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Original Research

Ovarian biometry and ovarian follicular fluid proteins during different phases of the estrous cycle in buffalo *(Bubalus bubalis*)

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Abstract

In the present study we examined the ovarian biometry and ovarian follicular fluid proteins in buffalo. Abattoir derived buffalo ovaries (n=82) were grouped into either follicular or luteal stages of estrous cycle based on the characteristics of the corpus luteum and morphometry of the ovary. The ovarian dimensions were recorded and the follicles were counted. The follicles were aspirated and follicular fluid samples were collected and follicular fluid proteins were estimated by SDS-PAGE analysis. The molecular weights (MWs) of protein bands were estimated by comparing their migration rates on the gel with those of protein markers with known MWs. No significant ($P<0.05$) difference was observed in the biometry of ovaries within the phases (follicular or luteal phases) and also between the phases of estrous cycle. The numbers of follicles were observed to be higher in the left ovary during follicular phases as compared to other phases. A total of 22 different protein bands were observed corresponding to MWs varying from 15 to 180 kilodaltons (kDa) during different phases of estrous cycle with some proteins being specific to some stage of cycle. High molecular weight proteins (mean 180-195 kDa) were identified during luteal phase compared to follicular phase whereas low molecular weight proteins were common during follicular phase. It was concluded that the biometry of buffalo ovaries is not different during reproductive cycle. High molecular weight proteins were common in the ovarian follicular fluid during luteal phases and low molecular weights proteins common during follicular phases.

Keywords: Buffalo, biometry, follicular fluid protein, SDS-PAGE.

Introduction

The ovaries and ovarian structures of buffaloes are inherently smaller in buffalo compared to cows and seasonal ovarian hypofunction and ovarian pathologies limit the breeding value of this important species [1]. There have been very few studies on the biometry of buffalo ovaries [2,3] and on the ovarian structures [3]. The biometry of ovaries appears important as most clinical evaluations utilize manual palpation of the ovaries and its structures for assessment of the stage of reproductive cycle [4,5].

Follicular fluid (FF) is an avascular compartment within the ovary, separated from the peri-follicular stroma by the follicular wall that constitutes a 'blood-follicle barrier' [6]. Follicular fluid is viscous in nature due to mucopolysaccharides. [7]. Follicular fluid contains many proteins, amino acids, sugars, enzymes, (collagenase, hylauronidase, transaminase, alkaline and acid phosphatases), salts, mucopolysaccharides, gonadotropins (LH, FSH, Prolactin), vitamins and steroids [8] and growth factors [9].

FF proteins influence the follicular development and oocyte maturation. FF when used for *in vitro* fertilization (IVF) and *in vitro* maturation (IVM) medium was found to have greater ability to stimulate nuclear and cytoplasmic maturation of oocyte, *in vitro* fertilization and development of embryos [10]. Buffalo FF alone without any supplementation induced good maturation of buffalo oocytes [11]. This positive response was because of the presence of various stimulatory proteins/peptides present in FF.

The identification and role of buffalo FF peptides/ proteins have not been elucidated completely. Detailed knowledge on protein pattern of follicular fluid would be helpful in elucidating the process of oocyte maturation. The present study examined the biometry of the ovaries and also estimated the follicular fluid proteins from buffalo ovaries during different phases of the estrous cycle in buffalo.

Materials and methods

Collection of samples

Buffalo genitalia were collected from abattoir immediately after slaughter and were brought to the laboratory in a thermocol box with ice packs. Genital organs without any gross pathology and at the desired stage were processed further.

Evaluating the stage of reproductive cycle of genitals

For determination of the stage of estrous cycle (Follicular /Luteal phase) the presence or/ absence of CL, color, size and consistency of CL and presence/absence of follicles on the surface of the ovary was utilized as described previously [12,13]. Briefly, the organs were divided into two groups (Follicular phase and Luteal Phase). Within this each group these were subdivided and classified into two groups

Right and Left ovary - Follicular phase
Right and Left ovary - Luteal phase

Follicular phase was characterized not only by the presence of regressed CL with no vasculature, cream color and hard texture in cut surface but also with at least one 10 mm or above diameter follicle.

Three characteristics were used for identification of luteal phase.

1. Gentalia with disorganized red color CL tissues and ovulation point not covered by surface epithelium.
2. Gentalia in mid luteal-phase had CL tissues that were soft in texture, full of blood vasculature, had covered ovulation point, incomplete folding pattern in cut surface with reddish brown color.
3. Presence of surface vascularization, complete folding in cut surface with moderately hard texture and brown CL color.

Ovarian dimensions

The weight of ovaries was recorded by placing individual ovary on a weighing pan (Sartorius Germany). The length, width and thickness of the ovaries were measured using a vernier caliper.

Follicular evaluations

a) The numbers of ovarian follicles were counted by visual observation.

b) Collection of follicular fluid (FF): In the laboratory, the ovaries were washed thoroughly twice with sterile normal saline before collection of follicular to avoid the contamination of the follicular fluid. Fluid from each follicle was drawn with the help of disposable sterilized syringe fitted with 22 gauge needle**.** For each group of follicles, different needle and syringes were used for the collection of fluid as per the method described [14,15].

Processing and storage of follicular fluid

The follicular fluid recovered from each group of follicles was pooled. The pooled samples of follicular fluid were subjected to centrifugation at 1500 rpm for 15 minutes (Remi C- 23, India), to remove the blood cells, oocytes and granulosa cells.

Phenyl methyl sulfonyl fluoride (PMSF) at the rate of 20mg/ml was added to the cell free FF to prevent proteolysis during storage. The cell free FF samples were stored at -20°C until used for further analysis.

SDS- Page Analysis

For qualitative estimation of protein the follicular fluid was processed as per method described previously [16] with some modification using commercially available kits (Hi – Media, India). Data was analyzed as per standard methods described previously.

Results

Ovarian Dimensions

The mean weight, length, width, thickness and number of follicles in right and left ovaries at different phases of the estrous cycle revealed highest weight and length of left ovary during the luteal phase and highest width and thickness of right ovary during luteal phase (Table. 1**)** whereas the highest number of follicles were observed for the left ovary during the follicular phase. However, no significant ($P<0.05$) difference was observed in the biometry of ovaries

within the phases (follicular or luteal phases) and also between the phases of estrous cycle (Table.1).

Ovarian follicular fluid Proteins

Follicular fluid Proteins with molecular weights ranging from 18.5 to 180 kilodalton (kDa) were found during the present study (Fig. 1, Table. 2). The molecular weights of protein that could be found using the present kit ranged from 11 to 245 kDa hence proteins below 11 kDa and above 245 kDa could not be assayed in the present study.

Comparison of proteins during different reproductive cycle stages.

During follicular phase the proteins with molecular weight of 40-45, 80-85, 180-200 kDa range were absent in both ovaries. In left ovary, proteins with molecular weights of 60-65, 100-120 kDa were absent during the follicular phase. In right ovary, proteins with 120-140 kDa were absent during the follicular phase.

During luteal phase the proteins with molecular weights of 35-40, 60-65, 90-100 and 100-120 kDa range were absent in both ovaries. In left ovary, 180-200 kDa were absent during the luteal phase. Proteins with molecular weights between 50-60 kDa were present in all the stages of estrous cycle.

Table. 1. Biometry of ovaries in different phase of estrous cycle.

Stage of estrous cycle	Ovary	Weight (gm)	Length (mm)	Width (mm)	Thickness (mm)	No. of follicles
Follicular phase	Right ovary	3.73 ± 0.42	20.96 ± 1.12	15.41 ± 0.95	11.30 ± 0.83	7.43 ± 0.95
	Left ovary	3.63 ± 0.49	20.96 ± 1.11	14.20 ± 0.81	10.95 ± 0.73	7.86 ± 1.27
Luteal phase	Right ovary	4.15 ± 0.39	23.31 ± 0.86	15.94 ± 1.03	11.94 ± 0.62	6.75 ± 0.89
	Left ovary	4.18 ± 0.38	22.38 ± 1.52	15.15 ± 0.70	11.72 ± 0.66	7.40± 1.03

Discussion

In the present study no significant ($P<0.05$) difference was observed in the biometry of ovaries within the phases (follicular or luteal phases) and also between the phases of estrous cycle. These findings are similar to those observed by Chandrahasan and Rajasekaran [17] who could not find any significant difference between the biometry of buffalo ovaries on right and left side and also

between stages of the cycle. These authors also mentioned that the mean number of visible follicles between right and left ovaries did not differ significantly in buffalo ovaries.

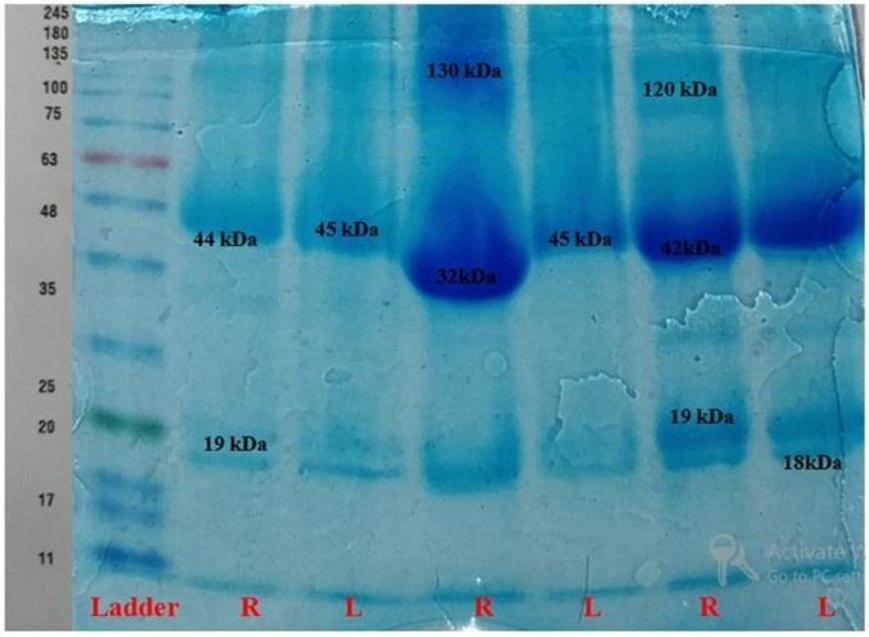

Fig. 1. Destained gel showing molecular weights of ovarian follicular fluid protein band fractions

Neelam and Saigal, [18] examined 53 adult buffalo ovaries during luteal and follicular phases of reproductive cycle. The mean weight and size of right and left ovaries did not differ significantly neither in follicular nor in luteal phase. The ovaries during luteal phase had significantly higher values for weight, volume, length and breadth but not for the thickness.

In the present study a total of 22 different proteins bands were observed corresponding to MWs varying from 15 to 180 kDa during different phases of the estrous cycle. Ovarian follicular fluid proteins with molecular weights between 90- 120 kDa were found during follicular phase but absent in luteal phase. Also four bands, below 25 kDa molecular weight protein were identified. A recent study [19] also found difference in the proteomics of follicular fluid from different sized buffalo ovarian follicles.

Chandrahasan and Rajasekaran [17] observed twenty-eight protein bands from ovarian follicular fluid proteins of which, 10 bands were of molecular weight higher than 97 kDa whereas only four bands were detected below 29 kDa. It was found that almost all these bands had their counterpart in blood serum and it suggests that almost all the follicular protein of the buffalo originated from blood serum.

In the present study mean molecular weights of follicular fluid proteins from the right ovary and left ovary during luteal phase were 42.5 ± 1.04 and 44 ± 1.08, respectively. Findlay et al. [20] identified and mentioned that 42.9 kDa peptide may be a form of inhibin subunit in bovine follicular fluid. Khan et al. [21] reported that there was no apparent difference in SDS PAGE pattern between follicular fluid from small, medium and large follicles of cyclic and acyclic buffaloes.

Conclusions

It was concluded that the biometry of buffalo ovaries is not different during reproductive cycle. In all the stages of estrous cycle proteins with molecular weights from 50-60 kDa are present in the ovarian follicular fluid with high molecular weights proteins common during luteal phases and low molecular weights proteins common during follicular phases.

Table. 2. Mean molecular weight follicular fluid proteins during different phases of estrous cycle.

MW of Protein (kDa)	Follicular Phase		Luteal Phase	
	Right Ovary	Left Ovary	Right Ovary	Left Ovary
15-25	19.5 ± 0.5^{a}	18.5 ± 0.5^{a}	18.5 ± 0.96^{a}	20.75 ± 2.95^{a}
35-40	35 ± 0.2^{b}	39.25 ± 0.47^{b}	-	-
40-45	-	-	42.5 ± 1.04^{c}	44 ± 1.08^{c}
50-60	52.67 ± 2.33^{d}	55.33 ± 0.33^{d}	55.67 ± 0.33^{d}	56 ± 1^{d}
60-65	62.33 ± 0.67^{e}	-	-	-
80-85	-	-	82.5 ± 7.5^{f}	84 ± 8^{f}
90-100	90 ± 0.1^{g}	90 ± 0.2^{g}	-	-
100-120	115 ± 7.9^{h}	-	-	-
120-140	-	125 ± 3.25^{i}	125 ± 0.33^{i}	121.5 ± 1.5^{i}
180-200	-	-	180 ± 0.23^{j}	-

Values with the same superscript in a row are not significantly different P (<0.05).

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References

[1] Purohit GN. Ovarian and oviductal pathologies in the buffalo. Asian Pac J Reprod 2014; ;3(2):156-168.
[2] Kumar S, Ahmed FA, Bhadwal MS. Biometry of female genitalia of Murrah buffalo. Indian J. Anim. Reprod. 2004; 25(2):143-145.
[3] Khandoker MAMY, Jahan N, Asad L, Hoque SAM, Ahmed S, Faruque MO. Qualitative and quantitative analysis of buffalo ovaries, follicles and oocytes in view of in vitro production of embryos. Bang J Anim Sci. 2011; 409 (1-2):23-27.
[4] Qureshi S, Khan IM and Chaudhry RA. Comparitive efficiency of rectal palpation and milk progesterone profiles in diagnosis of ovarian contents in buffaloes. Pak J Agric Res 1992; 13:196-200.
[5] Honparkhe M, Gandotra VK and Nanda AS. Ultrasonographic measurements in comparison with the rectal palpation and echotexture of reproductive organs of buffaloes (Bubalus bubalis) during different stages of the estrous cycle. Asian Aus J Anim Sci 2004; 17:919-923.

[6] Bagavandoss, P., Midgley, A.R. and Wicha, M. Developmental changes in the ovarian follicular basal lamina detected by Immunofluorescence and electron microscopy. J. Histochem. Cytochem. 1983; 31:633-640.
[7] Shalgi R, Kraicer P, Soferman N. Gases and electrolytes of human follicular fluid. J Reprod Fertil. 1972; 28:335-340.
[8] Schweigert FJ, Zucker H. Concentration of vitamin A, beta-carotene and vitamin E in individual bovine follicles of different quality. J Reprod Fertil. 1988; 82:575-579.
[9] Spicer LJ, Geisert RD. Concentrations of insulin-like growth factor-I, estradiol and progesterone in follicular fluid of ovarian follicles during early pregnancy in cattle. Theriogenology 1992; 37:749–760.
[10] Satitmanwiwat S, Changsangfah C, Faisaikarm T, Promthep K, Thammawung S, Saikhun K, Kaeoket K. Proteome profiling of bovine follicular fluid-specific proteins and their effect on in vitro embryo development. J. Vet. Med. Sci. 2017; 79(5):842–847.
[11] Gupta PSP, Nandi S, Ravindranatha BM and Sarma PV. Effect of buffalo follicular fluid alone and in combination with PMSG and M199 on in vitro buffalo oocyte maturation. Asian-Aust. J. Anim. Sci. 2001; 14:693-696.
[12] Roy SC, Suganthi UR, Ghosh J. Changes in uterine protein secretion during luteal and follicular phases and detection of phosphatases during luteal phase of estrous cycle in buffaloes (Bubalus bubalis). Theriogenology 2006; 65:1292-1301.
[13] Kumar D, Purohit GN. Uterine luminal fluid proteins in buffalo during follicular and luteal stages and early pregnancy. J. Anim. Health Prod. 2018; 6(1):41-46.
[14] Leroy JLMR, Vanholder T, Delanghane JR, Opsomer G, Van-Soom A, Bols PEJ, De Kruif A. Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim. Reprod. Sci. 2004; 80:201–211
[15] Sowmya N. Ovarian antral follicular dynamics in Ankamali pigs (M.V.Sc. Thesis) submitted to Karnataka Veterinary Animal and Fisheries Science University, Bidar, India.2010.
[16] Nandi M, Lewis GE . Thin-layer acrylamide gel electrophoresis. J. Clin. Path. 1970; 23:727-729.
[17] Chandrahasan C. Rajasekaran J. Biometry of buffalo (Bubalus bubalis) ovaries in relation to different stages of the oestrous cycle. Indian J. Anim. Reprod. 2004; 25(2): 87-90.
[18] Neelam B, Saigal RP. Gross morphological studies on the ovaries of Indian buffalo (Bubalus bubalis). Agric. Res. J. 2012; 42 (2):224 - 227.
[19] Marques NFS, Codognoto VM, de Souza FF, Scott C, Janini LCZ, Brochine S, Tironi SMT, et al. Proteomics of follicular fluid from buffaloes (Bubalus bubalis): Unraveling the secrets of follicular development, Livestock Sci. 2022, 260:104947.
[20] Findaly JK, Russell DL, Doughton B, Tsonis CG, Borchers C, Forage RG. Effect of active immunization against the amino-terminal peptide (alpha N) of the alpha 43 kDa subunit of inhibin (alpha 43) on fertility of ewes. Reprod. Fertil. Dev. 1994; 6:265-267.
[21] Khan FA, Das GK, Pande M, Singh R, Ghosh SK. Evaluation of follicular atresia and electrophoretic pattern of follicular fluid proteins in acyclic buffalo (Bubalus bubalis). Buffalo Bull. 2013; 32(4):265-269.