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ORIGINAL ARTICLE

Biochemical composition of blood plasma and follicular fluid in relation to follicular size in buffalo

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Abstract The aim of this study was to examine the biochemical composition of follicular fluid from different-sized follicles and its relationship with that of blood plasma in buffalo. Ovaries of adult and healthy buffaloes were collected after slaughter. Follicular fluid was aspirated from three size classes of follicles (4–5, 6–9, and 10–20 mm diameter). Blood samples were also collected from these buffaloes immediately before slaughter. The follicular fluid and blood plasma samples were analyzed for metabolites (glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea, and creatinine), ions (calcium and phosphorus), and enzymes (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase). The follicular fluid calcium, urea, creatinine, albumin, and ASAT and ALAT concentrations were not affected by the size of the ovarian follicles. Follicular fluid concentrations of phosphorus, cholesterol, triglyceride, ALK, and LDH decreased from small to large follicles. Phosphorus, albumin, and LDH concentrations in plasma were significantly lower than the levels in all follicle classes. The plasma concentrations of glucose, creatinin, cholesterol, triglyceride, protein, and glubolin were higher than in the small, medium, and large follicles. The concentration of glucose in the small follicles was significantly lower than in the medium and large follicles. Total protein concentration in fluid of small follicles was significantly higher than in the large follicles. The amount of globulin in medium follicles was higher than in the small and large follicles. The plasma concentration of ALK was significantly lower than in the small and medium follicles. ALK concentration difference between large follicles and plasma was not significant.

Keywords Buffalo · Biochemical composition · Follicular fluid · Blood plasma

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Introduction

Follicular fluid is in part exudates of serum and is also partially composed of locally produced substances, which are related to the metabolic activity of the follicular cells (Gerard et al. 2002). This metabolic activity, together with the barrier properties of the follicular wall, is changing significantly during the growth phase of the follicle (Gosden et al. 1988). Therefore, a different biochemical composition of the follicular fluid in different-sized follicles can be expected. Metabolic changes in blood serum may be reflected in the biochemical composition of follicular fluid (Leroy et al. 2004). As the oocyte and granulosa cells grow and get mature in a biochemical environment that changes from small to large follicles, the metabolite, ion, and enzymatic characteristics of follicular fluid and follicle or oocyte development are highly correlated (Iwata et al. 2006). Calcium plays an important role in the gonadotropic regulation of ovarian steroidogenesis (Carnegie and Tsang 1984). Marginal deficiency of phosphorus causes disturbance in the pituitary–ovarian axis including ovulation (Bhaskaran and Abdullakhan 1981; Das et al. 2002). In the mammalian ovary, the follicular fluid contains proteins and peptides that play an important role in the growth, development, and maturation of oocytes (Mukesh et al. 2004). Phosphatase enzymes have been implicated in both growth and atresia. Phosphatase enzymes are constituents

of follicular fluid from cows (Caravaglios and Cilotti 1957), pigs (McGaughey 1975), and women (Caucig et al. 1972). Alkaline phosphatase is associated with impending atresia (Ryan 1981; Wise 1987) and inversely correlated with follicle size (Wise 1987). Lactate dehydrogenase (LDH) plays an important role in carbohydrate metabolism, and its concentration in follicular fluid is an indicator of follicle requirements to energy (Trivedi and Lall 2004). Because blood profile changes during various reproductive states, it is imperative to study haematological constituents during these states. These changes in haematological constituents are important indicators of the physiological or pathological state of the animal (Ahmad et al. 2003). Before focusing on possible effects of metabolic changes of follicular fluid on oocyte quality, it is necessary to determine physiological concentrations of the most common metabolites in follicular fluid from different-sized follicles and to see to what extent the blood and FF levels of these metabolites are correlated (Arshad et al. 2005). The purpose of the present study was to determine the values of metabolites (glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea, creatinine), ions (calcium and phosphorus), and enzymes (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and LDH) in blood plasma and follicular fluid in relation to follicular size in buffalo.

Material and methods

Collection of ovaries and processing of follicular fluid

Adult buffaloes in good health and with normal reproductive tracts upon macroscopical examination after slaughter were used for this study. Ovaries were collected and immediately wrapped in plastic sheets, placed in an icebox, and taken to the laboratory within 1 hour after slaughter. In the laboratory, each ovary was cleaned of the extraneous tissue. Each ovary was examined for the presence of graafian follicles. Ovaries associated with pregnant buffaloes and those with any pathological lesions were not included in the study. The diameter of various follicles present on each ovary was measured with the help of Vernier calipers. These follicles were placed in three groups according to their diameter, i.e. small (4–5 mm), medium (6–9 mm), and large (10–20 mm). Then, fluid from each follicle was aspirated with the help of a disposable sterilized insulin syringe. For each buffalo and follicle class, a different needle and syringe were used. The fluid collected from various small follicles of each group from the same animal was pooled. Total of 60 samples, with 20 samples for each group, were used for analysis. Follicular fluid samples were stored at -20°C for further analysis. The follicular fluids from three different sizes of follicles were subjected to biochemical analysis (metabolites: glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea, and creatinine; ions: calcium and phosphorus; and enzymes: alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase and LDH).

Collection of blood samples

Jugular blood samples were collected from each buffalo immediately before slaughter in a test tube containing EDTA as an anticoagulant. These tubes were placed in an icebox beside the ovaries and were carried to the laboratory. In the laboratory, these samples were centrifuged at 3,000 rpm for 15 minutes; the plasma was separated and stored at -20°C for further analysis. Plasma samples were subjected to biochemical analysis (metabolites: glucose, total protein, albumin, globulin, cholesterol, triglycerides, urea, and creatinine; ions: calcium and phosphorus; and enzymes: alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and LDH).

Biochemical analysis

The follicular fluid of small, medium, and large follicles and blood plasma samples were analyzed for various metabolites, ions, and enzyme concentrations with photometry method using Auto Analyzer (RA-1000, Technicon).

Statistical analysis

The mean values (± SE) for concentrations of various biochemical compositions of follicular fluid of small, medium, and large follicles and blood plasma were computed. To see the magnitude of variation in concentrations of various biochemical constituents of follicular fluid and plasma, the data were subjected to one-way analysis of variance. Significance between means was tested using Duncan Multiple Range Test (Petrie and Watson 2006).

Results

The values of various biochemical constituents in fluid from small, medium, and large follicles and blood plasma are given in Table 1. The calcium, urea, creatinine, albumin, ASAT, and ALAT contents of the follicular fluid did not differ between follicle classes ($P>0.05$). The concentrations of phosphorus, cholesterol, triglyceride, ALK, and LDH in the follicular fluid decreased ($P<0.05$) as follicle size increased. The plasma concentrations of phosphorus, albumin, and LDH were lower ($P<0.05$) than in small, medium, and large follicles. Contrariwise, glucose,

Table 1 The concentrations of various biochemical compositions in fluid from small, medium, and large follicles and blood plasma in buffaloes

Biochemical composition	Small follicles (4-5 mm)	Medium follicles (6-9 mm)	Large follicles (10-20 mm)	Blood plasma
Calcium (mg/dl)	9.80±0.22[a]	9.63±0.45[a]	9.58±0.18[a]	9.52±0.17[a]
Phosphorus (mg/dl)	12.11±0.78[d]	10.85±0.65[c]	8.53±0.48[b]	6.85±0.33[a]
Glucose (mg/dl)	71.86±4.08[a]	91.50±5.63[b]	95.90±6.75[b]	138.42±8.60[c]
Urea (mg/dl)	30.63±3.47[a]	26.42±3.24[a]	24.61±2.50[a]	30.11±2.63[a]
Creatinin (mg/dl)	1.51±0.07[a]	1.53±0.09[a]	1.50±0.13[a]	1.94±0.11[b]
Cholesterol (mg/dl)	64.58±5.21[c]	52.37±4.77[b]	40.56±3.48[a]	94.21±6.49[d]
Triglicerid (mg/dl)	24.50±1.76[c]	19.00±1.12[b]	14.13±0.90[a]	39.00±2.50[d]
Protein (g/dl)	5.67±0.13[b]	5.53±0.14[ab]	5.14±0.18[a]	7.93±0.19[c]
Albumin (g/dl)	4.15±0.12[b]	3.45±0.12[b]	3.77±0.14[b]	2.42±0.16[a]
Globulin (g/dl)	1.51±0.06[a]	2.09±0.15[b]	1.40±0.15[a]	5.49±0.18[c]
ALK (IU/l)	597.86±17.38[c]	446.86±6.98[b]	255.71±13.51[a]	261.30±18.12[a]
LDH (IU/l)	269.14±5.69[d]	215.71±2.18[c]	167.00±5.27[b]	139.65±47.41[a]
ASAT (IU/l)	332.50±25.44[a]	326.50±19.34[a]	243.25±33.47[a]	230±50.65[a]
ALAT (IU/l)	165.43±30.51[a]	155.83±12.92[a]	128.62±28.03[a]	143±21.75[a]

Values with different superscripts in the same row differ significantly ($P<0.05$)

creatinin, cholesterol, triglyceride, protein, and glubolin concentrations were higher in blood plasma when compared with the follicular fluid. The amount of glucose in small follicles was lower ($P<0.05$) than that of medium and large follicles. The value of total protein in small follicles was higher ($P<0.05$) than in large follicles. Globulin level in medium follicle was higher ($P<0.05$) than that of small and large follicles. The plasma concentration of ALK was higher ($P<0.05$) than in small and large follicles. ALK concentration difference between large follicles and plasma was not significant ($P>0.05$).

Discussion

In this study, glucose concentration in blood plasma of buffaloes was 138.42±8.60 mg/dl, which was higher than that of the finding of Majeed et al. (1990; 40.46 mg/d). In a study by Arshad et al. (2005) and Shahzada (1995), glucose concentration in blood plasma was 123.55±3.5 and 40.93± 26.34 mg/dl, respectively. The difference in the plasma glucose levels of buffaloes in these studies might be due to differences in the nutritional conditions of animals used in these studies. Moreover, the experimental protocol adopted in different experiments can also affect the results (Arshad et al. 2005). We found that glucose concentration in small follicles was significantly lower than that measured in medium and large follicles, which confirms the results of Arshad et al. (2005) and Landau et al. (2000) for buffalo and Leroy et al. (2004) for cattle. The metabolism of glucose is less intense in larger follicles as compared with smaller ones, resulting in lower consumption of glucose from fluid of large follicles (Arshad et al. 2005). Our data also show that the glucose concentration in blood is significantly higher than that in small, medium, and large follicles. This implies that the principal source of follicular fluid glucose is blood, and very little glucose, if any, is synthesized locally by the granulosa cells of follicles (Arshad et al. 2005). Leese and Lenton (1990) has stated that glucose concentrations in follicular fluid in women is a result of both glycolysis taking place in mural granulosa cells and influx of same molecules from the plasma into the fluid. Leroy et al. (2004) also observed that the concentration of glucose in follicular fluid of small follicles was less than half of the level found in serum but only 21% lower in large follicles. Unlike the observations of Arshad et al. (2005) and Leroy et al. (2004), total protein concentration in fluid of small follicles was significantly higher than that in large follicles. In this study, the concentration of protein in blood plasma was significantly higher than the levels in all follicle classes, which was similar to results of Arshad et al. (2005) and Leroy et al. (2004). Similar to the results of Arshad et al. (2005), albumin content of the follicular fluid did not differ between follicle classes. The overall mean plasma glucose concentration was 2.42±0.16 g/dl, which is comparable to results of Arshad et al. (2005; 3.26±0.14 g/dl). Unlike the observations of Arshad et al. (2005), globulin concentration in the fluid of medium follicles was significantly higher than in small and large follicles and blood plasma. In the present study, calcium concentration differences between fluid of small, medium, and large follicles and blood plasma were not significant. In a study by Wise (1987) in cattle, calcium concentration increased significantly with advancement of follicular size. The amount of phosphorus decreased significantly from small to large follicles, which was in contrast to an earlier report in sheep (Nandi et al. 2007). The cholesterol

concentration of follicular fluid decreased with the increase in follicular size, which was in agreement with the findings of Thangavel and Nayeem (2004) in buffalo and Huang et al. (2002) in pigs and in contrary to the study of Leroy et al. (2004) in buffalo, Brantmeier et al. (1987) in cattle, Nandi et al. (2007) in sheep, and Thakur et al. (2003), Bordoloi et al. (2000), and Mishra et al. (2003) in goat. Cholesterol in follicular fluid derived from two sources, cellular de novo synthesis from acetate and uptake from plasma lipoprotein (Nandi et al. 2007). Ovarian alkaline phosphatase activity is quite high relative to other reproductive tissues (Rahi and Srivastava 1983). Alkaline phosphatase activity in the present study was in agreement with the findings of Nandi et al. (2007) in sheep and Bordoloi et al. (1999) and Mishra et al. (2003) in goats. Wise (1987) and Henderson and Cupps (1990) reported that as follicular diameter increased, alkaline phosphatase in follicular fluid was reduced in cattle. In contrast, no difference in alkaline phosphatase activity among follicles of different size was reported (Parmar and Mehta 1991). Alkaline phosphatase is a lysosomal enzyme that catalyzes 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 (Mishra et al. 2003). 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 (Kalmath 2000; Nandi et al. 2007). 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 (Nandi et al. 2007). Increased activity of LDH in follicular fluid indicated early follicular degeneration (Wise 1987; Nandi et al. 2007). In the present study the trend of higher LDH activity in small follicles was observed where atresia was more common, which was in accordance with an earlier study in cattle (Wise 1987). This suggested that as the follicle degenerated, there were distinct biochemical changes that accompanied the degenerative process (Nandi et al. 2007; Banks et al. 1976). In contrary to results of Leroy et al. (2004), in this study, urea concentration difference between various sizes of follicles and blood plasma was not significant. It is concluded that concentrations of various biochemical constituents of follicular fluid in buffalo may be changed with advancing the follicular growth, and also, the concentrations of biochemical constituents can be different between follicular fluid and blood plasma.

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References

Ahmad I, Gohar A, Ahmad N, Ahmad M (2003) Haematological profile in cyclic, non cyclic and endometritic cross-bred cattle. Int J Agric Biol 5:332–334

Arshad HM, Ahmad N, Rahman ZU, Samad HA, Akhtar N, Ali S (2005) Studies on some biochemical constituents of ovarian follicular fluid and peripheral blood in buffaloes. Pak Vet J 25 (4):189–193

Banks P, Barrley W, Britt LM (1976) The biochemistry of the tissues. John Wiley and Sons, New York, pp 291–295

Bhaskaran B, Abdullakhan CK (1981) Role of blood serum inorganic phosphorus in post parturient anoestrous cows. Livest Advis 9:33–36

Bordoloi PK, Sarmah BC, Dutta DJ, Deka BC (1999) Acid and alkaline phosphatase activity in follicular fluid of goat ovary. Indian J Anim Res 33:144–146

Bordoloi PK, Sarmah BC, Dutta DJ, Deka BC (2000) Follicular fluid cholesterol in goat ovary. Indian Vet J 77:638–639

Brantmeier SA, Grummer RR, Ax RL (1987) Concentrations of high-density lipoproteins vary among follicular sizes in the bovine. J Dairy Sci 70:2145–2149

Caravaglios R, Cilotti R (1957) A study of the proteins in the follicular fluid of the cow. J Endocrinol 15:273

Carnegie JA, Tsang BK (1984) The calcium calmodulin system; participation in the regulation of steroidogenesis activity at different stages of granulose cell differentiation. Biol Reprod 30:515–522

Caucig H, Friedrich F, Breitnecker G, Golob E (1972) Enzyme activity in the fluid of the human ovarian follicle. Gynecol Invest 3:215

Das S, Bandopadhya SK, Basu S, Ghosh BB, Dattagupta R (2002) Blood mineral profile of normal cyclic and repeat breeder crossbred cows under rural condition. Indian J Anim Reprod 23:167–169

Gerard N, Loiseau S, Duchamp G, Seguin F (2002) Analysis of the variations of follicular fluid composition during follicular growth and maturation in the mare using proton nuclear magnetic resonance (HNMR). Reprod 124:241–248

Gosden GR, Hunter HF, Telfer E, Torrance C, Brown N (1988) Physiological factors underlying the formation of ovarian follicular fluid. J Reprod Fertil 82:813–825

Henderson KA, Cupps PT (1990) Acid and alkaline phosphatase in bovine antral follicles. J Anim Sci 68:1363–1369

Huang WT, Lu SG, Tang PC et al (2002) Biochemical compositions of follicular fluid and the effects of culture conditions on the in vitro development of pig oocytes. Asian Aust J Anim Sci 15:1403–1411

Iwata H, Inoue J, Kimura K, Kuge T, Kuwayama T, Monji Y (2006) Comparison between the characteristics of follicular fluid and the developmental competence of bovine oocytes. Anim Reprod Sci 91:215–223

Kalmath GP (2000) Biochemical analysis of ovarian antral follicular fluid in buffaloes (M.V.Sc. Thesis). University of Agricultural Sciences, Bangalore, India

Landau S, Braw-Tal R, Kaim M, Bor A (2000) Preovulatory follicular status and diet affect the insulin and glucose contents of follicles in high yielding dairy cows. Anim Reprod Sci 64(3):181–197

Leese HJ, Lenton EA (1990) Glucose and lactate in human follicular fluid: concentrations and interrelationship. Hum Reprod 5:915–919

Leroy JL, Vanholder MRT, Dalanghe JR, Opsomer G, Van Soom A, Bols PEJ, de Kruif A (2004) Metabolite and ionic composition of follicular fluid from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim Reprod Sci 80(3–4):201–211

Majeed MA, Iqbal J, Chaudhry MN (1990) Blood chemistry of clinical metritis in Nili-Ravi buffaloes of two age-groups and at two stages of lactation. Pak Vet J 10(2):55–59

McGaughey RW (1975) A comparison of the fluids from small and large ovarian follicles in the pig. Biol Reprod 13:147

Mishra OP, Pandey JN, Gawande PG (2003) Study on biochemical constituents of caprine ovarian follicular fluid after superovulation. Asian Aust J Anim Sci 16:1711–1715

Mukesh V, Sharma MK, Dheer S (2004) Effect of follicular fluid proteins and gonadotropins on progesterone secretion by buffalo granulosa cells in vitro. Asian Aust J Anim Sci 17:1496–1500

Nandi S, Girish Kumar V, Manjunatha BM, Gupta PSP (2007) Biochemical composition of ovine follicular fluid in relation to follicle size. Develop Growth Differ 49:61–66

Parmar AP, Mehta VM (1991) Effect of seasons on enzyme profiles of follicular fluid and blood serum in Surti buffaloes. Indian J Anim Sci 61:1082–1084

Petrie A, Watson P (2006) Statistics for veterinary and animal sciences, 2nd edn. Blackwell, London. ISBN 1405127813

Rahi H, Srivastava PN (1983) Hormonal regulation of lysosomal hydrolases in the reproduction tract of the rabbit. J Reprod Fertil 67:447

Ryan RJ (1981) Follicular atresia: Some speculations of biochemical markers and mechanisms. In: N.B. Schwartz and M. Hunzicker-Dunn (Ed.) Dynamics of ovarian function. Raven Press, New York

Shahzada N (1995) Haematological, some serum biochemical and pathological aspects of repeat breeding Nili-Ravi buffaloes. MSc Thesis, Univ. Agri Faisalabad Pakistan

Thakur RS, Chauhan RAS, Singh BK (2003) Studies on biochemical constituents of caprine follicular fluid. Indian Vet J 80:160–162

Thangavel A, Nayeem M (2004) Studies on certain biochemical profile of the buffalo follicular fluid. Indian Vet J 81:25–27

Trivedi S, Lall SB (2004) Ovarian dehydrogenases of the non-pregnant, pregnant and lactating *Rhinopoma microphyllum kinneari* (Chiroptera: Rhinopomatidae)

Wise T (1987) Biochemical analysis of bovine follicular fluid: albumin, total protein, lysosomal enzymes, ions, steroids and ascorbic acid content in relation to follicular size, rank, atresia classification and day of estrous cycle. J Anim Sci 64:1153–1169