Ovarian Follicular Populations Prior to and during Superovulation in Cattle : Relationship with Superovulatory Response

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ABSTRACT : The present study examined the follicular populations prior to and during superovulation and investigated their relationship with superovulatory response in crossbred cattle. Eleven animals were administered i.m. 8 doses of Folltropin of 2.5 ml each (1.75 mg/ml) spread over 4 days beginning on Day 10 of oestrous cycle, and 30 and 20 mg Lutalyse was given alongwith the 5th and 6th injections of Folltropin, respectively, to induce luteolysis. The animals were artificially inseminated 48, 60 and 72 h after the first Lutalyse injection. The number of corpora lutea (CL) was recorded by palpation per rectum and embryos were recovered non-surgically on Day 6 (Day 0 = day of superoestrus). The ovarian follicular population was examined by transrectal ultrasonography 15 h prior to and 52 h after the first FSH injection, and then on the day of superoestrus and the day of flushing. The follicles were classfied on the basis of diameter as small (3-5 mm), medium (6-9 mm) and large ($\geq$ 10 mm). The total number of follicles increased significantly ($p < 0.01$) from 2.45 ± 0.35, 15 h prior to the first FSH injection to 8.09 ± 1.12, 52 h after the first FSH injection and then further to 13.27 ± 1.89 on the day of superoestrus. A positive correlation was observed between the number of small follicles 15 h prior to the first FSH injection ($r = 0.60$, $p < 0.05$), the number of large follicles 52 h after the first FSH injection ($r=0.59$, $p < 0.05$) and the number of CL. The follicular population prior to and during superovulation was, however, not significantly different between high (> 6 CL) and low responders ($\leq$ 6 CL). The present study suggests that the follicular populations undergo dynamic changes during superovulation and that follicular populations prior to superovulation have a limited application as an indicator of the superovulatory response.
(**Key Words**: Cattle, Superovulation, Ultrasonography, FSH, Embryo)

INTRODUCTION

The procedure commonly employed for ovarian superstimulation in cattle involves exogenous administration of gonadotropin during the mid-luteal phase of the oestrous cycle for stimulation of growth and subsequent ovulation of competent follicles, followed by treatment with prostaglandin for induction of regression of corpus luteum. However, variability and unpredictability in the superovulatory responses has remained a major limiting factor in embryo transfer programmes (Armstrong, 1993; Mapletoft and Pierson, 1993). The factors influencing this variability, which have been the focus of attention included breed differences and variations among individual animals, level of nutrition, season and age (Seidel et al., 1978; Bindon et al., 1980; Lerner et al., 1986; Brown et al., 1991; Murphy et al., 1991). However, in a major breakthrough in understanding the reasons for this variability, it was found that 70% of the variation in exogenous gonadotropin-induced superovulation response in cattle was due to differences in antral follicular populations (Monniaux et al., 1983). Use of real-time ultrasonography of the ovaries has enabled the study of ovarian follicular dynamics during superovulation (Grasso et al., 1989; Driancourt et al., 1991; Guilbault et al., 1991; Purwantara et al., 1993; Adams et al., 1994). A major determinant of the level of superovulatory response has been found to be the ovarian status of the animal at the time of initiation of superovulation. In addition to the effect of stage of oestrous cycle at the time of hormone treatment (Lindsell et al., 1986; Goulding et al., 1990; Macmillan et al., 1994), it has been found that the number, size, distribution and condition of antral follicles at the start of superovulatory treatment influences the subsequent superovulatory responses (Grasso et al., 1989;

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Received October 23, 1997; Accepted March 30, 1998

Romero et al., 1991). The objective of the present study was to examine the follicular populations prior to and during superovulation and investigate their relationship with superovulatory response in crossbred cattle.

MATERIALS AND METHODS

Animals and treatments

Eleven sexually mature, normally cycling, multiparous, nonlactating crossbred cows between 4.5 and 5.5 years of age, and weighing between 350 and 400 kg were used in the experiment. The animals were maintained under general herd managemental conditions in the National Dairy Research Institute, Karnal animal herd. For induction of superovulation the animals were administered i.m. 8 doses of Folltropin (Vetrepharm Inc., London Ontario, Canada) of 2.5 ml each (1.75 mg/ml) spread over 4 days beginning on Day 10 of oestrous cycle. For luteolysis, the animals were administered 50 mg of prostaglandin $F_2\alpha$ (PGF, Lutalyse, Upjohn, Belgium) 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. Embryos were recovered by flushing the animals non-surgically on Day 6 (Day 0 = day of superoestrus) with two-way Rusch Cathether. The number of corpora lutea (CL) was determined by palpation per rectum on the day of flushing.

Ultrasonography

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 methods 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 both for the left and right ovary 15 h prior to the first FSH injection, 52 h after the first FSH injection, on the day of superoestrus and on the day of flushing. The antra of follicles $\geq$ 10 mm were measured with the built-in callipers after freezing the ultrasound image, whereas the diameter of smaller follicles was 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 characterized as small (3-5 mm diameter), medium (6-9 diameter) and large ($\geq$ 10 mm diameter).

Statistical analyses

The data were log-transformed prior to analysis. In terms of the level of superovulatory response the animals were divided into high responders (> 6 CL) and low responders ($\leq$ 6 CL). The effects of the level of response, the day of treatment and follicular size were analysed by three-way analysis of variance (ANOVA) using Duncan's Multiple Range Test (DMRT). Correlation analysis was carried out to find out the relationship between the follicular populations on different days of experiment with the number of palpable CL. The differences in the numbers of CL palpated and embryos recovered between high and low responders were compared by Harvey' least square analysis (Harvey, 1976).

RESULTS

The follicular populations in terms of the mean ($\pm$ SEM) numbers of follicles of different size categories and the total number of follicles on different days of experiment in the left and right ovaries are presented in figure 1. As the number of small, medium and large follicles did not differ significantly between left and right ovaries, data from both the ovaries were pooled prior to further analysis. Following the initiation of superovulation, there was an increase in the number of follicles of all size categories resulting in a significant ($p < 0.01$) increase in the total number of follicles from 2.45 $\pm$ 0.35, 15 h prior to the first FSH injection to 8.09 $\pm$ 1.12, 52 h after the first FSH injection. The total number of follicles then rose further to 13.27 $\pm$ 1.89 on the day of superoestrus, after which it declined significantly ($p < 0.01$) to 5.27 $\pm$ 1.44 on the day of flushing.

The number of CL on the day of flushing, which was used as a measure of the level of superovulatory response was positively correlated ($r = 0.60$, $p < 0.05$) with the number of small follicles 15 h prior to the first FSH injection and the total number of follicles 52 h after the first FSH injection ($r = 0.59$, $p < 0.05$). The number of large follicles on the day of flushing was not correlated with the follicular population 15 h prior to and 52 h after the first FSH injection, and the number of large follicles on the day of superoestrus. The number of CL (12.00 $\pm$ 1.92, range 7-17) and embryos recovered (9.20 $\pm$ 1.50, range 5-13) were significantly higher ($p < 0.01$) in animals classified as high responders than in those classified as low responders (CL 3.50 $\pm$ 0.67, range 2-6, embryos recovered 1.17 $\pm$ 0.54, range 0-3). There was, however, no difference in the numbers of small, medium

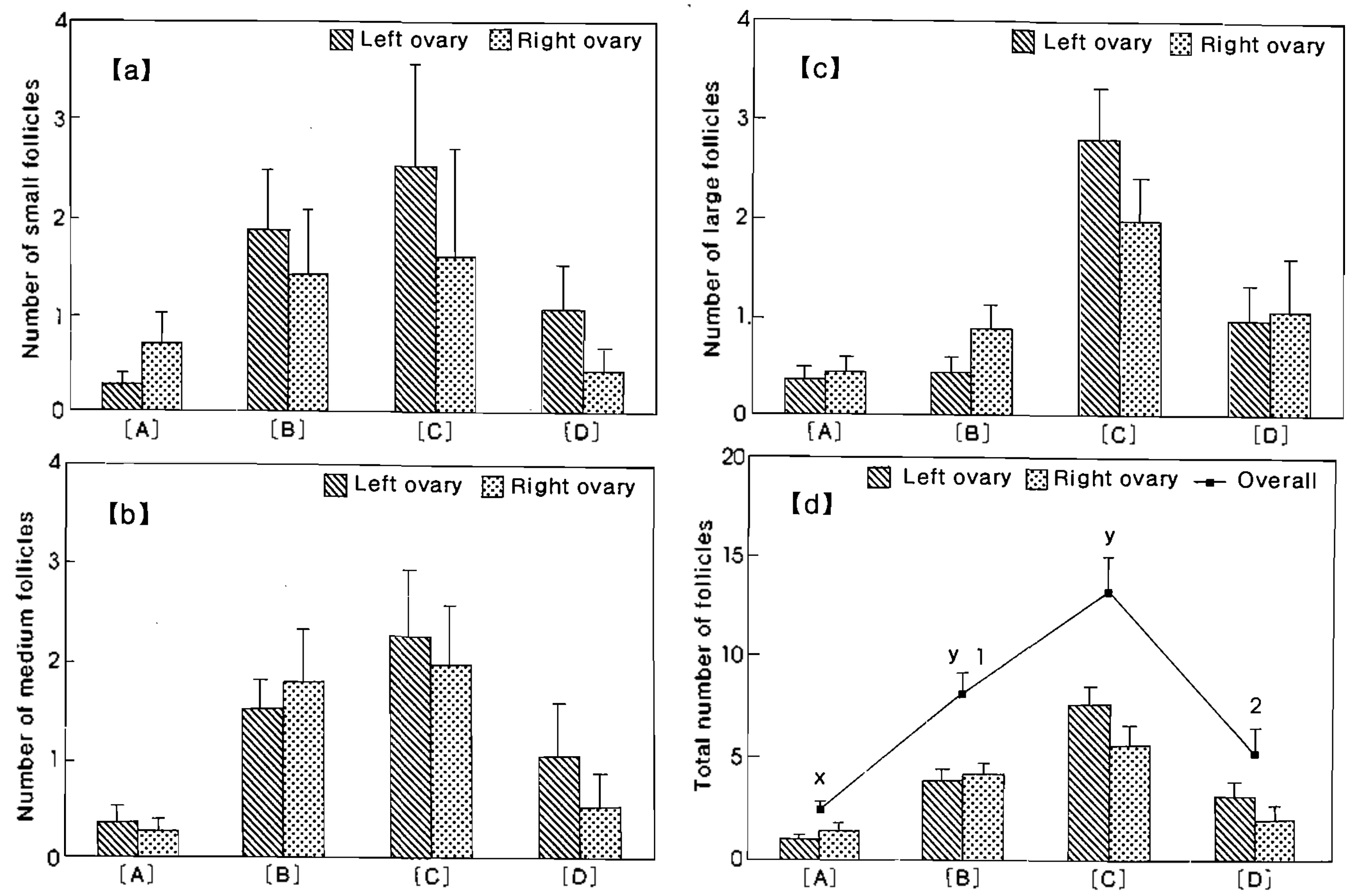

Figure 1. Mean (± SEM) number of small [a], medium [b] and large [c] follicles, and total number of follicles [d] in left and right ovaries, 15 h prior to (A) and 52 h after first FSH injection (B), on the day of oestrus (C) and on the day of flushing (D). x,y $p < 0.01$; 1,2 $p < 0.05$.

and large follicles, and the total number of follicles between the two groups 15 h prior to the first FSH injection (figure 2).

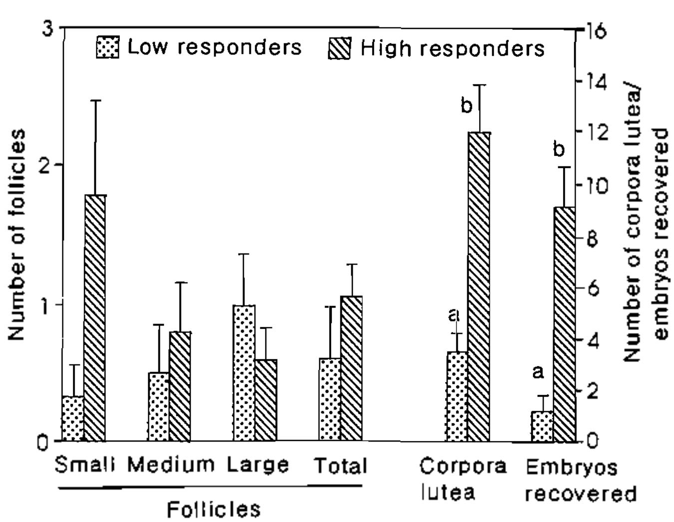

Figure 2. Mean (± SEM) number of follicles of different size categories 15 h prior to initiation of superovulation, and number of corpora lutea (CL) and embryos recovered among animals categorized as high (> 6 CL) and low responders (≤ 6 CL). a,b $p < 0.01$.

DISCUSSION

Our results of a lack of any significant difference between left and right ovaries prior to and during superovulation are in agreement with those of earlier reports (Purwantara et al., 1993). An increase in the number of follicles of different size categories as well as the total number of follicles from 15 h prior to the first FSH injection through the day of superoestrus and a sharp decline thereafter was as expected and is in agreement with earlier reports (Grasso et al., 1989; Purwantara et al., 1993; Desaulniers et al., 1995).

We observed a positive relationship between the number of small follicles 15 h prior to initiation of superovulation and the superovulatory response. Romero et al. (1991) also obtained a positive correlation between the number of follicles 3-6 mm in diameter at the start of superovulation and the number of embryos recovered. Others have also obtained a high correlation between the number of follicles < 5 mm in diameter and superovulatory response (Van der Schans et al., 1991). The number of small follicles and the total number of follicles, but not the numbers of medium and large follicles have been reported to be higher in responders (>2 CL) than in nonresponder (≤ 2 CL) superovulated cattle (Purwantara et al., 1993). However, a comparison of the follicular population at the start of superovulation, between high ($>$ 6 CL) and low responders (≤ 6 CL) did not provide a significant difference between the two groups in the present study. Purwantara et al. (1993) also observed that although differences in follicular populations existed between responders and nonresponders, these differences were not found for high (>20 CL), moderate (11-20 CL) and low responders (2-10 CL). This discrepancy can be explained in terms of the different mechanisms of action of gonadotropins. Administration of exogenous gonadotropins triggers follicle recruitment from follicles smaller in size than those in case of endogenous levels of gonadotropins. It has been reported that all healthy follicles >1.7 mm in diameter are mobilized following exogenous gonadotropin administration. These follicles, however, cannot be recorded by ultrasonography. Variations among individual animals in the population of follicles <2 mm in diameter could, therefore, be a major determinant to variability in superovulatory responses.

Exogenous administration of gonadotropin has been reported to reduce or prevent atresia among the follicles recruited by its administration, although it is probable that only very early stages of atresia may be reversed by FSH (McNatty et al., 1982; Monniaux et al., 1983). It is likely that some of the follicles recorded at the initiation of superovulation may be atretic and, therefore, may regress and not ovulate. In addition, some atretic follicles may luteinize rather than grow further, after rescue from atresia by exogenous gonadotropin (Purwantara et al., 1993). These reasons could also adversely affect the accuracy of correlating follicular status at the initiation of FSH treatment with subsequent ovulation rate.

In conclusion, the results of the present study suggest that the follicular populations undergo dynamic changes during superovulation and that follicular populations prior to superovulation can be used as indicators of superovulatory response only to a limited extent.

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