Glucose, cholesterol, total proteins and insulin-like growth factor type I values in the follicular fluid of female river buffaloes (*Bubalus bubalis*)

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Twenty six Murrah female river buffaloes, between 45 and 70 d post-partum, empty, multiparae, with an average live weight of 675 ± 56 kg, and average body condition of 3.5 points, in a 1 to 5 scale, were used to determine the concentrations of glucose, cholesterol, total protein and insulin-like growth factor type I(IGF-I) in the follicular fluid. The fluid was collected from dominant follicles, with diameters between 8 and 12 mm, by *in vivo* follicular aspiration. The oestrous cycle stage was not taken into account. The wave of follicular development was synchronized six days prior to the collection. Biochemical analyses of glucose and cholesterol were performed by the enzymatic colorimetric method with the utilization of commercial kits of Glicose (GOD-PAP) and Cholesterol (CHOD-PAP) (Kovalent), respectively. For the determination of total protein, the commercial kit total Protein (Kovalent), method Biuret, was employed. Readings were carried out through absorption spectrophotometry with visible light. Through the radioimmunoanalysis (RIA) technique the concentration of IGF-I was obtained using commercial kits of IRMA IGF-I (IMMUNOTECH). Descriptive statistics was used, by applying the PROC MEANS procedure of the SAS (2009) statistical package. Glucose concentrations (4.0 ± 0.75 mmol/L) and IGF-I (340 ± 129.83 ng/mL) showed higher values in female river buffaloes and dairy cows regarding those reported in other studies. However, cholesterol levels (0.51 ± 0.12 mmol/L) and total proteins (58.4 ± 4.43 g/L) were lower. Results indicate that there is a relationship between the concentration of biochemical indicators, the nutritional aspects, the diameter of the aspired follicles and the productive period.

Key words: *biochemical profile, follicular fluid, post-partum*

In specialized milk production systems, metabolic and nutritional problems influence significantly, although in many occasions not clinically noticed. These types of alterations compromise the productive and reproductive processes of the herds. Diverse studies developed in the last years demonstrate the existence of complex interactions between the nutritional balance and the metabolic alterations. The pre-partum feeding regime, the metabolic changes accompanying the beginning of lactation and the pre-partum body conditions led to modifications in the serum concentrations of different metabolites, which could influence on the good tissue and organ functioning (Hagawane *et al*. 2009).

The characteristics of the follicle fluid can be affected by the physiological conditions and the state of the follicle, its size, growth and atresia. Present research studies on this subject matter attempt to deepen in the processes appearing during the follicular development as the metabolic and biochemical changes produced during the course of the post-partum productive and reproductive stages (Khan *et al*. 2011). Studies developed by Leroy *et al*. (2008) demonstrate that the metabolic variations accompanying the beginning of lactation, as the result of the negative energy balance (NEB), provoke transformations in the biochemical composition of different metabolites present in the follicular fluid, and determine, in turn, the subsequent oocyte quality and later fertility of the animals.

The concentrations of these elements in the follicular fluid are known in humans (Leese and Lenton 1990), bovines (Orsi *et al*. 2005), goats (Herrick *et al*. 2006) and sheep (Nandi *et al*. 2007), which contributes to the knowledge of the nutritive elements and the metabolic requirements allowing a good reproductive performance. For the buffalo species few studies have been developed on this aspect, though there is limited information on the physiological processes occurring during the beginning of lactation and their relationship with the existing metabolic compounds in the follicular fluid. Thus, the objective of this study was to learn the levels of cholesterol, glucose, total proteins and insulin-like growth factor-I, in the follicular fluid of female river buffaloes, between 45 and 70 d post-partum, under dual-purpose systems.

Materials and Methods

The study was carried out at the buffalo experimental dairy unit of the Faculty of Veterinary Medicine and Zootechnics of the UNESP, Botucatú Campus, Brazil. Twenty six Murrah female river buffaloes, between 45 and 70 d post-partum, empty, multiparae, with an average live weight of 647 ± 56 kg, were used. Body condition was measured in a scale 1 to 5 points, which according to the estimation of Houghton *et al*. (1990), showed a mean of 3.5. Animal management was semi-intensive with a rotational paddock system, with

predominance of improved grasses of Brachiaria genus (*Brachiaria brizantha*) and Guinea (*Panicum maximum*). Supplementation consisted of 2 kg of concentrates with 16% of crude protein.

Milking was performed with the support of the buffalo calf, manually, once daily and between 6:00 and 7:30 a.m. Without considering the oestrous cycle stage, the fluid was obtained from dominant follicles, with diameters between 8 and 12 mm, by *in vivo* aspiration, for once, and according to the methodology described by Bols *et al*. (1996). Through the same technique, the development follicular wave was synchronized six days prior to the collection. After the aspiration, the samples were transported at 4ºC to the laboratory and stored at -20ºC in preservation tubes (Eppendorf) until further analyses.

The analytical methods for the determination of the studied elements are shown in table 1. The readings for glucose, total protein and cholesterol were made by absorption spectrophotometry with visible light (Semi-automatic Spectrophotometer Stardust MC 15). The determination of the insulin-like growth factor-I, was performed by radioimmunoanalysis, using a gamma counter in solid phase (RIA Cobra II). Values of the biochemical elements were analyzed by descriptive statistics, using the procedure PROC MEANS, of the statistical package SAS (2009).

Table 1. Systems of measurements and methods utilized for each biochemical element

Elements	Units (IUS)[1]	Analytical methods
Glucose	mmol/L	Glucose oxidase/Peroxidase
Cholesterol	mmol/L	Cholesterol oxidase/Peroxidase
Total protein	g/L	Biuret
IGF-I[2]	ng/mL	Radioimmunoanalysis

[1]IUS: International unit system
[2]IGF-I insulin-like growth factor-I

Results and Discussion

Glucose is an important energy source for ovary functioning. The hemato-follicular barrier is permeable to it. Table 2 shows mean values of the biochemical constituents obtained from the follicular fluid of the studied animals. Mean concentration of glucose was higher than in the results obtained by Nandi *et al*. (2008) (2.42 ± 0.31 mmol/L) in animals of the same species. However, studies developed by Villa *et al*. (2009) in sub-fertile cows (3.8 ± 0.19 mmol/L) and fertile (4.0 ± 0.19 mmol/L), showed lower values and similar to those of the present study, respectively. In general, these variations could be related to the nutritional factors, post-partum energy balance and diameter of the aspired follicles, since the hemato-follicular barrier is more permeable for follicles of greater size.

Cholesterol and total protein concentrations play roles in the ovary physiology, as precursors of steroidal hormones and growth factors of peptide origin, respectively. Both elements showed lower values than those reported by Arshad *et al*. (2005) from the ovaries of female buffaloes slaughtered during the season of lower partum concentration. Possibly, the utilization of unproductive females or with finished lactation, regarding the use of animals under production, as those employed in this study, could justify the concentrations found, due to the lipid and serum protein mobilization to the mammary gland for milk synthesis. The diameter of the follicles aspired in both studies could also be a determinant factor in the results obtained. Investigations developed by Thangaver and Nayeem (2004) demonstrated that dominant follicles show lower cholesterol concentration, which can be attributed to the greater conversion capacity of this element to hormonal steroid formation.

Hypophisiary gonadotropins are known to play an important role in growth control and follicular development. However, there are other factors which are directly related to follicle genesis processes. Within these, those insulin-like growth-I (IIGF-I) and II (IGF-II) also contribute specifically and during different stages to the regulation of cell proliferation and differentiation. In general, these growth factors stimulate, autocrinally

Table 2. Stadigraphs of the biochemical elements obtained from the ovary follicular fluid of river female buffaloes during post-partum

Elements	n	Medium	Mínimum	Maximum	VC (%)	SD
Glucose (mmol/L)	26	3.90	1.46	4.64	1.06	0.75
Cholesterol (mmol/L)	26	0.51	0.26	1.16	0.97	0.12
Total proteins (g/l)	26	58.40	49.20	67.60	82.70	4.43
IGF-I (ng/mL)	26	340.36	129.90	619.81	38.14	129.83

and paracrinally the functions developed by follicle stimulating hormones (FSH) and of luteinization (LH) in the follicular population. IGF-I levels found in the follicular fluid of the females buffaloes studied showed higher concentrations to those reported by Kinigsson *et al*. (2008) in the serum of high-productive dairy cows and during their production peak. These variations could be related to the high energy requirements that inhibit the IGF-I synthesis at hepatic level during the periods of higher milk production.

Although the highest IGF-I concentration in the follicle fluid of dominant follicles are synthesized in the liver, and is regulated by the growth hormone (GH) from the pituitary gland, other studies demonstrate the ovary capacity for producing IGF-I in theca cells and in the membrane of the granulose cells, which could contribute to the increase of the concentration of these factors in the follicular fluid, if compared with the circulation of the blood (Khalid *et al*. 2000). The follicular diameter also contributes to the increment of the concentrations of these growth factors. It is known that the pre-ovulatory follicles contain higher amount of IGH-I, due to the decrease in the bio-utilization of these compounds during the final stages of growth and atresia. Considering the species studied follicle aspiration with diameters between 8 and 12 mm could be related to the high concentrations of IGF-I obtained in the study.

Pre-partum nutritional deficiencies associated to the post-partum negative energy balance (NEB), compromise serum concentrations of glucose and insulin, which provokes decrease of the IGF-I levels in serum and follicular fluid. Regarding other investigations (Spicer and Geisert 1992), the high glucose concentrations found in the follicular fluid could justify that the animals did not show negative energy balance during the period under study, giving place to higher IGF-I values to those reported for milk production cows. There are studies indicating that the concentrations of these growth factors in the follicular fluid, if compared to the plasmatic levels, could be more resistant to nutritional changes, though the concentrations of hepatic and ovary origin could be under different control systems (Matoba *et al*. 2012).

This research study contributed to the knowledge of the values of some biochemical elements of the follicular fluid during post-partum, in a model buffalo dairy unit. Results show concentrations differing from other studies and domestic species, and depending mainly, from the pre-partum and post-partum nutritional stage, diameter of the aspired follicles, stage of the oestrous cycle and productive period. Further investigations evaluating, specifically, the relationship between de above mentioned aspects and the concentrations of the elements present in the follicular fluid, the productive and reproductive post-partum performance, besides other important indicators of the metabolic profile in animals destined to milk production, are recommended.

Acknowledgements

Thanks are due to A. Mendes Jorge, Dr.Cs for providing the animals for this study and to the Coordination Organization for Improving Higher Education Personnel (Coordinación de Perfeccionamiento del Personal de Nivel Superior, CAPES) for the financial support.

References

Arshad, H.M., Ahmad, N., Rahman, Z., Samad, H.A., Akhtar, N. & Ali, S. 2005. Studies on some Biochemical constituents of ovarian Follicular Fluid and Peripheral Blood in buffalo. Pakistan Vet. J. 25: 76

Bols, P., Vandenheede., van Soom, J., & Kruif, A. 1995. Transvaginal ovum pick-up (OPU) in the cow: a new disposable needle guidance system. Theriogenology 43: 677

Hagawane, S.B., Shinde, S.B. & Rajguru, D.N. 2009. Haematological and Blood Biochemical Profile in Lactating Buffaloes in and around Parbhani city. Vet. World 2: 467

Herrick, R., Michelle, L., Gardner, D., Behboodi, E., Memili, S., Blash, Y., Echelard, R. & Krisher, T. 2006. Metabolism, protein content, and *in vitro* embryonic development of goat cumulus-oocyte complexes matured with physiological concentrations of glucose and l-lactate. Mol. Reprod. Dev. 6:73

Houghton, P.L., Lemenager, R.P., Horstman, L.A., Hendrix, K.S. & Moss, G.E. 1990. Effects of body composition, pre and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. J. Anim. Sci. 68:143

Khan, F. A., Das, G.K., Pande, M., Pathak, M.K. & Sarkar, M. 2011. Biochemical and hormonal composition of follicular cysts in water buffalo (*Bubalus bubalis*). Anim. Reprod. Sci. 124: 61

Kinigsson, C., Savoini, G., Govoni, Invernizzi, G., Prandi, A., Kindahl, H. & Veronesi, C. 2008. Energy balance, leptin, NEFA and IGF-I plasma concentrations and resumption of post partum ovarian activity in Swedish red and white breed cows. Acta Vet. Scandinávica 50: 3

Leese, A & Lenton, A. 1990. Glucose and lactate in human follicular fluid: concentrations and interrelationships. Human Reprod. 5: 915

Leroy, J., van Soom, A., Opsomer, G., Goovaerts, I. & Bols, P. 2008. Mechanisms Linking Nutrition and Reduced Oocyti and Embryo Quality in High-yielding Dairy Cows. Reprod. Dom. Anim. 43: 623

Matoba, S., O'Hara, L., Carter, F., Nelly, A., Fair, T., Rizos, D. & Lonergan, P. 2012 The association between metabolic parameters and oocyte quality early and late postpartum in Holstein dairy cows. J. Dairy Sci. 95: 1257

Nandi, S., Girish Kumar, V., Manjunatha, M. & Gupta, P. 2007. The biochemical composition of ovine follicular fluid in relation to follicle size. Dev. Growth Differ 49: 66

Nandi, S., Girish Kumar, V., Manjunatha, B., Ramesh, H. S. & Gupta, P. 2008. Follicular fluid concentration of glucose, lactate and pyruvate in buffalo and sheep, and

their effects on culture oocytes, granulosa and cumulos cell. Theriogenology 69: 186

Orsi, N., Gopichandran, N., Leese, H., Picton, M. & Harris, E. 2005. Fluctuations in bovine ovarian FF composition throughout the oestrous cycle. Reproduction 13:129

SAS 2009. Statistical Analysis System. User´s Guide. Institute Inc. 4th Ed. Cary. N.C. USA 2:846

Spicer, L & Geisert, R. 1992. Concentrations of insulin-like growth factor-I, estradiol and progesterone in follicular fluid of ovarian follicles during early pregnancy in cattle. Theriogenology 37:74

Thangaver, A. & Nayeem, M. 2004. Studies on certain biochemical profile of the buffalo follicular fluid. Indian Vet. J. 81: 25

Villa, A., Pulgarín, F., Paula, A., Angarita, E. & Ceballos, A. 2009. Medidas corporales y concentracion sérica y folicular de lípidos y glucosa en vacas Brahman fértiles y subfértiles. Pesq. Agropec. Bras. 44: 68

Received: April 23, 2013