± 1.4) or by rectal palpation (6.9 ± 2.0 vs 16.3 ± 1.6) and mean progesterone concentrations (32.5 ± 19 vs 80.7 ± 16 ng/ml) after treatment were lower ($P < 0.01$) in the dominant than in the non-dominant group. Based on number of CL, two populations of heifers were identified in the dominant group, i.e. those that had a high (dominant-high, N = 4; >7 CL) or a low (dominant-low, N = 4; <7 CL) response to treatment. During treatment, the increases in number of follicles 7–10 mm and >10 mm in diameter occurred sooner and were of higher magnitude in the non-dominant than in the dominant-high or dominant-low groups ($P < 0.01$). At the expected time of ovulation 6–7 days after the start of treatment, there was a rapid decrease in number of follicles 7–10 mm and >10 mm in diameter in the dominant-high and non-dominant groups but not in the dominant-low group. Compared with the dominant-high group, differences in profiles of changes in diameter of largest (F_1) and second largest (F_2) follicles indicated that emergence of the dominant F_1 follicle before treatment was delayed by 1–2 days in the dominant-low group. These results suggest that the presence of a dominant follicle before superovulation treatment may decrease the superovulatory response and/or alter the maturation process of growing follicles during treatment, especially when emergence of the dominant F_1 follicle occurred within 3 days of the start of treatment.

Keywords: follicle; dominance; superovulation; ultrasonography; cattle

Introduction

The large variation in number of ovulations observed after a superovulatory treatment with gonadotrophins is a major limiting factor to embryo transfer activities in cattle. This variation has been attributed to various extrinsic factors related to the type of hormone preparation and to different injection schedules (Donaldson, 1984; Murphy *et al.*, 1984; Lindsell *et al.*, 1986; Guilbault *et al.*, 1987). However, it has also been reported that intrinsic factors related to ovarian status at the

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time of initiation of the superovulatory treatment can account for a large portion of the variability in superovulatory response (Monniaux *et al.*, 1983).

Evidence gathered over the past decade supports the concept that follicular development during the bovine oestrous cycle occurs in 2 or 3 waves (Matton *et al.*, 1981; Ireland & Roche, 1987; Pierson & Ginther, 1987) and that, during each wave, a single follicle becomes dominant whereas other follicles in the same wave regress (Sirois & Fortune, 1988; Savio *et al.*, 1988). Grasso *et al.* (1989) indicate that the presence of large follicles at the beginning of a superovulatory treatment is associated with a lower ovulation rate. Similarly, the superovulatory response is decreased when charcoal-extracted bovine follicular fluid is injected before the start of a superovulatory treatment (Lussier & Carruthers, 1989) and when superovulation is initiated after selection of the dominant ovulatory follicle at the end of the oestrous cycle (Pierson & Ginther, 1988). Therefore, it is likely that the presence of a dominant follicle can affect the subsequent superovulatory response since follicles smaller than 6 mm in diameter are recruited during a gonadotrophin treatment (Monniaux *et al.*, 1983; Grasso *et al.*, 1989). The objective of the present experiment was to determine the influence of the presence of a large dominant follicle on the ensuing superovulatory response both in terms of follicular growth and ovulation rate.

Materials and Methods

Animals and experimental design. The oestrous cycle of 17 dairy heifers between 17 and 23 months of age was synchronized with two i.m. injections of 500 µg cloprostenol (Estrumate: Coopers Agropharm Inc., Ajax, Ontario, Canada) given 11 days apart. Starting on Day 3 of the oestrous cycle (Day 0 = oestrus), ovarian activity was monitored daily by ultrasonography with a real-time linear scanning ultrasound diagnostic system (LS-300A: Tokyo Keiki Co. Ltd, Tokyo 144, Japan; 7.5 MHz transducer) as described by Grasso *et al.* (1989). Daily observations were videotaped and compared to observations of the previous days. This allowed monitoring of the growth and regression of follicles > 4 mm on an individual basis (Sirois & Fortune, 1988) and classification before start of a superovulatory treatment as dominant or non-dominant according to the following criteria. Dominant follicles had (1) a diameter of > 9 mm and (2) were in a growing phase (i.e. diameter increasing from day to day) or stable for < 4 days. Non-dominant follicles had (1) a diameter of < 10 mm or (2) were in a regressing phase (i.e. decreasing diameter from day to day) or stable for at least 4 days. These criteria were defined arbitrarily based on observations from Sirois & Fortune (1988) and from our own previous experiment (Grasso *et al.*, 1989).

Between Days 7 and 12 of the oestrous cycle, heifers were assigned to two groups according to the presence (dominant group; N = 9) or the absence (non-dominant group; N = 8) of a dominant follicle and were then superovulated. Superovulation was induced with 28 mg FSH-P (FSH-P: Schering Canada Inc., Pointe-Claire, Québec, Canada) administered in declining doses at 08:00 and 20:00 h over 5 days (0.4/5, 4.0/4.0, 3.25/3.25, 2.5/2.5 and 2.0/2.0 mg). On the evening of the 4th day of FSH-P treatment, luteolysis was induced with 500 µg cloprostenol. From Day 3 of the oestrous cycle and until 2 days after the end of superovulation, the numbers of follicles 4–6 mm, 7–10 mm and > 10 mm were determined daily by ultrasonographic observations recorded on videotape. The follicles identified as the largest (F_1) and second largest (F_2) were the follicles that had the largest and second largest diameter at the time of initiation of the superovulatory treatment. To avoid confounding of effects of day of the oestrous cycle and follicular status, heifers from dominant and non-dominant groups were matched for day of initiation of the superovulatory treatment. All heifers but one (No. 711) were superovulated during the first wave of follicular development. Heifer 711 was superovulated during the second wave of follicular development.

The number of corpora lutea (CL) was determined by ultrasonography and by transrectal palpation performed 7 days after the end of the superovulatory treatment. A peripheral blood sample was also collected at the same time to determine plasma progesterone concentrations (Guilbault *et al.*, 1988) in each cow. One heifer in the dominant group and 2 in the non-dominant group returned to oestrus before the induced luteolysis during treatment and were excluded from the experiment.

Validation of ultrasonographic observations. To assess the validity of the measures of follicular diameter, 52 follicles (3–19 mm) dissected from ovaries collected at slaughter were trimmed of stromal tissue and their diameter was measured using a vernier caliper. Each follicle was then placed in a water bath (22°C) and the diameter of its antral cavity was determined by ultrasonography. There was a linear relationship between the diameter of the antral cavity measured by ultrasonography and the diameter of the same follicles measured after dissection ($y = -1.70 + 0.83x$ where y = follicle diameter by ultrasonography and x = follicle diameter after dissection; $r = 0.96$, $P < 0.001$). In agreement with Quirk *et al.* (1986), diameters of dissected follicles were approximately 3 mm larger than the diameter of the antral cavity which is the portion of the follicle that is visible by ultrasonography. Furthermore, the total number of follicles > 3 mm in diameter was evaluated by transrectal ultrasonography in 13 cows superovulated with FSH-P (total = 22.1 mg) over 3.5 days. Ovaries from these cows were collected at slaughter and visible follicles were

dissected and counted. There was a linear relationship between the number of follicles > 3 mm assessed by ultrasonography and by dissection ($y = 0.35 + 1.0x$ where y = number of follicles by transrectal ultrasonography and x = number of follicles after dissection; $r = 0.75$, $P < 0.001$).

Statistical analysis. Data on the number of CL and on plasma progesterone concentrations evaluated 7 days after the end of treatment were compared by least squares analysis of variance using the Statistical Analysis System (SAS, 1985). Based on number of CL, heifers in the dominant group were divided retrospectively into two groups, i.e. those with a low ovulation rate (dominant–low; < 7 CL; $N = 4$) and those with a high ovulation rate (dominant–high; < 7 CL, $N = 4$). All data on follicular dynamics were analysed relative to the first day of superovulatory treatment (Day 0). Before and during the treatment, the design was a split plot in which heifers were nested by group and repeated measurements (ultrasonography) were taken over time. Changes in profiles of follicular dynamics before and during treatment among groups were compared by orthogonal contrasts as follows: (1) dominant *vs* non-dominant groups and (2) dominant–low *vs* dominant–high groups. Data (except F_1 and F_2 diameter) were transformed (logarithmic transformation) prior to statistical analyses.

Results

General

Profiles of growth and regression of the largest and second largest follicles before treatment are given individually in Fig. 1. Based on the criteria defining follicular dominance (see ‘Materials and Methods’) at the start of treatment, the dominant follicle of heifers in the dominant groups (low or high response) was > 9 mm and in a growing phase (Heifers 629, 633, 675, 703, 708) or stable for < 4 days (Heifers 697, 704 and 711). In contrast, no follicle > 9 mm in diameter was present when superovulation treatment was initiated in the non-dominant groups (Heifers 627, 670, 761, 762 and 774) or the follicle was stable for more than 4 days (Heifer 710).

Ovulation rate

Estimates of number of CL by ultrasonography or rectal palpation 7 days after oestrus were highly correlated with each other ($r = 0.95$, $P < 0.01$) and with plasma progesterone concentration ($r = 0.87$ and $r = 0.89$, $P < 0.01$, respectively). The mean number of CL evaluated by ultrasonography ($P < 0.01$) or by rectal palpation ($P < 0.001$) and mean plasma progesterone concentrations ($P < 0.01$) 7 days after oestrus were higher in heifers of the non-dominant than of the dominant groups (Table 1). All heifers in the non-dominant group had at least 9 or 11 CL as estimated by ultrasonography or palpation, respectively. In contrast, the number of CL varied widely in heifers of the dominant groups and ranged from 0 to 14 or 0 to 16 depending on the method used. Clearly, two populations of heifers could be identified in the dominant group, i.e. those that had either a high (dominant–high, $N = 4$; > 7 CL) or a low (dominant–low, $N = 4$, < 7 CL) response to superovulation. Ovulation rate and plasma progesterone concentrations were significantly lower in heifers of the dominant–low than the dominant–high group (Table 1). For the reasons given above, data on follicular development before and during superovulation were compared between heifers of the non-dominant, dominant–high and dominant–low groups.

Follicular dynamics before superovulation treatment

As expected, the profile of mean number of follicles > 10 mm in diameter before treatment differed ($P < 0.001$) between heifers according to whether they had a dominant follicle at the start of treatment (Fig. 2a). While there was a continuous and parallel increase in number of follicles > 10 mm in heifers of the dominant–high and dominant–low groups before treatment, the mean number of follicles > 10 mm increased in heifers of the non-dominant group before Day -2 but decreased after treatment was begun. Similarly, there was a parallel increase in diameter of the F_1 follicle from approximately 5 to 12 mm before treatment in the dominant–high and dominant–low groups (Fig. 2b). In the non-dominant group, however, (group \times day interaction; $P < 0.001$)

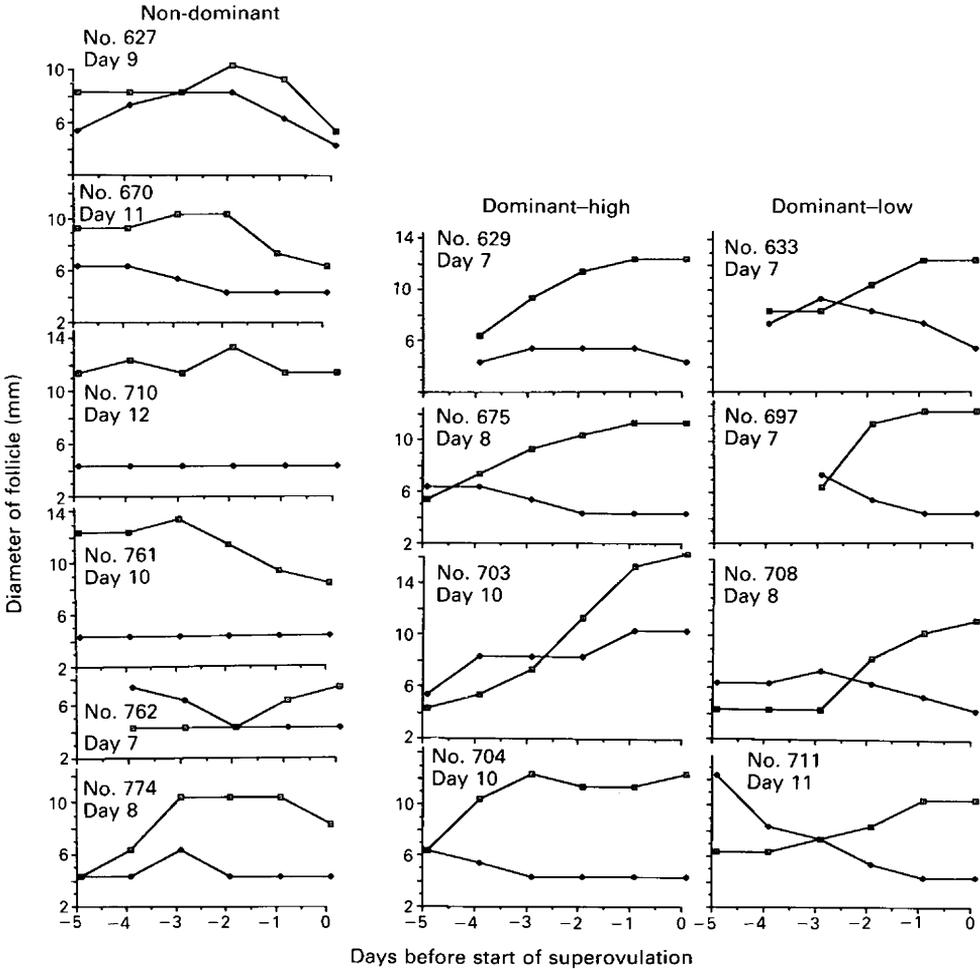


Fig. 1. Individual pattern of development of largest (F_1 ; \square — \square) and second largest (F_2 ; \blacklozenge — \blacklozenge) follicles before start of superovulation (Day 0) in heifers assigned to non-dominant ($N = 6$) and dominant ($N = 8$) groups. Heifers of the dominant group were subdivided into those that had a high (dominant-high; >7 CL; $N = 4$) or a low (dominant-low; <7 CL; $N = 4$) superovulatory response. Heifer number and day of the oestrous cycle when superovulation was started are indicated in each panel.

mean diameter of the F_1 follicle was 9 mm at 2 days before treatment and decreased progressively to <8 mm thereafter.

Overall, there was no difference in profile of mean diameter of the F_2 follicle in heifers in relation to the presence of a dominant follicle before treatment. This was due to the fact that profiles of the mean diameter of the F_2 follicle differed markedly ($P < 0.001$) between heifers of the dominant-high and those of the dominant-low groups (Fig. 2c). Indeed, before treatment, the mean diameter of the F_2 follicle remained constant and averaged 5.5 mm in diameter in the dominant-high group while it decreased from 9 to less than 5 mm in diameter during the same time interval in the dominant-low group.

The mean difference in diameter of F_1 and F_2 follicles increased progressively before treatment in the dominant-high and dominant-low groups. In contrast (group \times day interaction; $P < 0.001$), the difference in mean diameter of F_1 and F_2 follicles remained constant during the

Table 1. Number of ovulations (CL) and plasma progesterone concentrations 7 days after oestrus in Holstein heifers with or without a dominant follicle at the start of superovulation treatment

Exp. group	No.	No. of CL					
		Ultrasonography		Palpation		Progesterone (ng/ml)	
		Mean \pm s.e.	Range	Mean \pm s.e.	Range	Mean \pm s.e.	Range
Non-dominant	6	13.5 \pm 1.4	9–19	16.3 \pm 1.6	11–24	80.7 \pm 16	34.0–165.0
Dominant							
Dominant-high	4	11.2 \pm 1.7	8–14	11.5 \pm 1.9	9–16	54.9 \pm 19	41.0– 89.5
Dominant-low	4	3.0 \pm 1.7	0–6	2.2 \pm 2.0	0–5	10.0 \pm 19	0.2– 34.0
Orthogonal contrasts							
Non-dominant vs dominant		$P < 0.01$		$P < 0.001$		$P < 0.01$	
Dominant-high vs dominant-low		$P < 0.003$		$P < 0.001$		$P < 0.005$	

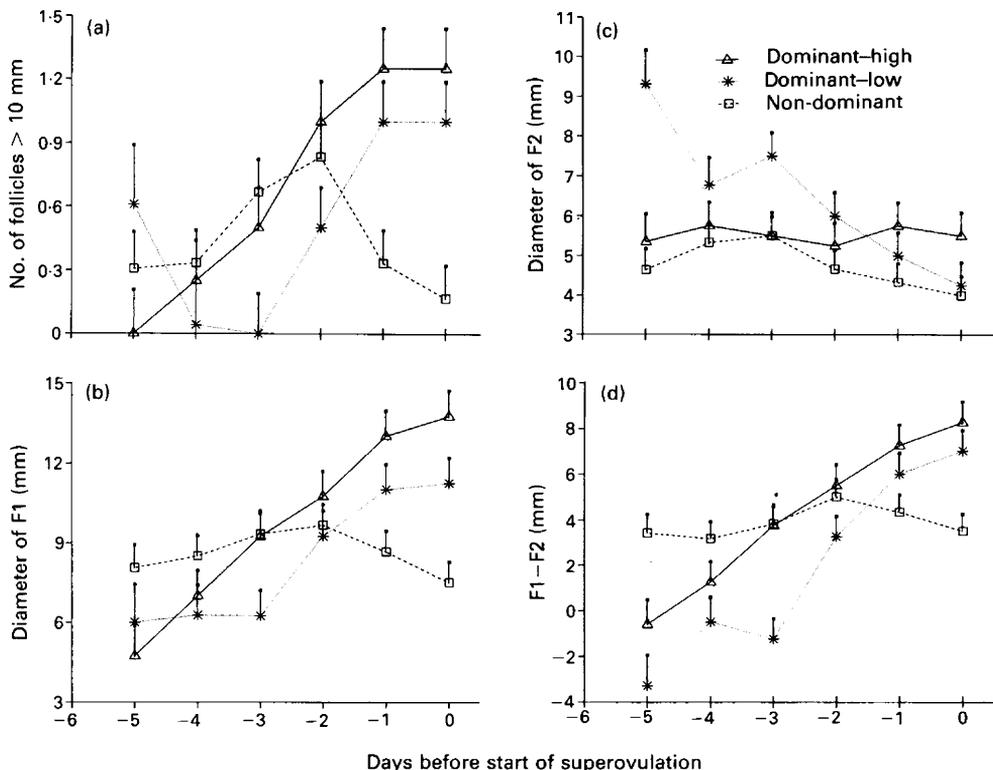


Fig. 2. Profiles of mean number of follicles > 10 mm, of mean diameter of largest (F_1) and second (F_2) largest follicles and of the mean difference between the two largest follicles ($F_1 - F_2$) before start of superovulation (Day 0) in heifers of the non-dominant ($N = 6$), dominant-high ($N = 4$), and dominant-low ($N = 4$) groups. F_1 and F_2 were defined as such based on their diameter on the day superovulation was started.

same period in the non-dominant group (Fig. 2d). The increases in mean difference in diameter of F_1 and F_2 follicles were parallel ($P > 0.1$) in the dominant-high and dominant-low groups. However, the absolute values between Days -5 and -3 were positive in the dominant-high group and negative in the dominant-low group and the increase in mean difference in diameter of F_1 and F_2 follicle was delayed in the dominant-low group. Neither the mean nor the profile of the mean number of follicles 4–6 mm and 7–9 mm before start of treatment differed ($P > 0.1$) between groups (data not shown).

Follicular dynamics during superovulation treatment

During treatment, the mean number of follicles 4–6 mm did not differ ($P > 0.1$) among groups. In the 3 groups, there was an increase during the first 4–6 days of treatment and a decrease thereafter ($P < 0.001$, Fig. 3a). However, the increases in number of follicles 7–10 mm and > 10 mm occurred sooner and were of higher magnitude in the non-dominant group than in the dominant-high or dominant-low groups ($P < 0.01$; Figs 3b, 3c). The profile of number of follicles 7–10 mm and > 10 mm during treatment was similar ($P > 0.1$) in the dominant-high and dominant-low groups. Numbers of follicles 7–10 mm and > 10 mm in diameter decreased rapidly 6–7 days after start of treatment (oestrus) in the dominant-high and non-dominant groups but were maintained at a constant level in the dominant-low group.

The number of follicles > 7 mm in diameter at the time of oestrus was correlated positively with the number of CL ($r = 0.64$, $P < 0.05$). When data from animals in the dominant-low group were excluded, the correlation coefficient increased to 0.83 ($P < 0.01$).

Discussion

The results clearly show that, when superovulation was initiated in absence of a dominant follicle, the ovulation rate of these heifers was higher and especially less variable than in those that had a dominant follicle. This is in agreement with previous results indicating that the presence of large follicles at the start of superovulation treatment is associated with a lower ovulation rate in cattle (Grasso *et al.*, 1989), primates (Goodman & Hogden, 1983) and ewes (Driancourt, 1987). It also supports the hypothesis that the ovarian status at the beginning of treatment accounts for a large portion of the variability in the superovulatory response (Monniaux *et al.*, 1983).

One of two sets of criteria is generally used to define follicular dominance, i.e. (1) asymmetric production of oestradiol by each ovary (ewe: Driancourt, 1987; primate: DiZerega *et al.*, 1980; cattle: Ireland & Roche, 1987), or (2) the presence of a large follicle which appears to suppress development of the other (ewe: Driancourt & Cahill, 1984; cattle: Sirois & Fortune, 1988; Savio *et al.*, 1988). In the present experiment, ovulation rates were homogeneous in the non-dominant group (> 9 CL), but were highly variable in the dominant group. Indeed, based on our criteria of follicular dominance, two populations of heifers in the dominant group clearly emerged; those that had either a high (> 7 CL) or a low (< 7 CL) response to superovulation. The use of morphological criteria related to the size of the largest follicle alone may not, therefore, be fully satisfactory to assess follicular dominance.

Based on the ability of a follicle to ovulate after induced luteolysis, Lavoit & Fortune (1990) concluded that morphological and functional dominances coincide when the dominant follicle of the first wave is actively growing but that such congruence is lost when its growth reaches a plateau phase. It is believed that the dominant follicle exerts an inhibitory effect over the growth and development of other follicles in the cohort (Sirois & Fortune, 1988; Savio *et al.*, 1988). Profiles of growth and regression of the 2 largest follicles before superovulation treatment in the present

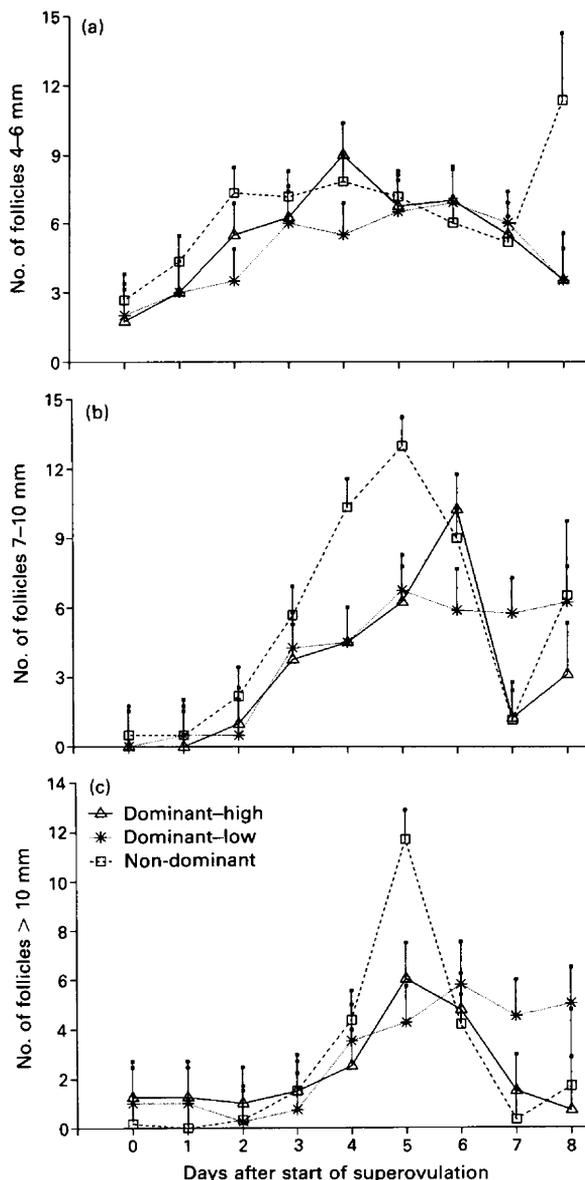


Fig. 3. Profiles of mean number of follicles 4–6 mm, 7–10 mm and > 10 mm after start of superovulation (Day 0) in heifers of the non-dominant (N = 6), dominant-high (N = 4) and dominant-low (N = 4) groups.

experiment provide evidence that the functional status of the dominant follicle in heifers of the dominant-low and dominant-high groups may have differed. Firstly, diameter of the F_2 follicle decreased constantly in the dominant-low group while it remained constant in the dominant-high group. Secondly, there was a parallel but delayed increase in the difference between the diameters of F_1 and F_2 follicles in the dominant-low group. This suggests that emergence of the F_1 follicle of the wave of follicular development was delayed by 1 or 2 days in the dominant-low group and that this follicle was functionally dominant. Hence, compared with the dominant-high group, initiation of

superovulation treatment within 3 days of emergence of the dominant follicle may have reduced the superovulatory response in the dominant–low group since, as reported by Lavoit & Fortune (1990), this newly growing F₁ follicle appears to be functionally dominant during this phase of development. On an individual basis (Fig. 1), emergence of the dominant F₁ follicle within 3 days of the start of treatment occurred in all heifers of the dominant–low groups but only in one (Heifer 703) of 4 heifers of the dominant–high group.

A two-step process involving recruitment of follicles and selection (or dominance) of one of these follicles over the others has been identified in ewes (Driancourt, 1987) and primates (Goodman & Hodgen, 1983) and has also been proposed for the control of preovulatory follicular growth in cattle (Ireland, 1987). It is likely that, during superovulation, the mechanisms by which one follicle becomes dominant are subverted while those involved in the process of follicular recruitment are still functional. During superovulation treatment in the present experiment, the entry of follicles in the pool of follicles 7–10 mm and > 10 mm occurred sooner and was of higher magnitude in heifers of the non-dominant than of the dominant (high- or low-response) groups. Similarly, in other studies, delayed and smaller entry of follicles into the pool of larger follicles (> 5–7 mm) occurs in superovulated cows that had a weak superovulatory response (Driancourt *et al.*, 1988), in those that had large follicles in the ovaries at the start of treatment (Grasso *et al.*, 1989) and in those that were superovulated after selection of the ovulatory follicle (Pierson & Ginther, 1988). Furthermore, administration of bovine follicular fluid which mimics the presence of large follicles, before start of a superovulatory treatment decreases the superovulatory response, delays the ovulatory LH and FSH surges and the post-ovulatory increase in progesterone concentrations (Lussier & Carruthers, 1989). Collectively, these results suggest that, in the absence of a dominant follicle, recruitment and passage of follicles from smaller (< 7 mm) to larger (> 7 mm) size classes during superovulation treatment may be facilitated. The suppressive action of large follicles on follicular recruitment may be mediated by a negative feedback at the pituitary level, decreasing FSH release, and/or by direct action of intraovarian factors at the ovarian level (Lussier *et al.*, 1988; Lussier & Carruthers, 1989; Grasso *et al.*, 1989; Guilbault *et al.*, 1989).

During superovulation, the similar increases in number of follicles 7–10 mm and > 10 mm in the dominant–low and in the dominant–high groups contrasts with their striking differences in ovulation rate. Compared with the rapid disappearance of follicles 7–10 mm and > 10 mm after prostaglandin-induced luteolysis in the dominant–high group, maintenance of unovulated follicles of this size in the dominant–low group suggests that ovulation did not proceed as efficiently in the latter group. Driancourt *et al.* (1988) observed that up to 50% of the large follicles may not ovulate following superovulation in cattle. In response to superovulation, more than 80% of the ovulations occurs over a 2-day-period and it has been suggested that the remaining ovulations represent follicles which were less mature or unable to respond normally to the LH surge (Yadav *et al.*, 1986; Pierson & Ginther, 1988). It is therefore possible that follicular maturation may have been altered in the dominant–low group and that ovulation did not proceed normally. In summary, the present results suggest that the presence of a dominant follicle before superovulation decreased recruitment of follicles during treatment and the number of ovulations after treatment. This was particularly evident when emergence of the dominant F₁ follicle in the follicular wave occurred within 3 days of the start of treatment. Characteristics of functional dominance and its effect on superovulatory responses need to be evaluated further in cattle.

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