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Short Communication

Studies on certain attributes of buffalo follicular fluid

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ABSTRACT

The aim of the present investigation was to determine average yield of follicular fluid, total protein, estradiol and progesterone profile in the follicular fluid of buffalo ovaries. The follicular fluid was collected by aspirating the visible surface follicles. The average yield of buffalo follicular fluid (buFF) per ovary was 0.45±0.03 ml which was higher during the months between November to March (0.54±0.02 ml) than April to September (0.38±0.02 ml). The mean concentration of total protein in pooled buFF samples was 4.86±0.41 g% (ranged between 3.0 to 6.7 g%). The mean concentration of estradiol and progesterone in pooled buFF samples were 3044.99±31.85 pg/ml and 74.31±2.94 ng/ml, respectively.

Key words : Buffalo, follicular fluid, yield, protein, steroid

Follicular fluid is a mixed secretion of follicles and partly of exogenous from plasma. The presence of follicular fluid (FF) testifies its potential importance in ovarian physiology. This small reservoir of fluid reflects the biochemical and endocrinological activity of the follicles and thus serves as a guide to its growth and differentiation (Edwards, 1974). It is a complex mixture of many substances viz., steroids, non steroidal and other compounds. The follicular fluid proteins are largely derived both from plasma and follicular cells (McNatty, 1978) and play an important role in several physiological and biochemical processess viz., oocyte maturation, folliculogenesis, ovulation, oocyte transport and various other follicle regulatory process related to reproduction (Edwards, 1974; Hafez and Hafez, 2000; Ali *et al.*, 2004). Follicular fluid proteins have also been found to influence the spermatozoan function (Kumar, 1995, 2002; Agarwal, 1997; Kumaresan *et al.*, 2003). The use of follicular fluid for augmentation of reproduction is fairly a recent development. Reports on the concentration of follicular fluid protein and steroids are available in cattle (Edwards, 1974; Johnson and Smith, 1985; Wise, 1987; Turzillo and Fortune, 1993). However, there are few reports pertaining to profile of protein (Khera, 1989; Meur *et al.*, 1995; Jindal *et al.*, 1997; Eissa, 1996) and steroids in buffalo follicular fluid (buFF) (Khera, 1989; Eissa, 1996), despite of its use to modulate reproduction in farm animals (Agarwal *et al.*, 1996; Singh, 1995; Kumar, 1997; Ghosh, 1998). The present study was, therefore, conducted to estimate the concentration of protein and steroids (oestradiol and progesterone) in buffalo follicular fluid recovered from abattoir ovaries.

Buffalo ovaries (n = 4437) were collected from the local abattoir for a period from April, 2002 to June, 2003 and transported to the laboratory on ice within 3-4 h of slaughter. Follicular fluid was aspirated from all visible surface follicles (irrespective of their size, dia. in mm) with the help of needle fitted with 2.0 ml syringe. The pooled follicular fluid was centrifuged at 3000 rpm for 15 minutes at room temperature. The supernatant was decanted and stoted at -20°C till further use. The follicular fluid from 10 batches was thawed and the concentration of protein was estimated using bovine serum albumin (BSA) as standard according to the method described by Lowry *et al.* (1951). The concentration of steroids (i.e. estradiol and progesterone) in pooled buFF for 6 batches was estimated using RIA technique by standard diagnostic kits (ImmunoTech, France).

A total of 4437 buffalo ovaries were processed at 94 different occasions and 1827 ml of follicular fluid was collected with an average yield of 0.45±0.03 ml per ovary. The monthwise collection of buFF was presented in Table 1. The average yield of buFF obtaind in the present study was lower than that reported in cattle which may be due to lower turnover rate and reduced number of antral follicles per

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Table 1: Collection of buffalo follicular fluid during different months of the year

Sl. No.	Month of collection		No. of Ovaries	Attributes	
				Volume of buFF (ml)	Average yield per ovary (ml)
1.	January	2003	185	99.0	0.54
2.	March	2003	358	210.5	0.59
3.	April	2003	539	221.0	0.41
		2002			
4.	May	2003	782	292.0	0.37
		2002			
5.	June	2003	901	321.0	0.36
		2002			
6.	July	2002	706	228.0	0.32
7.	September	2002	338	141.5	0.42
8.	November	2002	363	177.0	0.49
9.	December	2002	265	137.0	0.52
	Total		4437	1827.0	0.45±0.03

ovary in buffaloes (Parmer and Mehta, 1992) than cattle (Ty *et al.*, 1989). The mean yield of follicular fluid per ovary was relatively higher during the months between November to March (0.54±0.02 ml) than that obtained during April to September (0.38±0.02 ml). This may probably because of greater availability of ovarian follicles during the winter (breeding season) than summer months in buffaloes (Agarwal and Tomer, 2003).

The mean concentration of total protein was 4.86±0.41 g per 100 ml of buFF and ranged between 3.0 to 6.7 g%. Our finding substantiates the values reported previously in the same species (Khera, 1989; Meur *et al.*, 1995; Jindal *et al.*, 1997) and also in cattle (Wise, 1987). The mean concentration of estradiol and progesterone in pooled buFF samples were 3044.99±31.85 pg/ml (~3.1 ng/ml) and 74.31±2.94 ng/ml, respectively. The estradiol concentration was within the range (0.9 - 14.0 ng/ml) previously reported in buFF (Khera, 1989; Eissa, 1996). But the concentration of progesterone estimated in the present study was higher than that of the value (8.32 - 42.5 ng/ml) recorded by earlier workers (Khera, 1989; Eissa, 1996). The estimated concentration of steroids (estradiol) in buFF in the present study was higher than that of peripheral blood plasma/serum (30-35 pg/ml) of buffaloes. The steroid concentration has been reported to be 500 x 10^3 times higher in oFF (McNatty *et al.*, 1981) and 1000 times higher in pFF (Eiler and Nalbandov, 1977) as compared to their respective species blood plasma. The concentration of steroids particularly in reference to estradiol, has been reported excessively high (400 x 10^4 ng/ml) in eFF (Nambo *et al.*, 2002). Interestingly, estradiol concentration in the present study was only 88-103 times higher in buFF than the value reported in buffalo blood plasma around the time of estrus (Agarwal and Tomer, 2003). It may be speculated that lower estradiol concentration in buFF may lead to its low exchange to peripheral circulation, contributing poor expression of behavioural signs of estrus in this species.

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