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STUDIES ON SOME BIOCHEMICAL CONSTITUENTS OF OVARIAN FOLLICULAR FLUID AND PERIPHERAL BLOOD IN BUFFALOES

H. M. Arshad, N. Ahmad, Zia-ur-Rahman[1], H. A. Samad, N. Akhtar and S. Ali

Department of Animal Reproduction and [1]Department of Veterinary Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan

ABSTRACT

This project was designed to study some biochemical constituents of ovarian follicular fluid (FF) and peripheral blood in buffaloes. For this purpose, ovaries were collected from adult buffaloes immediately after slaughter, FF was aspirated and stored at -4°C. Blood samples were also collected from these buffaloes before slaughter, plasma was separated and stored for further analysis. Samples were classified into two groups according to the follicle diameter i.e. small (3-10 mm) and large (11-20 mm). The FF and plasma samples were analyzed for various biochemical constituents, including glucose, cholesterol, total proteins, albumin, globulin, sodium, potassium, calcium, magnesium, zinc, triiodothyronine and thyroxine contents, using commercial kits.

The results showed that small follicles had significantly lower ($P<0.05$) glucose contents than large follicles, while blood plasma had significantly higher ($P<0.05$) glucose contents than fluid from both classes of follicles. The differences in concentrations of cholesterol, total proteins, albumin and globulin between small and large follicles were non-significant. The concentrations of these compounds were higher in the blood than in FF, except albumin, which was higher in FF than in the blood. Contents of electrolytes and trace elements did not vary between the two follicle classes. However, the plasma levels of these electrolytes and trace elements, except potassium and zinc, were significantly higher ($p<0.05$) than their levels in FF. The level of potassium was significantly higher ($P<0.05$) in FF than in the plasma, while serum zinc level did not differ from FF. The differences in concentrations of triiodothyronine and thyroxine in fluid from small and large sized follicles were non-significant. Likewise, the levels of these hormones were non-significantly higher in blood plasma than in FF. It was concluded that FF levels of glucose differed between small and large follicle groups, while blood levels of most of constituents were higher than their levels in FF.

Key words: Biochemical composition, thyroid hormones, follicular fluid, plasma, buffaloes.

INTRODUCTION

Within the ovarian follicle, developing oocyte is surrounded by the follicular fluid (FF). Besides meeting nutritional requirement of the growing oocyte, FF also maintains proper environment for growth and maturation of the oocyte. Follicular fluid is an avascular compartment within the mammalian ovary, separated from the perifollicular stroma by the follicular wall that constitutes a 'blood-follicle barrier' (Bagavandoss *et al.*, 1983). Besides a transudate of serum, FF is partially composed of locally produced substances, which are related to the metabolic activity of 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 (Bagavandoss *et al.*, 1983; Wise, 1987; Gosden *et al.*, 1988). Therefore, a different biochemical composition of the FF in different sized follicles can be expected.

Changes in biochemical constituents of blood are important indicators of physiological state of an animal (Perveen and Usmani, 1993). Similarly, structural and functional integrity of an oocyte is a pre-requisite for its fertilization by a sperm. O'Callaghan and Boland (1999) has suggested that low fertility in high-yielding dairy cattle is mainly a problem of inferior oocyte and embryo quality, rather than being the result of a disruption in gonadotropin secretion. Since changes in concentrations of gonadotropin secretion, steroids and growth factors in FF of dairy cows have been linked with alterations in oocyte quality (Izadyar *et al*., 1997; Driancourt and Thuel, 1998), it is quite possible that metabolites which are present in the FF can influence oocyte quality.

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 FF from different sized follicles and to see to what extent the blood and

FF levels of these metabolites are correlated. Therefore, the present project was planned to study some biochemical, hormonal and trace elements profiles of fluid collected from follicles of different sizes. Attempts were also made to determine if there is any relationship between biochemical, hormonal and trace elements profiles of plasma with those of FF.

MATERIALS AND METHODS

Collection of ovaries

Ovaries of buffaloes slaughtered during the period from March to June, 2004 were collected. These ovaries were wrapped in plastic sheets, placed in an icebox and taken to the laboratory within 2 hours after slaughter. No pre-slaughter information regarding the nutritional or reproductive status of these buffaloes was available.

Ovarian examination

Each ovary was examined for the presence of Graafian follicles. The diameter of various follicles (3-20 mm in diameter) present on each ovary was measured with the help of vernier calipers. These follicles were placed in two groups according to their diameter, i.e. small (3-10 mm) and large (11-20 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 was used. The fluid collected from various small follicles of each group from the same animal was pooled. Thus, a total of 20 samples, with 10 samples for each group, were available for analysis. This fluid was stored at –4°C for further analysis.

Collection of blood samples

About 10 ml of jugular blood samples were collected from each buffalo at the time of slaughter in a test tube containing EDTA (ethylene diamine tetra acetic acid) as an anticoagulant. These tubes were placed in an icebox and carried to the laboratory. In the laboratory, these samples were centrifuged at 3000 rpm for 10 minutes, the plasma was separated and stored at –4°C for further analysis.

Biochemical analysis

The FF and blood plasma samples were analyzed for various biochemical metabolites (glucose, cholesterol, total proteins, albumin and globulin), electrolytes and trace elements (sodium, potassium, calcium, magnesium and zinc) and thyroid hormones (triiodothyronine and thyroxine), using appropriate commercial kits. Glucose was determined by following enzymatic kinetic colorimetric test. Cholesterol contents were measured by using enzymatic colorimetric method of Linear Chemic B279. Total protein contents were measured through colorimetric method using total proteins kit (Cat No. 530, Bio Rays). Albumin was measured by colorimetric method, using albumin kit (Cat. No. 530, BioRays). The globulin concentrations in FF and plasma were measured by subtracting albumin concentration from total proteins concentration.

Follicular fluid and blood plasma samples were also analyzed for electrolytes and trace elements. For this purpose, samples were subjected to wet digestion following method of Richards (1968). Concentrations of sodium and potassium were determined using the flame photometric method, while concentrations of zinc, calcium and magnesium were measured using atomic absorption photometer. Triiodothyronine and thyroxine concentrations were determined by using enzyme immunoassay kits (BioCheck BC-1007).

Statistical analysis

The mean values (± SE) for concentrations of various biochemical constituents of follicular fluid of small and large follicles and the blood plasma were computed. In order to see the magnitude of variation in concentrations of various biochemical constituents of FF and plasma, the data were subjected to one-way analysis of variance using completely randomized design (Steel and Torrie, 1982). Significance between means was tested using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Mean concentrations of various biochemical constituents in fluid from small and large follicles and blood plasma are given in Table 1.

Glucose

The overall mean concentration of glucose in blood plasma was 123.55 ± 3.5 mg/dL. Serum glucose concentration determined in buffaloes by Majeed *et al.* (1990) was 40.46 mg/d, which is much lower than the value observed in the present study. In a later study, Shahzada (1995) confirmed the above observation, as he recorded the serum glucose concentration of 40.93 ± 26.34 mg/dL in healthy buffaloes. The difference in the plasma glucose levels of buffaloes among these studies might be due to differences in the nutritional status of animals used in these studies. Moreover, the experimental protocol adopted in different experiments can also affect the results.

Fluid from small follicles had significantly lower ($P<0.05$) glucose concentration (30.75 ± 5.50 mg/dL) than that from large follicles (42.95 ± 4.54 mg/dL). This shows that glucose concentration increases as the follicle diameter increases. Similar observations were made by Landau *et al.* (2000), who reported that preovulatory and subordinate follicles contained 1.02 and 0.450 mg/mL of glucose, respectively ($P<0.0001$). Leroy *et al.* (2004) also observed an increase in FF glucose level, and a decrease in its metabolite lactate, as follicular size increased. This could mean that glucose metabolism is less intense in larger follicles as compared with smaller ones, resulting in lower consumption of glucose from fluid of large follicles

Table 1: Mean (± SE) concentrations of various biochemical constituents in fluid from small and large follicles and blood plasma in buffaloes

Biochemical constituents	Small follicles	Large follicles	Blood plasma
Glucose (mg/dL)	30.75 ± 5.50c	42.95 ± 4.54b	123.55 ± 3.51a
Cholesterol (mg/dL)	102.12 ± 4.11b	108.19 ± 5.25b	134.30 ± 4.55a
Total proteins (g/dL)	6.30 ± 0.32b	6.41 ± 0.30b	7.68 ± 0.34a
Albumin (g/dL)	3.94 ± 0.26a	4.10 ± 0.14a	3.26 ± 0.14b
Globulin (g/dL)	2.82 ± 0.31b	2.43 ± 0.24b	4.39 ± 0.39a
Sodium (mg/dL)	33.31 ± 0.76b	35.18 ± 0.77b	47.24 ± 0.56a
Potassium (mg/dL)	4.94 ± 0.10a	4.98 ± 0.09a	4.57 ± 0.04b
Calcium (mg/dL)	3.11 ± 0.29b	2.72 ± 0.07b	3.84 ± 0.13a
Magnesium (mg/dL)	5.07 ± 0.19b	5.22 ± 0.23b	8.02 ± 0.30a
Zinc (mg/dL)	0.14 ± 0.02a	0.22 ± 0.05a	0.31 ± 0.09a
T3 (ng/mL)	3.28 ± 0.30a	2.90 ± 0.26a	2.48 ± 0.31a
T4 (ug/dL)	14.96 ± 0.97a	16.10 ± 1.09a	14.00 ± 1.47a

Values with different letters within a row differ significantly ($p<0.05$).

(Leroy *et al.,* 2004). An increasing amount of FF is a second explanation for the increase in glucose in large follicles, since in large follicles a relatively smaller number of granulosa cells consume glucose from a relatively larger amount of FF (McNatty *et al.,* 1978; Gosden *et al.,* 1988). An other reason for higher glucose content in large follicles could be the increased permeability of the blood follicle barrier during follicular growth (Bagavandoss *et al.,* 1983). Consequently, equilibrium between the vascular component and FF can be achieved more easily in larger than small follicles (Leroy *et al.,* 2004).

The results of the present study also indicated that the glucose level in blood plasma was significantly higher ($P<0.05$) than the FF in both the groups (Table 1). This implies that the principal source of FF glucose is blood and very little glucose, if any, is synthesized locally by the granulosa cells of follicles. Leese and Lenton (1990) has stated that glucose and lactate concentrations in FF 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 FF of small follicles was less than half of the level found in serum but only 21 percent lower in large follicles.

Cholesterol

Cholesterol plays a significant role in the physiology of the ovary, as it is the precursor of steroid hormones secreted by this organ. In the present study, cholesterol concentrations in FF of large follicles were higher than that of small follicles, however, the difference was non-significant (Table 1). These results are not in agreement to those reported for dairy cows by Leroy *et al.* (2004), who observed a significant increase of the total cholesterol contents from small to large follicles. Whether this discrepancy is due to species differences, or otherwise, is not clear.

Leroy *et al.* (2004) also noted that serum concentrations of cholesterol were significantly higher ($P<0.05$) than in small, medium and large sized follicles. The average cholesterol levels in all follicular classes were 41 percent of level found in serum. Similar findings were observed in the present study, where plasma cholesterol level was higher ($P<0.05$) than that found in small or large follicles.

The mean cholesterol concentration in plasma of buffaloes observed in this study was 134.30 ± 4.55 mg/dL. Shahzada (1995) reported significantly lower cholesterol concentration (63.90 ± 20.07 mg/dL) in normal as compared to that of repeat breeder buffaloes (68.87 ± 26.60 mg/dL).

Total proteins

The difference in total protein contents between the two follicular classes was non-significant (Table 1). This indicates that the follicular contents of total proteins do not change with the follicular growth. Similar findings have been reported in dairy cows by Leroy *et al.* (2004).

In the present study, it was also observed that the total protein contents of blood plasma were significantly higher than those of small, as well as large, follicles. Similarly, Leroy *et al.* (2004) observed that serum contents of total proteins were significantly higher ($P<0.05$) than in small, medium and large follicles. According to these workers, the average total protein concentrations found in all follicular classes were 75-80 percent of the concentration found in serum. According to Wise (1987), the high correlation between total protein contents in follicular fluid and serum suggests that a substantial part of the protein contents in follicular fluid originates from the serum.

Albumin

The albumin contents in the fluid from small follicles did not differ from those of large follicles (Table 1). This indicates that follicular growth does not seem to have any effect on its albumin contents. This study also indicated that blood plasma contained significantly lower albumin level than FF ($p<0.05$).

This higher albumin level in FF than in plasma suggests an active inward transport of this compound from blood into the follicles which may be required for binding some chemicals as well as minerals inside the follicular fluid for various physiological functions including growth and maturation of follicles.

The overall mean plasma albumin level in buffaloes was 3.26 ± 0.14 g/dL. These values are comparable to some earlier reports. Hamza and El-Abdin (1976) recorded the albumin level in blood of normal healthy buffalo as 3.4 g/100 mL, while Majeed *et al.* (1983) calculated it as 2.70 ± 0.04 g/100 mL.

Globulin

Globulin has a significant importance in the body due to its immunity producing activity. The mean globulin concentration in small sized follicles was 2.82 ± 0.31 g/dL, while for large sized follicles, this value was 2.43 ± 0.24 g/dL, the difference between two follicular classes was non significant. A significant difference was observed between levels of globulin in FF and plasma, the latter contained higher globulin than the former. This shows that the level of albumin was higher, while that of globulin was lower, in FF than the blood. The globulin present in FF, though in small quantity, might be necessary for protecting the follicle from external environments.

Electrolytes and trace elements

The mean plasma sodium level was 47.24 ± 0.56 mg/dL, while Singh *et al.* (1983) recorded the blood sodium level in buffaloes as 134.66 ± 2.72 meq/liter. There was no effect of follicular size on FF sodium contents, although the value was higher in large than in small follicles. However, plasma sodium concentration was significantly higher than that in FF of small, as well as large, follicles (Table 1).

Potassium levels in small and large sized follicle classes in buffaloes were 4.94 ± 0.10 and 4.98 ± 0.09 mg/dL, respectively., the difference was non significant. In a recent study on dairy cows, Leroy *et al.* (2004) recorded the value of potassium in small, medium and large sized follicle classes as 10.1 ± 0.21, 7.90 ± 0.28 and 6.00 ± 0.23 mM, respectively. Similarly, plasma potassium level in this study was 4.57 ± 0.04 mg/dL. Leroy *et al.* (2004) reported blood potassium concentration of 5.00 ± 0.10 mM in dairy cows.

In our study, potassium level in FF was significantly higher ($P<0.05$) than the plasma levels. Similar observations were made in cows by Leroy *et al.* (2004). This concentration gradient for potassium between serum and follicular fluid suggests an active inward transport of this cation (Gosden *et al.*, 1988). According to these workers, no correlation of follicular potassium contents with serum was found, indicating that potassium levels in follicular fluid may also be the result of local metabolism. It is also important to consider postmortem changes which can induce potassium concentration by leakage from damaged cells.

The mean calcium concentration in plasma of buffaloes included in this study was 3.84 ± 0.13 mg/dL and the values of plasma calcium in small and large sized follicular group of animals were 3.11 ± 0.29 and 2.72 ± 0.07 mg/dL, respectively ($P>0.05$). There was a significant difference between plasma and FF calcium levels, the values being higher in the blood than in FF from either follicle class.

The mean plasma magnesium concentration in buffaloes was 8.02 ± 0.30 mg/dL. Prasad and Rao (1998) investigated the blood magnesium level as 1.88-25 mg/100 mL in anoestrus and repeat breeder crossbred cows, while 2.38 µg/100 mL in normal cows. In buffaloes included in the present study, there was significantly higher ($P<0.05$) concentration of magnesium in plasma as compared to FF from each sized follicle. However, the difference in FF magnesium concentration between small and large follicles was non significant.

Leonhard (2000) has reviewed that zinc ions are involved in the process of cell division, development and differentiation and in the control of gene expression. Zinc deficiency can affect spermatogenesis as well as embryonic development and parturition. In this study, zinc contents in the fluid from small and large follicles differed non-significantly. Similarly, zinc contents in the plasma and follicular fluid were the same (Table 1).

Thus, the concentrations of none of the electrolytes or trace elements included in this study differed between small and large follicles. Whether these elements play any significant role in the follicular development in the buffalo is not clear. The concentrations of sodium, calcium and magnesium were higher in blood plasma than in FF, while reverse was true for potassium. Zinc concentrations did not differ between blood and FF. The physiological significance of this profile of electrolytes and trace elements in plasma and FF remains to be investigated.

Thyroid hormones

In this study, triiodothyronine levels in small sized follicles was 3.28 ± 0.30 ng/mL and 2.90 ± 0.26 ng/mL for large sized follicle class. For thyroxine, these levels were 14.96 ± 0.97 and 16.10 ± 1.09 µg/dL in small and large follicles, respectively. The differences in the contents of two hormones between the two follicle classes were non-significant (Table 1).

Mean plasma value of triiodothyronine in buffaloes was 2.48 ± 0.31 ng/mL, while for thyxoine this value was 14.00 ± 1.47 µg/dL. According to Campos (1995), blood concentrations of triiodothyronine averaged 1.36, 1.34 and 1.38 nmol/L, while for thyroxine these values were 40.56, 38.72 and 39.39 nmol/L for crossbred, Brownswiss and Holstein cows, respectively.

Manalu *et al.* (1997) analyzed blood thyroid hormones of goat during different stages of pregnancy. Mean concentrations of triiodothyronine and thyroxine were lower in aborting (68.25 and 47.76 ng/mL), twin bearing (75.26 and 39.31 ng/mL) and single bearing goats (71.76 and 45.78 ng/mL) than in non-pregnant

goats (111.62 and 19.02 ng/mL). However, it is clear that levels of triiodothyronine observed in non-pregnant goats were higher than those recorded in the buffaloes in this study. This might be due to species differences.

Based on the findings of the present study it was concluded that FF glucose concentration was higher in large than in small follicles. Moreover, blood levels of most of biochemical constituents were higher than their levels in FF.

REFERENCES

Bagavandoss, P., A. R. Midgley and M. Wicha, 1983. Developmental changes in the ovarian follicular basal lamina detected by immunofluorescence and electron microscopy. J. Histochem. Cytochem., 31: 633-640.

Campos, G. R., 1995. Triiodothyronine and thyroxine values and reproductive performance in cattle of different breed types. Acta-Agronomica, Universidad Nacional de Colombia, 45(2-4): 140-145.

Driancourt, M. A. and B. Thuel, 1998. Control of oocyte growth and maturation by follicular cells and molecules present in follicular fluid. A review. Reprod. Nutr. Develop., 38: 345-362.

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

Gosden, R. G., R. H. F. Hunter, E. Telfer, C. Torrance and N. Brown, 1988. Physiological factors underlying the formation of ovarian follicular fluid. J. Reprod. Fert., 82: 813-825.

Hamza, S. M. and Y. Z. El-Abdin, 1976. Studies on some biochemical constituents and enzymes in the serum of normal non-pregnant dairy Egyptian buffaloes. J. Egyptian Vet. Med. Assoc., 36(1): 169.

Izadyar, F., H. T. Van-Tol, B. Colenbrander and M. M. Bevers, 1997. Stimulatory effects of growth hormone on in vitro maturation of bovine oocytes is exerted through cumulus cells and not mediated by IGF-1. Mol. Reprod. Develop., 47: 175-180.

Landau, S., R. Braw-Tal, M. Kaim and A. Bor, 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, H. J. and E. A. Lenton, 1990. Glucose and lactate in human follicular fluid: concentrations and interrelationship. Hum. Repord., 5: 915-919.

Leonhard, M. S., 2000. Why do trace elements have an influence on fertility? Tierarztliche-Praxis-G.–Ausgabe-G – Grosstiere-Nutztiere, 28(2): 60-65.

Leroy J. L. M. R., T. Vanholder, J. R. Dalanghe, G. Opsomer, A. Van Soom, P. E. J. Bols and A. de Kruif, 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, M. A., S. Manawar, A. Ahmed, M. A. Toor and Zia-ur-Rahman, 1983. Effects of sex and age on the serum proteinogram in growing buffalo calves. Pakistan Vet. J., 3(2): 65-69.

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

Manalu, W., M. Y. Sumardai and N. Kusumorini, 1997. Maternal serum concentrations of total T3, T4 and cortisol in different stages of pregnancy during late pregnancy in Ettawah-cross does. Asian Austr. J. Anim. Sci., 10(4): 385-390.

McNatty, K. P., D. M. Smith, A, Makris, R. Osathanondh and K. J. Ryan, 1978. The microenvironment of human antral follicle: interrelationships among the steroid levels in the antral fluid, the population of granulosa cells, and the status of the oocytes *in vivo* and *in vitro*. J. Clin. Endocrinol. Metab., 49: 851-860.

O'Callaghan, D. and M. P. Boland, 1999. Nutritional effects on ovulation. Anim. Sci., 68: 299-314.

Perveen, S. and R. H. Usmani, 1993. Peripartum profiles of certain haematological and biochemical parameters in normally calving buffaloes. J. Anim. Hlth. Prod., 12-13: 55-60.

Prasad, K. S. and S. V. N. Rao, 1998. Blood mineral profiles of anoestrus and repeat breeder crossbred cows: a field study. Vet. Bull., 58(7): 6658.

Richards, L. A., 1968. Diagnosis and Improvement of Saline and Alkaline Soils. 1st Ed., Agri. Handbook No. 60. IBH Co., New Delhi, India.

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

Singh, K., D. Nandan, K. L. Gera and I. S. Chendna, 1983. Biochemical constituents in the plasma and urine of normal and urolithiasis affected bovine. Indian J. Anim. Sci., 53(9): 1016-1018.

Steel, R. G. D. and J. H. Torrie, 1982. Principles and Procedures of Statistics, A biometrical approach. 4th Ed., McGraw Hill Book Co., New York, USA.

Wise, T., 1987. Biochemical analysis of bovine follicular fluid: albumin, total proteins, lysosomal enzymes, ions, steroid and ascorbic acid contents in relation to follicular size rank, atresia classification and day of oestrous cycle. J. Anim. Sci., 64: 1153-1169.