Influence of the Dominant Follicle on the Superovulatory Response in Cattle

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ABSTRACT : Nine cows were superovulated by administration of 8 injections of Folltropin each (2.5 ml/injection, 1.75 mg/ml) i.m spread over 4 days, beginning on Day 10 of oestrous cycle, and 30 and 20 mg prostaglandin $F_{2\alpha}$ was given along with the 5th and 6th injections of Folltropin, respectively, to induce luteolysis. The animals were artificially inseminated 48, 60 and 72 h after the first prostaglandin $F_{2\alpha}$ injection. The number of corpora lutea was recorded by palpation per rectum and by ultrasonography on Day 6 (Day 0 = day of oestrus). The ovaries were examined daily by ultrasonography on Days 3-9 of the oestrous cycle for following the growth and regression of the largest follicle, which was considered the morphologically dominant follicle. The animals were classified into two groups depending upon the presence (n = 4) and absence of a dominant follicle (n = 5). There was a high correlation (r = 0.97, $p < 0.001$) between the number of corpora lutea observed by palpation per rectum and that determined by ultrasonography. Mean (±SEM) number of corpora lutea determined by ultrasonography (11.20 ± 3.71 vs 3.25 ± 0.75) and by palpation per rectum (10.40 ± 3.91 vs 2.25 ± 0.75) was significantly higher ($p < 0.05$) in the nondominant group compared to that in the dominant group. There was no difference in the numbers of follicles 2-3 mm (13.80 ± 4.49 vs 8.00 ± 1.08), 4-6 mm (7.00 ± 1.87 vs 3.50 ± 1.33), and the total number of follicles ≥ 2 mm (22.00 ± 5.95 vs 12.50 ± 1.26) between the two groups, one day prior to initiation of superovulation. There was, however, a significant ($p<0.01$) positive correlation between the number of corpora lutea with the numbers of follicles 2-3 mm (r = 0.83), 4-6 mm (r = 0.80) and the total number of follicles ≥ 2 mm (r = 0.89) observed one day prior to initiation of superovulation. The results of this study indicate that the presence of a dominant follicle adversely affects the superovulatory response in cattle.
(**Key Words:** Cattle, Dominant Follicle, Superovulation, Ultrasonography)

INTRODUCTION

Large variability and unpredictability in the superovulatory responses has been a major limiting factor in the embryo transfer programmes (Mapletoft and Pierson, 1993). This variability has been reported to be due to both extrinsic factors viz. FSH preparation, mode of administration, diet etc. (Murphy et al., 1984; Lerner et et., 1986; Murphy et al., 1991) and intrinsic factors viz. ovarian status (Monniaux et al., 1983). Use of real-time ultrasonography has shown that in cattle follicular development occurs in two or three waves during oestrous cycle. Each wave of follicular growth is characterized by the synchronous development of a cohort of follicles, followed by selection of a dominant follicle and subsequent regression of subordinate follicles. The first wave begins at the start of the oestrous cycle and is marked by development of a dominant nonovulatory follicle. The second wave begins at midcycle and in an oestrous cycle with two waves, culminates in the development of a dominant ovulatory follicle (Savio et al., 1988; Sirois and Fortune, 1988). Presence of the dominant follicle of the first wave, which can be detected from Day 3 onwards (Day 0 = day of oestrus) at the time of initiation of superovulation has been reported to adversely affect superovulatory response in some studies (Guilbault et al., 1991; Huhtinen et al., 1992; Bungartz and Niemann, 1994; Taneja et al., 1995), whereas others have found no effect of the presence of a large follicle, ≥ 10 mm in diameter and termed as morphologically dominant follicle (Wilson et al., 1990; Staigmiller et al., 1995). The objec-tive of the present study was to examine the effect of presence of a dominant follicle on superovulatory response in cattle.

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MATERIALS AND METHODS

Animals and treatments

Nine sexually mature, normally cycling and multiparous dairy cows between 8 and 10 years of age were used in the experiment. The animals were maintained under general herd managemental conditions in the National Dairy Research Institute animal herd. Oestrous cycles were synchronized by treatment with prostaglandin $F_{2\alpha}$ (25 mg Lutalyse, Upjohn, Belgium). For induction of superovulation, the animals were administered i.m Folltropin, a porcine pituitary follitropin extract with low LH:FSH ratio (Vetrepharm Inc., London, Ontario, Canada) in 8 injections of 2.5 ml each (1.75 mg/ml) spread over 4 days, beginning on Day 10 of oestrous cycle (Day 0 = day of oestrus). For luteolysis, the animals were administered 50 mg of Lutalyse, i.m in two divided doses of 30 and 20 mg with the 5th and 6th injections of Folltropin, respectively. The animals were artificially inseminated 48, 60 and 72 h after the first injection of PGF. The number of corpora lutea (CL) was determined by palpation per rectum and by ultrasonography on Day 6 (Day 0 = day of oestrus).

Ultrasonography and experimental design

Ovarian follicular changes were monitored with a real-time B-mode ultrasound scanner (Tokyo Keiki. LS-1000) equipped with a linear array 5 MHz transducer designed for transrectal placement. The animals were restrained by making them stand in an animal crate, without use of any chemical method for restraining the animals. The transducer was inserted after evacuating the rectum and ultrasonography was performed as described earlier (Manik et al., 1994). Observations on the number of follicles and their sizes were recorded daily on videotape starting on Day 3 of the oestrous cycle up to one day prior to initiation of superovulation. Observations recorded daily were compared with those of the previous day in order to monitor the growth and development of individual follicles. The antra of follicles $\geq$ 10 mm were measured with the built-in callipers after freezing the ultrasound image, whereas the diameters of smaller follicles were measured against the in-built centimeter scale displayed on the screen alongside the ultrasound image. This was done to minimize the errors during freezing of image. The diameter of nonspherical follicles was calculated by taking the average of the longest and widest measured points of the follicle. The follicles were categorized on the basis of their diameter as 2-3 mm, 4-6 mm, 7-9 mm and $\geq$ 10 mm.

Based on the observations from Day 5 to Day 9, animals were retrospectively assigned to either of the two groups according to the presence (dominant group n = 4) or absence of a dominant follicle (nondominant group n = 5). The criteria for identification of a dominant follicle were based on those defined by Grasso et al. (1989) and Guilbault et al. (1991). Briefly, dominant follicles had 1) a diameter of > 9 mm and 2) were in a growing phase or were stable for < 4 days. Nondominant follicles had 1) a diameter of < 10 mm or 2) were in a regressing phase or were stable for at least 4 days.

Statistical analyses

Data on the numbers of follicles of various size categories and the number of CL determined by ultrasonography and palpation per rectum were compared between the dominant and nondominant groups by Harvey's least square analysis (Harvey, 1976) after log transformation. Correlation analysis was carried out to correlate the numbers of follicles of various size categories one day prior to initiation of superovulation with the number of CL, and the numbers of CL determined by ultrasonography with that observed with palpation per rectum.

RESULTS

The profiles of growth and regression of the largest follicle before initiation of superovulation in individual animals are shown in figure 1. Based on the criteria defined for follicular dominance, detailed in 'Materials and Methods' the dominant follicle of cows in the dominant group was > 9 mm and in a growing phase (Animals 1044, 910 and 3147) or stable for < 4 days (Animal 1036). In the nondominant group, no follicle > 9 mm was present at the initiation of superovulation (Animals 1256, 144, 1313 and 1046) or the dominant follicle was stable for at least 4 days (Animal 4479). The number of CL determined by palpation per rectum and by ultrasonography on Day 6 (Day 0 = day of oestrus) was used as a measure of the magnitude of superovulation response. There was a high correlation ($r = 0.97$, $p < 0.001$) between the number of CL observed by palpation per rectum and that determined by ultrasonography. The number of CL was significantly higher ($p < 0.05$) in the nondominant group compared to that in the dominant group, following use of either method for determination of the number of CL (figure 2). There was no difference in the numbers of follicles 2-3 mm, 4-6 mm and the total number of follicles $\geq$ 2 mm in the two groups, one day prior to initiation of superovulation (figure 3). There was, however, a significantly ($p < 0.01$) positive correlation

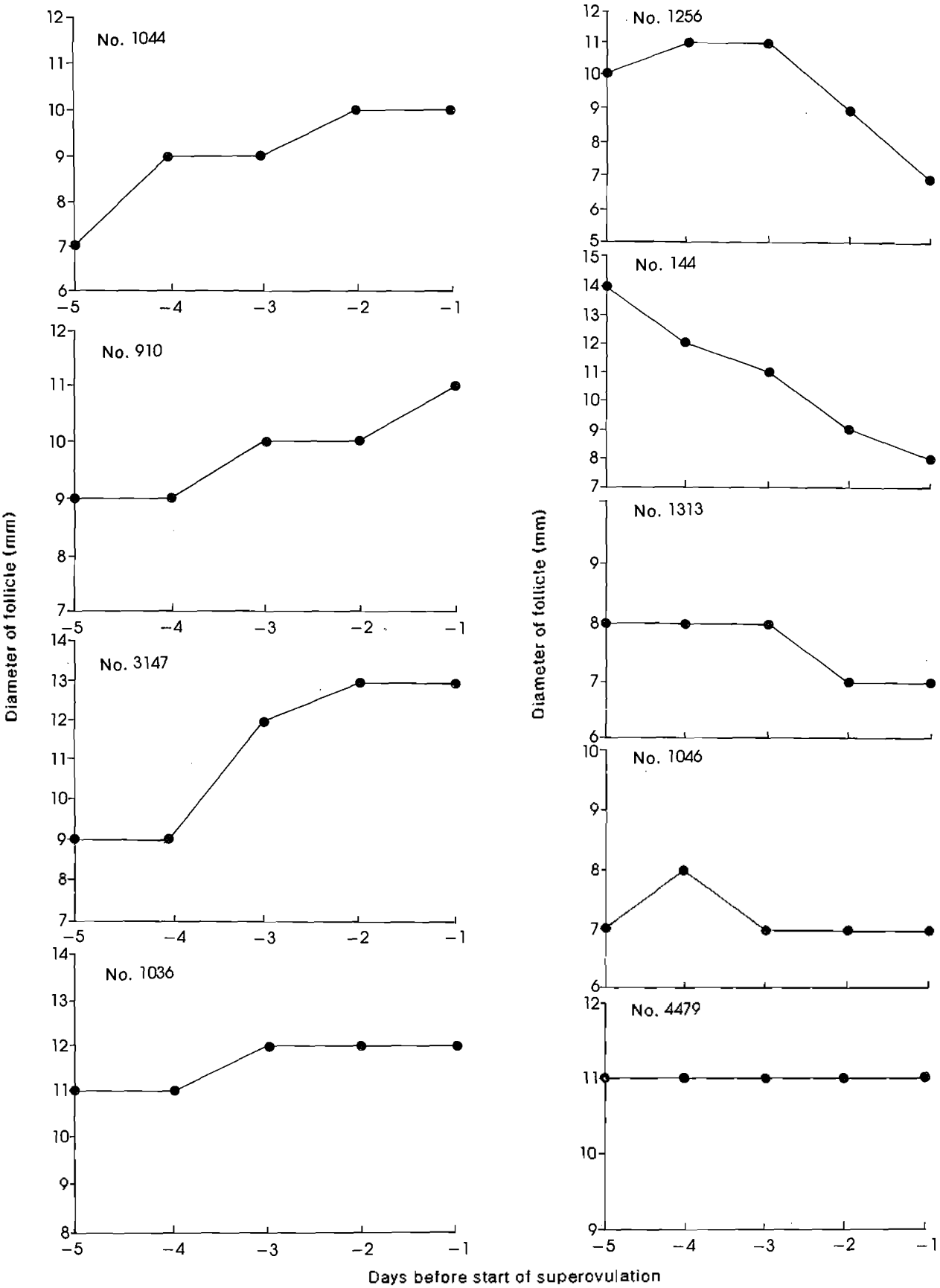

Figure 1. Pattern of development of largest follicle before start of superovulation (Day 0) in individual cows assigned to dominant (Animal Nos. 1044, 910, 3147 and 1036) and nondominant group (Animal Nos. 1256, 144, 1313, 1046 and 4479).

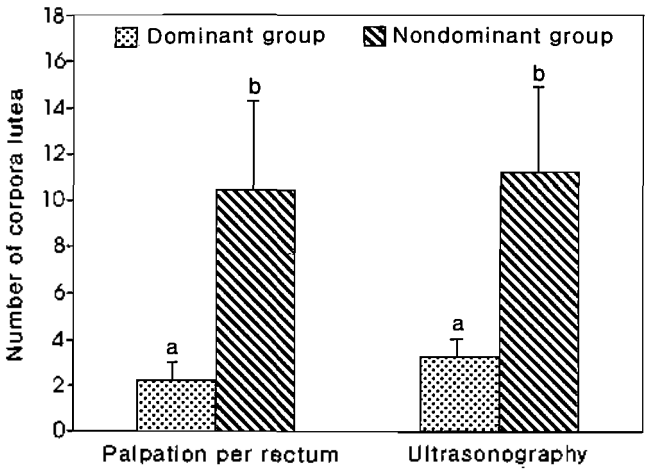

Figure 2. Mean (± SEM) number of corpora lutea determined by palpation per rectum and ultrasonography in cows with or without a dominant follicle (a, b differ significantly $p < 0.05$).

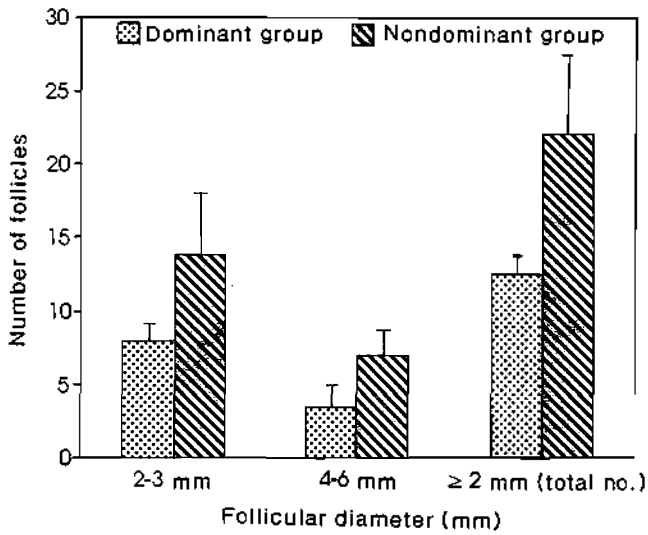

Figure 3. Mean (± SEM) number of follicles of various size categories one day prior to initiation of superovulation in cows with or without a dominant follicle.

between the number of CL and the numbers of follicles 2-3 mm ($r = 0.83$), 4-6 mm ($r = 0.80$) and the total number of follicles ≥ 2 mm ($r = 0.89$) observed one day prior to initiation of superovulation.

DISCUSSION

The results of the present study indicate that the superovulation respone was higher when superovulatory treatment was initiated in the absence of a dominant follicle in cattle. This is in agreement with earlier reports in cattle (Grasso et al., 1989; Guilbault et al., 1991; Bungartz and Niemann, 1994) and buffalo (Taneja et al., 1995). In our study, as well as in most of these studies the dominant follicle was identified by daily monitoring of growth and regression profiles of the largest follicle for at least 5 days prior to the initiation of superovulation. In their attempt to determine if a single examination of the ovaries by ultrasound could detect a large follicle, which they termed as morphologically dominant follicle capable of influencing the response to superstimulation treatment, Staigmiller et al. (1995) found no evidence that this follicle did indeed alter the ovulation rate or the number of transferable embryos. Wilson et al. (1990) obtained similar results. Gray et al. (1992) also did not find any effect of the presence of a large follicle that had started to regress on the number of embryos collected. One possible reason for this discrepancy could be the differences in the functional status of the dominant follicle at the time of initiation of superovulation. The dominant follicle of the first wave has a growing phase marked by active period of growth, a static phase when its size remains nearly constant and a regressing phase marked by a reduction in size (Ginther et al., 1989). Earlier studies based on ultrasonography indicated that the morphological and functional dominance coincide when the dominant follicle of the first wave is actively growing but such congruence is lost at the end of the growth phase (Lavoir and Fortune, 1990). Use of both ultrasound analysis and ratios of oestradiol/progesterone concentra-tions has confirmed that there are two different periods of growth of multiple follicles ≥5 mm in diameter, the period between Days 1-3, which is the selection phase for development of the early dioestrus dominant nonovulatory follicle and the period between Days 10-12, which is not only the selection phase for development of the next dominant follicle, but also the period when the first dominant follicle ceases to function (loses dominance), becomes oestrogen-inactive and begins to undergo atresia (Sunderland et al., 1994). Days 4-9 mark the period of functional dominance corresponding with the presence of a single large (≥ 10 mm) oestrogen-active follicle that has greater concentrations of oestradiol and ratio of oestradiol/progesterone than those for other co-existing follicles (Sunderland et al., 1994). Therefore, any single observation recorded on or after Day 9 indicating the presence of a large ≥ 10 mm follicle does not account for the functional status of the follicle. As this follicle may have lost its functional dominance, though it may still be the largest follicle, it may not be able to effect a significant adverse influence on superovulatory response.

The mechanism by which a functional dominant follicle influences superovulatory response may involve

suppression of FSH. The concentrations of oestradiol and inhibin, both of which suppress FSH secretion have been reported to increase as the dominant nonovulatory follicle increases in size (Martin et al., 1991). A rise in FSH occurs 2-4 days prior to the emergence of a new wave of follicular development (Adams et al., 1992; Sunderland et al., 1994). Any suppresion of this rise may adversely influence the recruitment of follicles for the next wave. Evidence for this hypothesis has been provided by studies in which removal of the inhibitory influence of the dominant follicle by ovariectomy on Days 3, 5 and 8 of oestrous cycle was found to increase FSH concentrations and the number of small follicles, and advance the emergence of new dominant follicle (Adams et al., 1992). Additionally, administration of bovine follicular fluid which delays the rise in FSH has been found to delay the emergence of a new dominant follicle (Adams et al., 1992).

Whereas the numbers of follicles 2-3 and 4-6 mm at the initiation of superovulation were found to be similar in the dominant and nondominant groups in the present study, as also reported earlier in some studies (Grasso et al., 1989; Rouillier et al., 1990; Guilbault et al., 1991), others have observed the number of small follicles to be lower in the presence of a dominant follicle (Taneja et al., 1995). Bungartz and Niemann (1994) found the number of 3-8 mm follicles at the initiation of superovulation to be highly dependent upon the presence or absence of a dominant follicle. Rouillier et al. (1990) observed that the functional status and the ability of small follicles to respond to superovulation was adversely affected by the presence of dominant follicle.

The magnitude of superovulatory response was found to be dependent upon the number of small follicles (2-3 and 4-6 mm) available for recruitment at the time of initiation of superovulation in the present study, which is in agreement with earlier reports (Monniaux et al., 1983; Romero et al., 1991). A reduction in the number of recruitable follicles in the dominant group could, therefore, be a reason for the reduced superovulatory response in this group.

In conclusion, the results of this study indicate that the presence of a dominant follicle adversely affects the superovulatory response in cattle.

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