

Ultrasonic monitoring and biometry of ovaries and ovarian structures during superovulation following transvaginal follicle ablation in Murrah buffaloes

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Abstract

Five Murrah buffaloes were studied ultrasonographically to record the biometry of the ovarian structures and superovulatory response during superovulation (SOV) following follicle ablation. Transvaginal ultrasound-guided follicle aspiration / ablation (FA) was done at the middle of the oestrous cycle (day 10) on follicle of over 10 mm diameter, using 5.0 MHz convex-array intravaginal transducer using a B-mode scanner. The animals were simultaneously subjected to superovulation with either FSH (Follitropin V) or FSH+PMSG. Ovarian size and ovarian structural changes of these five superovulated buffaloes were monitored on a) the 10th day post estrus (Day of Follicle Ablation and start of superovulation protocol,) b) 3rd day post FA+SOV (the 3rd day of superovulation programme) and c) 6th day post FA+SOV (Day before Flushing). Before FA+SOV programming, the length and width of the left ovary was recorded to be 29±0.1 and 21±0.1 mm while the right ovary measured 28±0.1 and 20±0.1 mm and the average size of CL and follicles was 18.50±0.28 and 11.50±0.5 mm, respectively. There was significant increase in the size of ovaries on

3rd day post FA+SOV and 6th day post FA+SOV (day before flushing). A greater number of ovarian structures (CL and follicles) were found at 6th day post FA+SOV than during 3rd day post FA+SOV indicating late ovulations and an-ovulations. The average size of the follicle showed increase on day before flushing due to cystic ovarian condition in a few buffaloes. Late ovulation and a lower number of recruited follicles during superovulation may be the reason for lower response in Murrah buffaloes.

Keywords: Murrah buffalo, Follicle ablation, Ultrasonography, Superovulation, Ovary

Introduction

The applications of ultrasound include the ability to monitor follicular characteristics, ovarian activity, and aid in follicle ablation, aspirations of follicular contents including oocyte retrieval and it is widely recognized and used as a key tool in reproductive management and research.

Superovulation is the key factor in any

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multiple ovulation and embryo transfer program. Considering the negative feedback effect of existing dominant follicle on recruitment and development of more follicles in response to superovulatory hormonal regimen, it is anticipated that deletion of this retardant should result in improved growth of a new wave of follicles to be ovulated in response to the superovulatory treatment. Within this framework, transvaginal oocyte recovery by puncture and aspiration of antral follicles has become a routine procedure in most laboratories where superovulation is part of the services offered to breeders. This technique of follicle ablation consists in the transvaginal recovery of the oocytes by follicular aspiration under ultrasound guide.

Buffaloes (*Bubalus bubalis*) in general are known to be poor responders to superovulation protocols in comparison to cattle. The main problem encountered during superovulation with different hormones in buffalo is the presence of anovulatory follicles leading to few and poor quality embryos (Madan *et al.*, 1996), besides influencing the recruitment of new follicles. Several reports suggest a lower follicular population in the buffalo ovaries (Totey *et al.*, 1991). It is essential to know the number of follicles recruited and CL available in buffaloes before and during superovulation and embryo collection programme. Ovarian size may also determine the embryo recovery rate as excessive enlargement of ovaries may

hamper ovum pick-up by the fimbria during ovulation of multiple follicles. Hence an attempt has been made to study ovarian size and structures using an ultrasound scanner in Murrah buffaloes during superovulation.

Materials and methods

The study was carried out at the Central Institute for Research on Buffaloes, Hisar, Haryana, India during April 2012. Twelve female Murrah buffaloes were managed under a semi-intensive system of management at the Farm of Central Institute for Research on Buffaloes. During the day time, the animals were allowed to graze on natural pastures of the farm land. The animals were fed with 2 kg of concentrate ration per day per animal.

The estrum were synchronized with one injection of prostaglandin, (inj. Lutalyse - 5 ml i/m; Hoechst, India), animals were checked with ultrasonography at 72 h for the presence of pre-ovulatory follicle in ovary and uterine tone. The animals reporting for estrus (n=5) were selected for follicle ablation and Transvaginal ultrasound-guided follicle ablation was done at the middle of the oestrous cycle (day 10) on follicles of size greater than 10 mm with 5.0 MHz convex-array intravaginal transducer using a B-mode scanner (Figure 1 and 2).

These five buffaloes were simultaneously superovulated using FSH or FSH+PMSG. Superovulatory hormone (s) was given from the 10th day of the

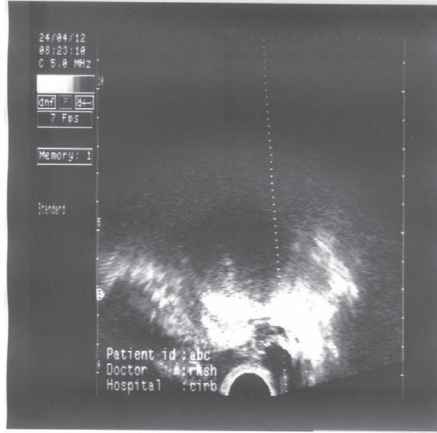


Figure 1: Ovary and structural changes prior to follicle ablation



Figure 2: Ovary and its structural changes post to follicle ablation.

estrous cycle for 5 days in a tapering dose rate. Luteolysis was induced with prostaglandin injections administered along with the 7th and 8th FSH dose. All the animals reporting to estrus (at 48-72 h post PGF2 α) were inseminated twice, at 60 and 72 h after the first injection of PGF2 α .

Ultrasound examinations of the ovaries were done with a 7.5 MHz linear-array transducer for intrarectal use and used to record the ovarian structures and superovulatory response in these animals during the superovulation programme. The length and breadth of ovaries and the number and diameters of corpora lutea and follicles of superovulated buffaloes were monitored ultrasonographically, recorded and compared on a) the 10th day post heat (Day of FA+SOV), b) 3rd day post FA+SOV and c) 6th day post FA+SOV (Day before Flushing).

Statistical Analysis

The means and standard errors for all variables were calculated and are presented. Differences between the ovarian size and the numbers and size of follicles and corpus luteum before and after follicle ablation and superovulation were tested by Student "t" test.

Results and Discussion

The ovaries were subjected to ultrasonographic biometry and examined for the presence of follicles or corpus luteum. Moreover, the numbers and size of follicles and CL were measured and recorded (Table 1).

The total follicle population was recorded, which appeared as anechoic rounded structures, while CL appeared as granular structures with greater echogenicity (Fig. 3 and 4). The means and standard errors for all variables were calculated and are presented (Table 2).

Table 1: Ultrasonographic ovarian biometry during superovulation

Particulars	Length (mm)		Width (mm)	
	Lt. Ovary	Rt. Ovary	Lt. Ovary	Rt. Ovary
10 th day(Day of FA+SOV)	29± 0.1 ^a	28± 0.1 ^a	21± 0.1 ^a	20± 0.1 ^a
3 rd Post FA+SOV	34.63 ± 1.77 ^b	32.50 ± 2.19 ^b	25.50 ± 2.37 ^b	24.75 ± 2.39 ^b
6 th Post FA+SOV (Day before Flushing)	38.71 ± 1.69 ^b	35.00 ± 3.42 ^b	30.57 ± 2.02 ^b	28.86 ± 2.41 ^b

Means in the same column within categories with different superscript differ significantly (p<0.05).

Table 2: Ultrasonographic biometry of corpus luteum and follicles during superovulation programme.

Particulars	Number available		Average Size (mm)	
	CL	Follicle	CL	Follicle
10 th day(Day of FA+SOV)	1.00±0.0 ^a	4.8±0.52 ^a	18.50±0.28 ^a	11.50±0.5 ^a
3 rd Post FA+SOV	0.6±0.5 ^b	14±2.5 ^b	14.88±0.15 ^b	14.42±1.07 ^b
6 th Post FA+SOV (Day before Flushing)	3.8±0.2 ^c	9.2±2.2 ^c	16.21±0.79 ^c	22.25±2.28 ^c

Means in the same column within categories with different superscript differ significantly (p<0.05).



Figure 4: Ovaries and structural changes on the Day before flushing.

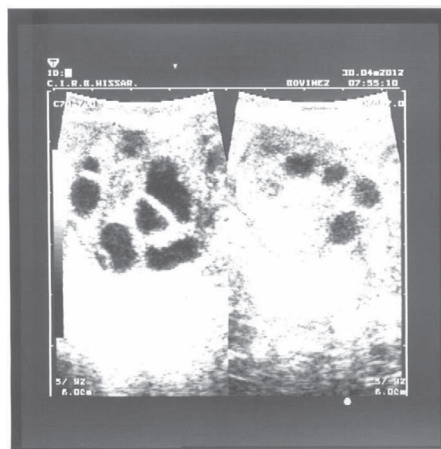


Figure 3: Ovaries and structural changes on the 3rd post FA+SOV.

Significant changes were observed in the length and width of ovaries prior to and post follicle ablation and superovulation. The smallest ovary measured 28 x 20 mm (length x width), while the biggest ovary measured 38 x 30 mm on the day before flushing. The size of ovary, as observed with ultrasonography in the present study, was higher than that reported by Chandrahasan and Rajsekaran (2004) in non-descript buffaloes and by Kumar *et al.* (2004) in Murrah Buffaloes.

Use of FSH+PMSG significantly increased the size of the ovary on 3rd day post FA+SOV. Similarly, significant increase ($P<0.05$) in the size of the ovary was observed on the 6th day post FA+SOV (day before flushing). The changes in ovary size and structures post follicle ablation and superovulation are shown in Figures 3 and 4.

On the 10th day post estrus (Day of FA+SOV), all buffaloes were having a distinct CL and varied numbers and sizes of follicles in their ovaries. The number of corpora lutea significantly increased to 3.8 ± 0.2 on the day before flushing compared to those recorded on the 10th day (1.00 ± 0.00) (Table 2). Similarly, there was an increase in the average number of follicles in response to superovulation from 11.50 ± 0.5 on 10th day to 22.25 ± 2.28 on the day before flushing.

These findings indicate that the buffaloes in this study had late ovulations (post-estrus), and that the number of recruited follicles even with superovulation

treatment was low. Buffaloes are regarded to have a lower reproductive efficiency and several reports suggest lower follicular population in buffalo ovaries (Madan, 1990 and Totey *et al.*, 1991).

Overall, there were significant variations in terms of the presence of ovarian structures on the 10th day post-estrus, 3rd day post FA+SOV and the day before flushing. Buffalo ovaries remain partially active at all times, as evident from the results of Rohilla *et al.* (2005), who reported mean numbers of as many as 7.7 ± 0.3 follicles in anoestrus Murrah buffaloes while Chandrahasan and Rajsekaran (2004) found a 3.41 ± 0.11 follicles in buffalo ovaries, though Madan (1990) showed that buffaloes have a lower population of primordial follicles at the 10th day of the estrous cycle.

The size of the follicle observed in this study was comparable to the ultrasonographic studies by Honparkhe *et al.* (2003) and Rohilla *et al.* (2005). The average size of the largest follicle on the flushing day (22.25 ± 2.28 mm) was greater as compared to the 10th day (11.50 ± 0.5 mm). This increase in size of the ovaries may be due to the presence of persistent cystic follicles recorded on the day of flushing in three buffaloes. The size of follicles and CL was larger than those reported by Honparkhe *et al.* (2003) and Chandrahasan and Rajsekaran (2004).

In conclusion, Murrah buffaloes have large-sized ovaries which react with

increased activity in response to superovulatory treatment with highly significant increase in the numbers of follicles and CL. All stimulated follicles, however, failed to ovulate. It provides important information which can be used to plan future superovulation programme for Murrah buffaloes. Also, it was observed that ultrasound serves as an important tool for detailed, reliable and accurate study of ovarian responses to superovulation in buffaloes.

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