Comparative Follicular Dynamics in Superovulated Crossbred Cows and Water Buffaloes

R. S. Manik[1], S. K. Singla[1] and M. L. Madan[2]
Embryo Biotechnology Centre, National Dairy Research Institute
Karnal-132 001, India

ABSTRACT : To understand the causes for poor response to superovulation in water buffalo compared to crossbred cows, follicular events, before start of superovulation, during superovulation and after superovulation were compared. Follicular development was monitored a day before start of superovulation, daily upto superestrus and on the day of flushing. A real time B mode diagnostic instrument equipped with a linear array, 5 MHz transducer was used in five crossbred cows and five Murrah buffaloes. Crossbred cows yielded significantly ($p < 0.01$) higher number of corpora lutea than buffaloes (21 vs 10). The mean number of small size (2 to 5 mm); medium size (6 to 9 mm) and large size ($\geq$ 10 mm) follicles, a day before start of superovulation were almost similar or even slightly higher in buffalo. Though initial shift in the mean number of follicles was higher in buffalo than cow, yet, from Day 2 to Day 3 of the treatment, the average increase in medium (3.2 vs 1.2) and large size (5.0 vs 2.0) follicles was higher in cows than buffaloes. The mean number of medium and large size follicles was 9.8 and 14.4 in cows and 6.4 and 7.6 in buffaloes. On the day of flushing, the number of large size follicle was more in buffaloes than cows, indicating the ovulation problem in this species. The major conclusion from this investigation was that, a day before start of superovulatory treatment, the number of small and medium size follicles was slightly higher in buffaloes, even then superovulatory response was better in cows, due to shift, recruitment and passage of follicles from smaller size to larger size from Day 2 of treatment. Ovulation problem in buffaloes was also responsible for lower superovulatory responses as revealed by the presence of higher number of large size follicles on the day of flushing.

(**Key words** : Superovulation, Cows, Buffaloes, Follicles, Ovulation)

INTRODUCTION

Unpredictable response to superovulation by administration of exogenous hormones limits widespread adoption and exploitation of full potential of embryo production in cattle. The variability may be influenced by breed and individual animal factors (Betteridge, 1977; Crister et al., 1980; Bindon et al., 1986), the level of nutrition (Dunn, 1980), season and lactational stage (Shea et al., 1984; Brown et al., 1995), age (Hasler et al., 1981; Lerner et al., 1986). The use of gonadotrophins such as follicle stimulating hormone (FSH) from batches with different biological activities result in unpredictability in the production of number of good quality embryos (Newcomb et al., 1979; Goulding et al., 1991). Superovulatory treatment starting between Day 9 and 12 of the oestrous cycle results in more number of viable embryos recovered compared with regimes which start at Days 3, 6 or 13 (Lindsell et al., 1985; Boland et al., 1991). These effects are due to differences among follicular population at different stages of oestrus cycle.

However, in buffalo the superovulatory responses are so low that the results of multiple ovulation and embryo transfer (MOET) are being compared with single ovulation and embryo transfer (SOET) (Singla and Madan, 1990), besides repeatability. More understanding of follicular dynamics is, therefore, required before, during and after superovulation to improve upon existing responses. Real time ultrasound imaging of the ovaries holds promise not only to understand sources of individual animal variability but even between species too. The present experiment was designed to understand the causes of low superovulatory responses in buffaloes by comparing follicular events before, during and after superovulation both in crossbred cows and water

[1] Address reprint requests to R. S. Manik.
[2] D.D.G. (A.S.), Indian Council of Agricultural Research, New Delhi, India.

Received May 21, 1997; Accepted July 28, 1997

buffaloes.

MATERIALS AND METHODS

Animals

A total of 10 (5 Crossbred cows + 5 Murrah buffaloes (*Bubalus bubalis*) multiparous, non lactating animals between 8 to 10 years of age and weighing 450 to 550 kg were used. The crossbred cows were "Karan Swiss" i.e. cross between Brown Swiss and Sahiwal (Zebu). These animals were kept in loose housing system and were maintained at the National Dairy Research Institute, Karnal, Haryana, India. They were kept under uniform nutritional and managemental conditions and were in good physical and reproductive health during the course of investigation. The animals which displayed at least two estrous cycle of normal duration (18 to 24 d) were used in the experiment. Standard management practice included visual inspection thrice a day and parading of vasectomized buffalo bull every four hours for detection of estrus in buffaloes.

Estrus synchronization and superovulation treatment

A total of 10 animals in each species i.e. crossbred cows and Murrah buffaloes were palpated per rectum for presence of corpora lutea. Seven animals each having a corpus luteum were injected with single dose (25 mg) of prostaglandin F_2 (PGF) (Lutalyse, Upjohn, Belgium). Five animals each, exhibiting behavioural and ovulatory estrus (confirmed by palpation and ultrasound monitoring) were included in the study. These animals were injected (i.m.) with one vial of ovagen containing two units of FSH (Immunochemical Products Limited, New Zealand) through eight divided injections spread over four days, beginning on Day 12 of oestrus cycle. For luteolysis, the animals were treated with lutalyse twice alongwith fifth (30 mg) and sixth (20 mg) FSH injections. They were artificially bred at 48, 60 and 72 h after the first injection of PGF. These animals were then flushed non-surgically on Day 6 using two-way Rusch cathetor.

Ultrasound examination schedule

Ultrasound examinations were performed using a real time B mode diagnostic instrument equipped with a linear array, 5 MHz transducer designed for transrectal placement. All the observations were carried out by the same person and using the same methodology as already described by our laboratory (Manik et al., 1994). The number of follicles and their size were recorded prior to start of superovulation and then once daily upto estrus, which will be hence called super estrus (SE) and finally on the day of flushing. The antra of follicles larger than 10 mm were measured with built in callipre after freezing the ultrasound image. The diameter of the most of the smaller follicles were estimated using the centimeter scale displayed on the sereen alongside the ultrasound image. The diameter of follicles which were not sphericle was estimated by averaging the longest and widest measured points of the follicles. The observations were also recorded on a video tape to review it subsequently and diagram of each ovary, depicting the relative location of follicles were made at the time of examination.

Statistical analysis

Follicle number in different size category were classified in three parts i) before start of superovulation, ii) during superovulation, iii) on the day of super eatrus for statistical analysis. Analysis of variance (ANOVA) was used to compare means (Snedecor and Cochran, 1967). Comparison of superovulatory responses in terms of number of corpora lutea and embryo recovered was performed by using 't' test.

RESULTS

Number of corpora lutea, ovulation rate and embryos recovered

There was significant difference ($p < 0.01$) in the mean number of corpora lutea (CL) in cows and buffaloes. In all there were 21 CL in cows and 10 CL in buffaloes. Out of five, in three buffaloes there was no corpus luteum, however, large and medium size follicles were present on the day of estrus in these animals (table 2). Seven CL's were present in buffalo No. 1 and three in buffalo No. 2, irrespective of more number of large and medium size follicles present on day of superestrus in buffalo No. 2. In all eight embryos were recovered, seven from animal No. 1 and one from animal No. 2, giving the recovery rate of 80 percent. Unlikely in buffaloes, all the cows had corpora lutea on the day of superestrus (table 1). Maximum of nine CL were in animal number 3 in which maximum number of medium and large size follicles were present. In cows the maximum increase in large size follicles was in animal No. 2, i.e., from 0 to 18 with an increasing trend in each animal. A total of fifteen embryos were recovered (table 1).

Follicular status before superovulatory treatment

The number of follicles according to their size in each animal are given in table 1 and 2 for crossbred cows and buffaloes respectively. All the three category of sizes did

Table 1. Classification of follicles according to size before, during and after superovulation treatment in crossbred cattle

Cow No.	Follicle size (mm)	No. of follicles before, during and after treatment						Corpus luteum	Embryo recovered
		−1	0	1	2	3	5		
	2-5	13	10	15	5	0	0	3	1
1	6-9	2	1	0	7	4	6		
	≥ 10	1	1	3	3	11	16		
	2-5	18	14	11	5	7	7	2	2
2	6-9	1	2	6	4	6	4		
	≥ 10	0	0	1	5	18	20		
	2-5	9	12	15	10	6	0	9	9
3	6-9	0	0	5	5	23	27		
	≥ 10	0	0	0	4	3	16		
	2-5	6	9	7	2	3	0	4	0
4	6-9	2	2	3	6	4	6		
	≥ 10	1	1	2	8	12	15		
	2-5	5	3	4	2	2	0	3	3
5	6-9	1	3	3	3	4	6		
	≥ 10	0	0	0	2	3	5		

Table 2. Classification of follicles according to size before, during and after superovulatory treatment in buffalo

Buffalo No.	Follicle size (mm)	No. of follicles before, during and after treatment						Corpus luteum	Embryo recovered
		−1	0	1	2	3	5		
	2-5	9	9	7	5	4	0	7	7
1	6-9	2	2	2	4	4	7		
	≥ 10	1	1	3	1	3	3		
	2-5	13	11	13	10	3	2	3	1
2	6-9	2	4	6	11	18	14		
	≥ 10	0	0	1	1	1	8		
	2-5	9	9	6	8	0	0	0	0
3	6-9	1	1	6	2	6	7		
	≥ 10	0	0	1	2	3	4		
	2-5	9	10	13	3	2	2	0	0
4	6-9	0	0	5	4	0	0		
	≥ 10	1	1	1	6	10	10		
	2-5	13	12	12	4	4	3	0	0
5	6-9	2	3	6	5	4	4		
	≥ 10	0	0	1	3	6	13		

not differ between these two species. The mean number of small size (2 to 5 mm) follicles were 10.6 vs 10.2, medium size (6 to 9 mm) 1.4 vs 1.2; large size (≥ 10 mm) 0.4 vs 0.4 in buffaloes and cows respectively (figure 1). The mean number (12.4 vs 11.8) of follicles irrespective of size was slightly higher in buffalo than cows. The one large size (≥ 10 mm) follicle was present both in buffalo and cow No. 1 and 4.

Follicular status during superovulation

The number of small size follicles decreased from first day of treatment (Day 0) to last day of treatment (Day 3) in each buffalo and cow (tables 1 and 2). The mean number of these follicles was 10.2 and 2.6 (figure 2). The corresponding values for cows were 9.6 and 3.6. In all the cows too, there was decreasing trend in small size follicles from first day of treatment to last day of

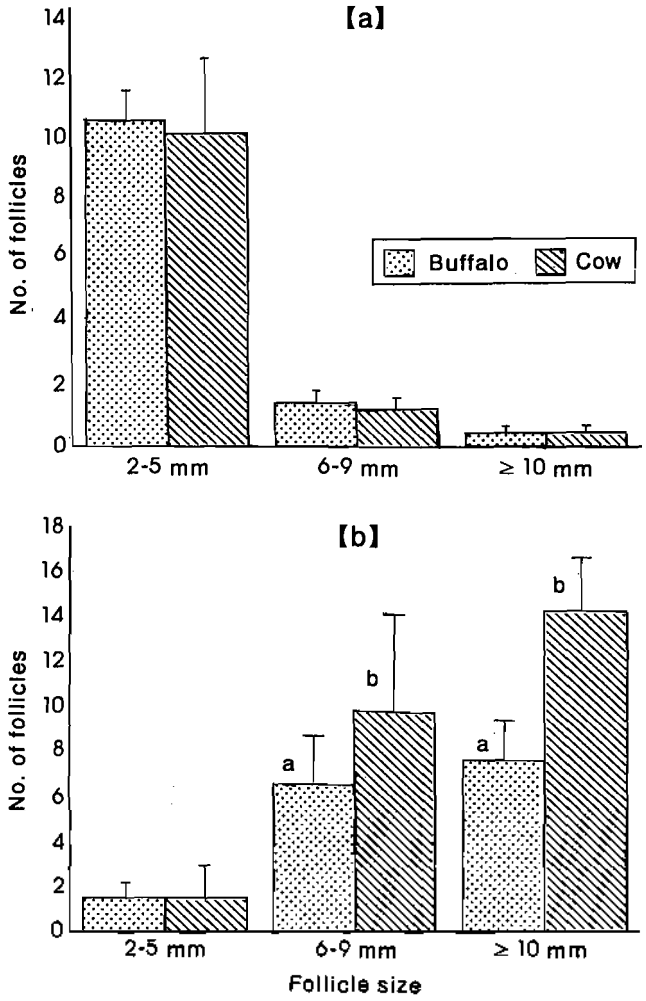

Figure 1. Profiles of the mean (± sem) number of follicles in cow and buffalo [a] a day before superovulation and [b] on day of superestrus. The bars with different superscripts differ ($p < 0.01$).

treatment similar to buffaloes. The mean number of medium size follicles increased from 2.0 to 6.4 in buffalo and 1.6 to 8.2 in cows from first day of treatment to last day of treatment (figure 2). These differences were statistically significant ($p < 0.01$). In buffalo No. 2 this increase was as high as from 4 to 18, on the other hand it remained zero in buffalo No. 4. In cows, the maximum increase was in animal No. 3 i.e. from 0 to 23 and the remaining animals also showed increasing trend (table 1). The mean number of large size follicles increased from 0.4 to 4.6 in buffaloes and 0.4 to 9.4 in cows from first day of treatment to last day of treatment ($p < 0.01$). The animal No. 4 where there was no medium size follicle had a maximum increase in large size follicles from 1 to 10 and animal No. 2 this increase was minimum i.e. from 0 to 1. However, in cows the maximum increase was in animal No. 2 (from 0 to 18) and minimum in animal No. 3 and 5.

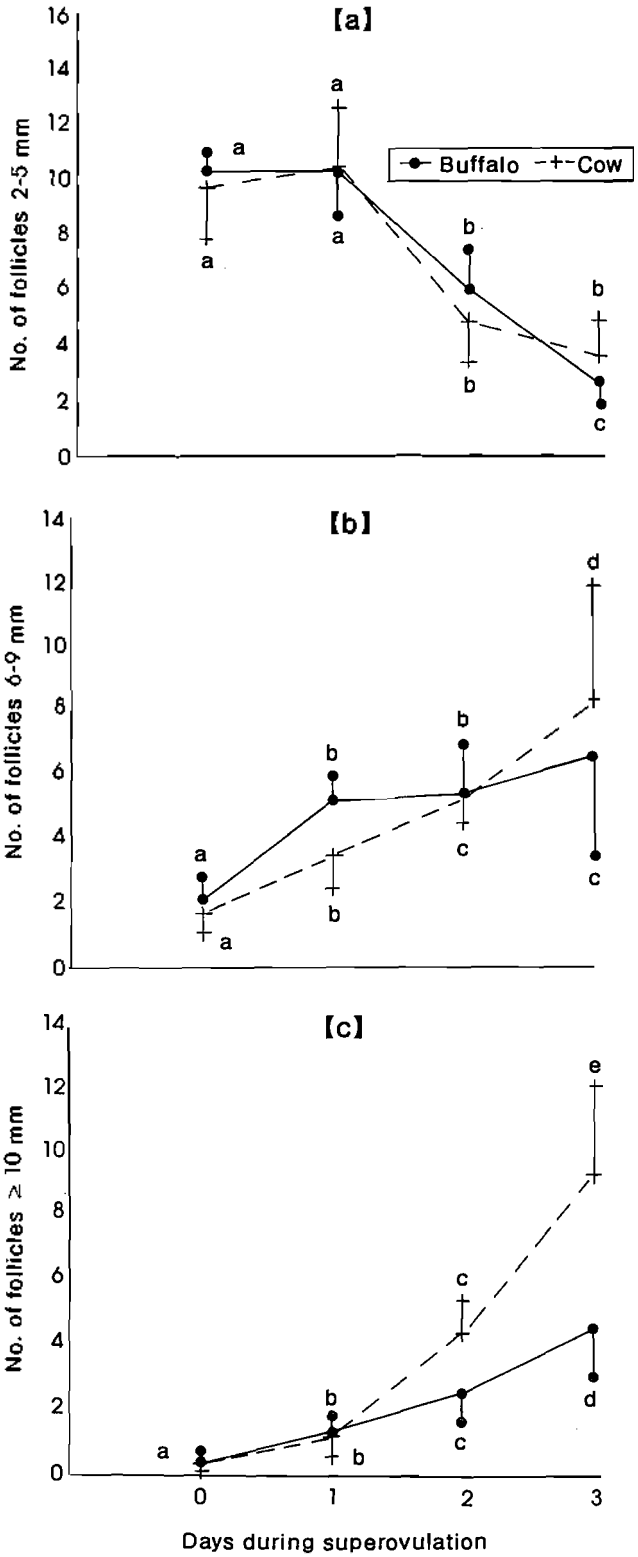

Figure 2. Profiles of the mean (± sem) number of follicles during superovulation in cow and buffalo. The points with different superscript differ ($p < 0.01$).

Follicular status on days of super estrus and day of flushing

The number and size of follicles on the day of estrus matters a lot for number of ovulations. The mean number (1.4) of small size follicles was lowest and similar both in cows and buffaloes. However, the number of medium and large size follicles varied in these species ($p < 0.01$). The corresponding values for medium size follicles were 6.4 and 9.8 and large size 7.6 and 14.4 in buffalo and cows respectively (figure 1). On an average, there was increase in the number of large size follicles in buffaloes (7.6 to 10.0) whereas in cows it decreased (14.4 to 13.0) on the day of flushing from the day of super estrus.

DISCUSSION

Results from this investigation showed that under the condition of our experiment the number of follicles at the time of induction of superovulatory treatment though were almost similar both in cattle and buffalo, yet, the superovulatory response in cattle was much higher than buffaloes. It has been demonstrated in cows (Monniaux et al., 1983) that the number of ovulations following gonadotrophin is highly related to the number of healthy follicles (> 1.7 mm) present before initiation of treatment. Lower follicular population in buffalo is due to the low exit of reserve of primordial follicles (Mauleon and Mariana, 1977). However, this was not the situation in present investigation before induction of superovulatory treatment. Because buffalo had a mean number of 10.6 small size and 1.4 medium size follicles, which were slightly higher than cows and large size follicles were same in both the species. Another factor which might determine the superovulatory response is the presence or absence of dominant follicle before start of superovulatory treatment. Many workers have reported both in cattle (Guilbault et al., 1991; Huhtinen et al., 1992) and buffalo (Taneja et al., 1995) the reduced superovulatory response in presence of dominant follicle. However, these workers started monitoring of ovarian activity starting on Day 3 of oestrus cycle to classify the presence or absence of dominant follicle, which was not under the scope of the present experiment. Bungartz and Niemann (1994) attempted to assess the presence or absence of dominant follicle by single ultrasound examination taking into account the presence of number of small size (3-8 mm) follicles in midcycle. They clearly stated that animal with more than 10 follicles of 3 to 8 mm in diameter can be considered to have no functional dominant follicle in midcycle. We examined our data taking this criteria into account in both the species and concluded that even if the large size follicle which was present in buffalo No. 1, which had seven CLs and cow No. 1, which had three CLs was non functional dominant follicle. Buffalo and cow No. 4 had functional dominant follicle, according to this criteria but no embryos could be recovered from both of these animals, irrespective of four CLs present in cow No. 4. Only one animal in each species had a functional dominant follicle, thus forming the homogenous groups. It clearly shows that follicular status during or after superovulation might have been responsible for poor superovulatory response in buffalo under this experiment.

Follicular dynamics during superovulation answers to some extent as to why superovulatory response was better in cows than buffaloes inspite of higher follicular numbers before start of SOV in buffaloes. From Day 2 to Day 3 of treatment the average increase in medium (3.2 vs 1.2) and large size (5 vs 2) follicles was higher ($p < 0.01$) in cows. On the other hand initial shift in mean number of follicles was higher in buffalo than cattle (figure 2). During superovulation the increase in the number of large size follicles is attributed to the passage of follicles from smaller to larger classes (Grasso et al., 1989; Mauleon and Mariana, 1977). This was not dependent on the number of small size follicles because their number were almost similar (Desaulniers et al., 1995) or even higher (Desaulniers et al., 1995a) in superovulated heifers and matured cows. In present investigation the number of small size follicles was almost comparable in both the species.

Two days after PG injection i.e. on the day of super estrus the decline in the small size follicles was of similar magnitude but there was linear increase in cow (medium 5.0 to 9.8; large 4.4 to 14.4) than buffalo (medium 5.2 to 6.4; large 2.6 to 7.6) from Day 2 of the treatment to day of superestrus. Further on the day of flushing, increase in the number of large size follicles in buffalo might reflect the presence of more number of unovulatory follicles. The ovulation problem in buffalo has already been reported both in normal estrus (Manik et al., 1994a) and induced estrus (Manik et al., 1992) animals. In conclusion, results from our experiment suggest that it was the process of shift, recruitment and passage of follicles from smaller to larger size from Day 2 of treatment and thereafter upto superestrus which contributed toward lower response in buffaloes. Not only that, the ovulation rate was also lower which was reflected by the presence of large size follicles on the day of flushing. Further ultrasound examinations alongwith endocrine estimatioins might suggest some means to solve the problem of ovulation in this species.

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