Effect of Removal of Follicles through Repeated Transvaginal Follicle Aspiration on Subsequent Follicular Populations in Murrah Buffalo (*Bubalus bubalis*)

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ABSTRACT : This study was conducted to investigate the effects of removal of all ovarian follicles through repeated transvaginal follicle aspiration (TVFA) on the subsequent follicular populations in buffaloes. This information is crucial for determining the optimum time interval between successive aspirations for recovering oocytes from live buffaloes through Transvaginal Oocyte Retrieval (TVOR). The oestrus of cycling buffaloes (n=5) were synchronized by a single PGF injection schedule. All the ovarian follicles were removed once every 7 days for 6 weeks through TVFA, starting from Day 7 of the oestrous cycle (Day 0 = day of oestrus). The number and size of individual ovarian follicles was recorded at Day 3 and Day 5 (Day 0 = day of TVFA) through transrectal ultrasonography. The follicles were classified on the basis of their diameter as small (3-5 mm), medium (6-9 mm) and large (≥10 mm). There was no difference in the number of small and medium follicles, and the number of total follicles between Day 3 and Day 5. However, the number of large follicles was significantly higher ($p<0.05$) at Day 5 than that at Day 3. There was a significant ($p<0.05$) decrease in the proportion of small follicles and an increase ($p<0.05$) in the proportion of large follicles from Day 3 to Day 5, with no change in the proportion of medium follicles. The number of total follicles at Day 3 or Day 5 did not differ during the 6 TVFA sessions. It can be concluded that an interval of 3 days is more suitable than that of 5 days between successive aspirations for recovering oocytes through TVOR in a twice weekly schedule and that repeated removal of follicles through TVFA does not adversely affect the number of total follicles 3 or 5 days after TVFA. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 5 : 632-636)***

Key Words : Buffalo, Follicles, Ovum Pick-up, TVOR

INTRODUCTION

Because of the limited availability of superior germplasm (Misra et al., 1990), the major focus of the reproductive technologies under development for buffalo is on its faster multiplication. Application of Embryo Transfer Technology (Singla et al., 1996; Misra, 1997) to buffaloes has had a very limited success because of some peculiar problems in this species (Madan et al., 1996). The primary reasons for this is that the superovulation responses are very poor in buffaloes, which result in a recovery of only 2.6 total and 1.4 transferable embryos per flush (Misra, 1997). The emphasis in this species is, therefore, shifting to development of *in vitro* technologies like *in vitro* embryo production (Palta and Chauhan, 1998) through a combination of *in vitro* maturation, fertilization and culture (IVMFC) of oocytes. The only means available for producing offspring of known genetic background is to retrieve oocytes from live animals of high genetic merit through Transvaginal Oocyte Retrieval (TVOR) and subject them to IVMFC for *in vitro* production of embryos. These embryos can then be transferred to suitably synchronized recipients for producing offspring of high genetic merit.

TVOR, which involves Transvaginal Follicular Aspiration (TVFA) i.e., aspiration of ovarian follicles from live animals through the vaginal route for collection of oocytes has been applied successfully in buffalo and cattle to prepubertal (Revel et al., 1995; Taneja et al., 2000; Techakumphu et al., 2004), cyclic (Boni et al., 1996; Neglia et al., 2003) pregnant (Meintjes et al., 1995) and problem animals (Looney et al., 1994; Hasler et al., 1995; Manik et al., 2002a). Although repeated TVOR has no adverse effects on follicular populations (Boni et al., 1996; Manik et al., 2002a), it may sometimes cause adhesions.

Although a number of factors like the breed, species, age and reproductive status of the animal; type, diameter and bevel of the needle used for follicle aspiration; vacuum pressure; gonadotropin pretreatment etc. are known to affect the oocyte yield through TVOR, the factor that has the highest influence on the oocyte yield per unit time, is the time interval between successive aspirations (Gibbons et al., 1994; Broadbent et al., 1997; Garcia and Salahhedine, 1998; Goodhand et al., 1999). TVOR has been carried out either every 7 days i.e., once weekly or after an interval of 3 or 4 days i.e., twice weekly in cattle. In buffalo, however, no study has been conducted to find out the optimum interval between successive aspirations. Since TVOR involves aspiration of all follicles, therefore, the next TVOR can be carried out only after new follicles have been recruited from the pool of antral follicles and when their number is such that a sufficient oocyte harvest can be expected. Thus, the interval between successive aspirations is dependent upon

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the rate at which follicles are recruited. There is no information available on follicular recruitment after the removal of follicles through TVFA in buffaloes. The present study was, therefore taken up to study the effects of removal of follicles through repeated transvaginal follicle aspiration on subsequent follicular populations.

MATERIALS AND METHODS

Animals and management

The animals taken for the study were healthy, cycling Murrah buffaloes between 7-12 years of age (n = 11), which had not been subjected to follicular aspiration previously. These were maintained under general feeding and management conditions at the National Dairy Research Institute (NDRI) animal farm. The experiment was completed between February and April.

Experimental design

The oestrus of the animals were synchronized by a single $PGF_{2\alpha}$ injection schedule. For this, all the animals were administered a single i.m injection of 25 mg of Lutalyse, a $PGF_{2\alpha}$ analog (Dinoprost tromethamin). Five out of the total 11 animals, which exhibited oestrus 72 h after PGF injection were subjected to transvaginal follicular aspiration (TVFA). Occurrence of oestrus was confirmed by rectal palpation and by behavioral oestrus signs. The animals in which the follicular populations were recorded had been previously subjected to transvaginal follicular aspiration (TVFA) starting from Day 7 of the oestrous cycle (Day 0 = day of oestrus). Each day of follicle removal through TVFA was marked as Day 0. The number and size of individual ovarian follicles was recorded on Day 3 and Day 5 after each TVFA session through transrectal ultrasonography. The study continued for a total of 6 TVFA sessions.

TVFA was carried out as described earlier (Manik et al., 2002a). Briefly, each animal was given a general anesthetic (Rompun, 0.8 ml/animal) about 15 min before TVFA. The animals were then moved to suitably designed crates, which allowed minimal movement. A few minutes before TVFA, each animal was given an epidural anesthesia of 5 ml/animal Xylocaine. TVFA was performed using an ultrasound machine (Aloka SSD-500) with a 5 MHz transvaginal transducer with stainless steel dorsal needle guide and single lumen 19-gauge, 60 cm long sterile needle with an ultrasound Echo Tip (Cook Veterinary Products, Qld, Australia) and a constant vacuum pressure of 50 mm Hg obtained through a vacuum pump (Karl Storz-Endoskpe, West Germany).

Measurement of size and number of follicles

The size and number of individual ovarian follicles was recorded at Days 3 and 5 (Day 0 = day of TVFA) through transrectal ultrasonography, which was performed using a real time B-mode diagnostic instrument (Tokyo-Keiki LS-1.000) equipped with a linear array 5 MHz transducer, as described by Manik et al. (1994) and as used routinely in the laboratory. Briefly, the animals were restrained by holding them in suitably designed crates, without use of any chemical tranquilizer. The rectum was evacuated and the anal and vulvar area were washed thoroughly. The acoustic face of the probe was covered with the coupling gel after which the transducer was inserted in the rectum. It was manipulated per rectum till the ovary could be scanned from different angles so that all follicles ≥3 mm diameter could be monitored. The diameters of follicles ≥10 mm were measured with the built-in calipers 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 images. The diameters of non-spherical follicles were calculated by taking the average of the longest and widest measured points of the follicle. The number and diameter of all follicles with an antral diameter of ≥3 mm was recorded at each examination. The follicles were then classified on the basis of their diameter as small (3-5 mm), medium (6-9 mm) and large (≥10 mm).

Statistical analyses

The number of small, medium and large follicles and the number of total follicles were compared between Day 3 and Day 5 (Day 0 = day of TVFA) by paired 't' test after log transformation of data. The number of total follicles at Days 3 and 5 were compared between different weeks by one way ANOVA after log transformation of data. The proportions of small, medium and large follicles of the total follicles were compared between Day 3 and Day 5 by paired 't' test after arcsin transformation of data.

RESULTS

Effect of removal of all follicles through TVFA on follicular populations at days 3 and 5

The mean (±SEM) number of follicles of small, medium and large categories and the total number of follicles for all the 6 TVFA sessions are presented in Figure 1. The number of small and medium follicles, and the number of total follicles was not different between Days 3 and 5. However, the number of large follicles was significantly higher ($p<0.05$) at day 5 than that at day 3.

Relative distribution of follicles of different size categories

The mean (±SEM) number of small, medium and large

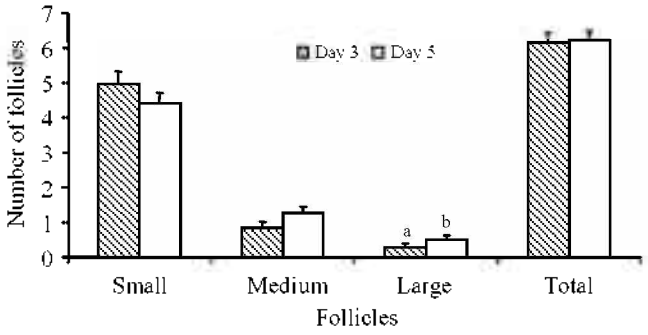

Figure 1. Mean (±SEM) number of small (3-5 mm), medium (6-9 mm) and large (≥10 mm) follicles, and the total number of follicles at days 3 and 5 (day 0 = day of TVFA). [a, b] Bars with different superscripts differ significantly ($p<0.05$).

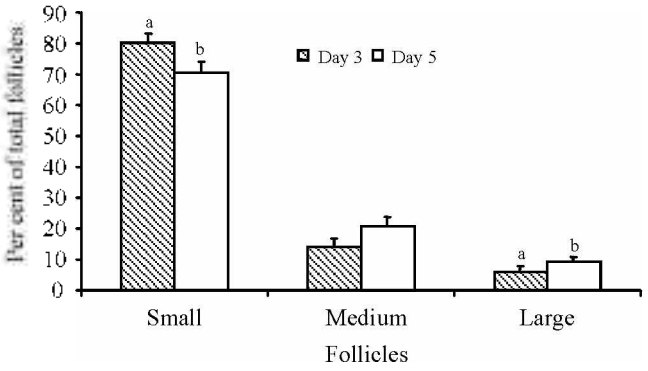

Figure 2. Mean (±SEM) number of small (3-5 mm), medium (6-9 mm) and large (≥10 mm) follicles as percent of the number of total follicles at days 3 and 5 (day 0 = day of TVFA). [a, b] Bars with different superscripts differ significantly ($p<0.05$).

follicles as per cent of the number of total follicles at days 3 and 5 for the 6 TVFA sessions are presented in Figure 2. There was a significant ($p<0.05$) decrease in the proportion of small follicles and an increase ($p<0.05$) in the proportion of large follicles between day 3 and day 5. The proportion of medium follicles also increased between days 3 (14.1± 2.73%) and 5 (20.6±3.25%). The differences were, however, not statistically significant.

Effect of repeated TVFA on follicular populations

The number of total follicles at different TVFA sessions during the 6-week period varied between 5.20±0.76 and 7.00±0.28 at day 3 and between 5.20±0.66 and 7.00±0.28 at day 5 (Figure 3). The differences between weeks were not significantly different both for day 3 and day 5.

DISCUSSION

The results of the present study suggest that there is no difference in the number of total follicles present 3 or 5 days after removal of all follicles through TVFA.

Very few reports are available on the effects of removal

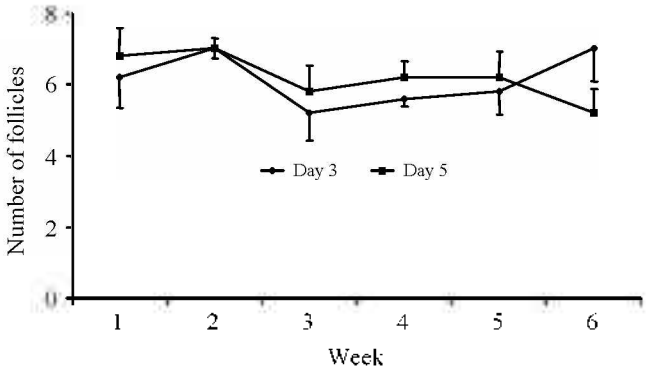

Figure 3. Mean (±SEM) number of total follicles at days 3 and 5 (day 0 = day of TVFA) over 6 weeks.

of all follicles through TVFA on the subsequent follicular dynamics. This is probably because the follicular aspiration experiments are focused mainly on maximizing the recovery of oocytes. To our knowledge, there is only one report available in buffalo in which the number and size distribution of follicles has been recorded subsequent to removal of all follicles through TVFA (Boni et al., 1996). Our results are in agreement with this report in which the authors reported that in Italian Mediterranean buffaloes subjected to TVFA every 3 to 5 days for 2 or 4 months, the number of total follicles was similar 3, 4 and 5 days after removal of all follicles through TVFA. In a similar study, Boni et al. (1997) who subjected cows to twice weekly TVFA for 3 months observed that the number of total follicles was not different 3, 4 and 5 days after removal of all follicles through TVFA. Garcia and Salahheddine (1998) who carried out daily ultrasonographic examination of ovaries in cows subjected to TVFA observed that emergence of new follicular growth was initiated within 2 days after the puncture procedure and suggested that TVFA induces and synchronizes subsequent follicular wave emergence. The results of our study confirm these results and suggest that the new follicular wave started after removal of all follicles during previous TVFA reaches its full numerical development within 3 days of TVFA and that there is no further increase in the number of follicles from day 3 to day 5 after TVFA.

Our results suggest that in buffaloes, even with a 3-day interval between successive TVFA sessions, the follicle number can reach a maximum. Increasing the interval from 3 to 5 days does not increase further the total number of follicles. Since the number of oocytes that can be collected is dependent upon the number of follicles that are available for aspiration, and because a maximum number of follicles are available 3 days after the previous TVOR, the oocyte yield per unit time can be maximized in buffaloes by keeping a 3-day interval between successive TVOR sessions.

A significant decrease in the proportion of small

follicles, a significant increase in the proportion of large follicles and a non-significant increase in the proportion of medium follicles between days 3 and 5, as observed in the present study could be due to growth of follicles from small through medium to large categories. Our results are in agreement with those of Boni et al. (1997) who reported that the proportion of Class I (2-9 mm) follicles decreased whereas that of Class II (>9-14 mm) follicles increased from day 3 to day 5 in cows subjected to TVFA twice weekly. Boni et al. (1996) also observed an increase in the proportion of large follicles between days 3 and 5 in Mediterranean buffaloes subjected to TVFA twice weekly, although there was no change in the proportion of small and medium follicles between these two days.

The number of small, medium and large follicles and the total number of follicles, as observed in the present study in the Murrah breed of buffaloes is similar to that reported for slaughterhouse buffalo ovaries (Madan et al., 1996; Kumar et al., 1997) or that in Mediterranean buffaloes (Boni et al., 1996). It is however much lower than that reported for cattle subjected to TVOR (Boni et al., 1997; Broadbent et al., 1997; Goodhand et al., 1999). Although the pattern of follicular growth and development during oestrous cycle is similar in buffaloes and cattle, the number of antral follicles has been reported to be much lower in buffaloes compared to that in cattle (Le Van Ty 1989; Manik et al., 2002b). This may be primarily because of species differences. However, the role of nutrition cannot be ruled out since the availability of nutrition has been found to alter the growth characteristics of follicles and to have adverse effects on follicular turnover in cattle (Mackey et al., 1999). Moreover, in cattle, the planes of nutrition and body condition have also been known to affect follicle numbers (Dominguez, 1995).

There is no information available on the effects of repeated TVFA on the number or size of follicles present 3 or 5 days later. Our results suggest that that there was no effect of once weekly repeated TVFA on the number of total follicles at day 3 or day 5 during the 6-week period. These results are in agreement with earlier studies in which it has been reported that repeated TVFA for long periods of time has no effect on the number of follicles recorded on the day of TVFA in Mediterranean buffaloes (Boni et al., 1996) and cattle (Boni et al., 1997; Broadbent et al., 1997).

CONCLUSION

An interval of 3 days is more suitable than an interval of 5 days between successive TVOR sessions since the follicle number does not increase between day 3 and day 5. Thus, the oocyte yield per unit time can be increased by subjecting animals to TVOR more frequently by reducing the time interval between successive aspirations from 5 to 3 days. The availability of a higher proportion of small follicles at day 3 would be an additional advantage since a more homogeneous population of immature oocytes can be recovered from them. Moreover, TVOR could be carried out over long period of time at 3-day intervals since it does not adversely affect the number of follicles available for aspiration.

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