RESEARCH ARTICLE
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Some biochemical constituents in follicular fluid of indigenous cows of Assam

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Received: 28-07-2014, **Revised:** 02-10-2014, **Accepted:** 09-10-2014, **Published online:** 19-11-2014

doi: 10.14202/vetworld.2014.976-979. **How to cite this article:** Deka SS, Kalita DJ, Sarma S, Dutta DJ (2014) Some biochemical constituents in follicular fluid of indigenous cows of Assam, *Veterinary World* 7(11): 976-979.

Abstract

Aim: Estimation of some biochemical constituents in follicular fluid of non-descript cows of Assam.

Materials and Methods: Twenty five pairs of ovaries were collected from local slaughter house and the follicular fluid was aspirated from small (2-4 mm), medium (4-6 mm) and large (6-9 mm) follicles. Aspirated fluid samples were centrifuged at 4000 rpm for 20 min in a refrigerated centrifuge to remove granulosa cells and other cell debris. Supernatant was used for estimation of glucose, total protein, cholesterol, acid phosphatase and alkaline phosphatase. Data generated in the study were analyzed statiscally by SPSS (version 16.0). SPSS South Asia Pvt. Limited, Kacharakanahalli, Bangalore, 560043).

Results: A non significant difference was recorded in case of total protein and cholesterol of follicular fluid of small, medium and large sized follicles of cow. However, the glucose level significantly ($p<0.01$) increased with the increase of follicular size where as the activity of acid phosphatase and alkaline phosphatase significantly ($p<0.01$) decreased with increase of size.

Conclusion: Certain biochemical constituents and enzyme activities of follicular fluid changes with the growth of follicles in non-descript cows of Assam. The glucose concentration increased with the growth of the follicles while acid phosphatase and alkaline phosphatase levels had an inverse relation with the size of the ovarian follicles.

Keywords: biochemical parameters, follicles, follicular fluid, oocytes maturation, phosphatase.

Introduction

Follicular fluid is an avascular component within the mammalian ovary, separated from the peri follicular stroma by the follicular wall that constitutes a “blood-follicle barrier” [1]. Follicular fluid is composed of transudate of serum and locally produced substances, which are related to the metabolic activity of follicular cells. The increase in size of follicles from early antral to graafian stage is due to the accumulation of follicular fluid in antral cavities [2]. Metabolite composition of the follicular fluid, which presents the intrafollicular environment, may be an important factor affecting oocyte maturation and subsequent early embryo development [3]. The constituents of follicular fluids are considered as a regulating factor in follicular development and steroidegenesis [4,5].

Follicular fluid compositions are frequently studied to increase knowledge of follicular development, oocyte maturation and follicular atresia [6]. Follicular fluid provides an important environment for maturation of oocytes in ovary and the concentration of different biochemical components of the follicular fluid is major driving force for the physiological, biochemical and metabolic aspects of the nuclear and cytoplasmic maturation of the oocyte and the release of the egg from the ruptured follicle and fertilization [7]. The knowledge of the biochemical composition of follicular fluid can also provide useful information about the requirements, growth and maturation of oocyte and which may be used as a provisional guide for formulating suitable culture media for *in vitro* cell culture and maturation in a particular species.

Considering all these vital role of follicular fluid, present study was undertaken to elucidate the concentration of certain biochemical components namely glucose, total protein, cholesterol, acid phosphatase and alkaline phosphatase in relation to follicle size in non-descript cows of Assam.

Materials and Methods

Ethical approval

The experiment was carried in accordance to guidelines laid down by the Institutional Animal Ethics Committee (770/ac/CPCSEA/FVSc/AAU/IAEC/11-12/91) and in accordance to social laws and regulation.

Sample collection

The study was performed at College of Veterinary Sciences, Khanapara, Assam from January 2012 to June 2012. The ovaries were collected from apparently healthy 25 adult, indigenous cows of unknown reproductive history from local slaughter house. Prior to slaughter, the animals were examined per rectal and animals with no apparent clinical abnormalities in reproductive tracts were selected for the study. Post slaughter the ovaries were observed to determine the reproductive stage. The presence of corpus luteum in the ovaries suggested that the animals were cyclic and in diestrus stage. The non-cystic ovaries were excised immediately after slaughter and transported to the laboratory in normal saline (4°C) containing 0.06 g penicillin G per 100 ml in thermo flask. The follicles were washed thoroughly after collection with normal saline and the diameters of the follicles were taken following standard protocol and categorized as small (2-4 mm), medium (4-6 mm) and large (6-9 mm). The follicular fluid was aspirated from all these three categories of follicles by holding them with forceps and using 1 ml syringe with 20 gauge needle. According to the size of the follicles, the fluids were pooled in three groups and centrifuged at 4000 rpm for 20 min in a refrigerated centrifuge to remove granulosa cells and other cell debris. The supernatant was used for analysis of different biochemical parameters immediately.

Biochemical analysis

The pooled follicular fluid as per the size of the follicles were used for analysis of biochemical parameters namely glucose, total protein, cholesterol, acid phosphatase and alkaline phosphatase. The biochemical parameters were estimated using commercially available kits (Coral Company, Mumbai, Maharashtra, India) following manufacturer's protocol.

Statistical analysis

Data generated from the study were analyzed by (analysis of variance, Latin square design). Computer assisted software; SPSS (version 16.0) SPSS South Asia Pvt. Limited, Kacharakanahalli, Bangalore, 560043 was used for analysis of data.

Results

The concentration of different biochemical constituents (mean±standard error values) are presented in Table-1.

Total protein

The mean total protein concentration in follicular fluid of small, medium and large follicles was found to be 59. 94±1.45, 57.10±3.53 and 53.76±0.99 mg/ml respectively. Statistical analysis revealed that there was no significant difference of total protein concentration between different sized follicles.

Cholesterol

The mean concentrations of cholesterol in follicular fluid of small, medium and large size follicles and were found to be 2.57±0.41 (mg/ml), 2.73±0.46 (mg/ml) and 2.36±0.62 (mg/ml) respectively with no significant difference in cholesterol levels of different categories of follicles.

Glucose

The glucose concentrations (mg/ml) in small, medium and large follicles was found to be 0.32±0.04, 0.47±0.03 and 0.63±0.02 respectively. The level of glucose observed in follicular fluid of large follicles was highly significant ($p<0.01$) from follicular fluid of small and medium size.

Acid phosphatase

The acid phosphatase activity was found to be 5.87±0.52, 2.65±0.43 and 2.84±0.38 KA units in small, medium and large follicles respectively. The activity was significantly higher ($p<0.01$) in the follicular fluid of small follicles as compared to medium and large follicles. However, no significant difference in activity of acid phosphatase was observed between the follicular fluid of the medium and large follicles.

Alkaline phosphatase

Alkaline phosphatase activity (KAU) in bovine follicular fluid was found to be 55.49±5.27, 25.37±3.75 and 11.97±0.33 KA unit in follicular fluid of small, medium and large follicles and there was significant difference in activity of alkaline phosphatase in small, medium and large follicles. However, the activity of small follicles was highly significant ($p<0.01$) from medium and large follicles.

Discussion

Total protein

The mean total protein concentration in follicular fluid of small, medium and large follicles was found to be 59.94±1.45, 57.10±3.52 and 53.76±2.99 mg/ml respectively. In the present investigation, a non-significant decreasing trend of total protein concentration was recorded with increase size of the follicles. The total protein concentration in ovarian follicular fluid of our experiment was correlated with the previous findings [8-11] indicating that protein concentration may not have a specific impact on the process of follicular development. However, in contrast to our findings a

Table-1: Biochemical constituents and phosphatase activity (mean±SE) in fluid of different sized bovine follicles.

Parameters	Size of the follicles			F value
	Small	Medium	Large	
Total protein (mg/ml)	59.94±1.45	57.10±3.52	53.76±2.99	1.221
Cholesterol (mg/ml)	2.36±0.62	2.57±0.41	2.73±0.46	0.1293
Glucose (mg/ml)	0.32±0.04^{a}	0.47±0.03^{b}	0.63±0.02^{c}	22.6818**
Acid phosphatase (KAU/ml)	5.87±0.52^{c}	2.65±0.43^{ab}	2.84±0.38^{a}	16.3854**
Alkaline phospahatse (KAU/ml)	55.49±5.27^{c}	25.37±3.75^{b}	11.97±0.33^{a}	35.5157**

Means±SE in the same row with different superscripts are significant ($p<0.01$); **Significant at $p<0.01$; SE= Standard error.

non significant increase in total protein of large follicles was reported by several workers [12-15]. The maintenance of equilibrium between plasma and follicular fluid is necessary in respect of protein concentration [16] and this justified for approximately equal protein concentration in different size follicles in our present findings. A non significant low concentration of protein in the large follicles of the present experiment might be due to the utilization of the high amount of protein in metabolic activities of the follicular cell during steroid synthesis.

Cholesterol

The mean concentrations of cholesterol in follicular fluid of small, medium and large size follicles were found to be 2.36±0.62, 2.57±0.41, 2.73±0.46 mg/ml respectively. Statistical analysis of the cholesterol concentration revealed non-significant variation among the follicular fluid of different size follicles. However, the mean level of cholesterol concentration in follicular fluid was observed highest in large follicles followed by medium and small follicles. Increase trend of cholesterol concentration with follicular size was in agreement with the earlier findings [5,6,14] in cattle. Cholesterol has a significant role in ovarian functions as the precursor for steroid synthesis and the follicular fluid contains only high-density lipoprotein, therefore, the avascular granulosa cells of the follicles totally depend on the cholesterol from high-density lipoprotein, which is derived from the blood plasma by crossing the basement membrane of granulosa cells. Cholesterol is the precursor for steroid synthesis and increased concentration of cholesterol in large follicles reflected the increasing demand of cholesterol for steroid synthesis in this category of follicles [10].

Glucose

The glucose concentration (mg/ml) was observed highest in large follicles (0.63±0.02) followed by medium (0.47±0.03) and small (0.32±0.04). The difference in concentration of glucose was highly significant ($p<0.01$) from one another. The findings were in accordance with earlier workers [13,17-22] who observed that with the increase of follicle size, the glucose levels in the fluid also increase in cows, buffaloes and sheep. In a study on dromedary camels it was revealed that glucose concentrations were significantly higher in the large follicles than in small ones, irrespective of the reproductive status [23]. Glucose metabolism is less intense in larger follicles as compared with smaller ones, resulting in lower consumption of glucose from fluid of large follicles [18]. Perhaps this is because the large follicles can filter and reserve the high concentrations of glucose from the blood for utilization in their development to the mature Graafian follicle [12]. Another reason for higher glucose concentration in large follicles might be due to increased permeability of glucose in the blood follicle barrier during follicular growth [1].

Phosphatase

Acid phosphatase activity (KAU) was significantly higher ($p<0.01$) in the follicular fluid of small follicles (5.87±0.52) as compared to medium (2.65±0.43) and large follicles (2.84±0.38). Alkaline phosphatase activity (KAU) in bovine follicular fluid was found to be highest in small follicles (55.49±5.27) followed by medium (25.37±3.75) and large follicles (11.97±0.33). Statistical analysis revealed that alkaline phosphatase activities were highly significant ($p<0.01$) from one another. The activity of acid phosphatase was significantly higher ($p<0.01$) in the follicular fluid of small follicles as compared to medium and large follicles. However, no significant difference in activity of acid phosphatase was observed between the follicular fluid of the medium and large follicles. Similar result pertaining to activity of acid phosphatase was also recorded in buffalo follicular fluid [24-28]. It is thought that follicular acid and alkaline phosphatase activity might be an excellent indicator of atrophy due to lysosomal enzyme effect upon phosphorylated receptor, which would lead to atresia. It is reported that the high concentration of phosphatase activity in follicular fluid of small antral follicles might limit their activity to respond to gonadotropin stimulation [29]. Wise [9], also recorded the correlation of phosphatase in follicular fluid with follicular progesterone, androgens, dehydroepiendosterone and testosterone level. Acid and alkaline phosphatase are lysosomal enzymes that catalyze various reactions in the body and are involved in the active transport of phosphates across the cell membrane, synthesis of protein and DNA turnover in nucleus [6]. A higher concentration of alkaline phosphatase activity in small antral follicles was seen in goats [30] which might limit their ability to respond to gonadotropin and leading to atresa. The higher alkaline phosphatase activity in the initial stages of follicular development might be due to a progesterone and androgen dominant environment that exits in the small follicle, in that a higher concentration of progesterone and androgen could be conducive to phosphatase activity [31]. The decreased follicular fluid alkaline phosphatase activity with the development of the follicle in the present study could be due to the shift in the follicular hormonal milieu from androgen to estrogen dominant, with the development of the follicle.

Conclusion

In this study, some biochemical constituents of bovine follicular fluid of slaughter house ovaries with follicles categorized as small (2-4 mm), medium (4-6 mm) and large (6-9 mm) were estimated in indigenous cows of Assam. The glucose concentration increased with the growth of the follicles while acid phosphatase and alkaline phosphatase levels had an inverse relation with the size of the ovarian follicles. Thus from the present experiment it can be concluded that the concentration of certain biochemical constituents and enzyme activities fluctuate with follicle size and growth process.

Authors' Contributions

SSD, DJK, SS and DJK planned and designed the study. SSD collected the samples, performed test and analysed the data. All authors participated in draft and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors owe a sincere and earnest thankfulness and indebtedness to Assam Agricultural University, Guwahati and Department of Biotechnology, Government of India for providing the facilities and financial support respectively to conduct the research works smoothly.

Competing Interests

The authors declare that they have no competing interests.

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