Biochemical analysis of glucose level and superoxide dismutase activity in buffalo follicular fluid and blood Serum

(Received: 03. 05. 2018; Accepted: 15.05.2018)

Tawfik H. N. [1], Kandil O. M. [1], Hemeida N. A. [2], Abdoon A. S. S. [1], Elkhadrawy H. H. [1] and Abdel-Aziz S. A. [3]

[1]Dept of Animal Reproduction & Artificial Insemination, Veterinary Research Division, National Research Centre (NRC), Cairo,

[2] Theriogenology Department Cairo University, Faculty of Veterinary Medicine, Cairo, Egypt

[3]Biochemistry and Chemistry of Nutrition Department, Cairo University, Faculty of Veterinary Medicine, Cairo, Egypt.

ABSTRACT

The reducing reproductive efficiency is one of the critical problems of the water buffalo, so the recent studies have indicated alterations in the follicular fluid and blood serum composition during such condition. The present study, therefore, aims at assaying the changes of follicular fluid glucose level and superoxide dismutase (SOD) activity in different size follicles and assaying these parameters in follicular fluid and blood serum during different phases of estrous cycle in the buffalo. Blood samples and ovaries were collected from 20 cyclic buffaloes of known estrous phase (follicular or luteal). Follicular fluid was aspirated from small sized ovarian follicles (2-4 mm) and medium sized follicles (5-8 mm). Glucose level and SOD activity were significantly higher ($P < 0.05$) in medium sized follicles (85.89 ± 1.59 mg/dl and 53.66 ± 0.76U/ml, respectively) than in small sized follicles (72.09 ± 0.84 mg/dl and 49.30 ± 0.41U/ml, respectively). Moreover, both the follicular fluid glucose level and SOD activity were significantly higher ($P < 0.05$) during the follicular phase than the luteal phase either in medium or small follicles. Also, the blood serum SOD activity was higher ($P < 0.05$) during the follicular phase (45.47 ± 0.50 U/ml) than in the luteal phase (38.46 ± 0.43 U/ml). In conclusion, Glucose level and SOD activity were significantly high in medium follicles follicular fluid and in serum during follicular phase of estrous cycle in buffalo.

Key words; *Buffalo, follicular fluid, glucose, SOD activity.*

INTRODUCTION

The buffaloes are economically very potential livestock animals and distributed in 43 countries in the world. However, only four countries, India, Pakistan, China and Egypt, are producing more than 98% of the world buffalo's milk and around 73% of the world buffalo's meat (Soliman and Bassiony, 2011). The biggest problem concerning buffaloes facing the world is the reducing reproductive efficiency (Das and Khan, 2010). The follicular fluid provides a most important microenvironment for oocyte protection and maturation development, because it is the product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretary activity of granulosa and theca cells of ovary (Fortune, 1994).Therefore, metabolic alterations in blood serum could be reflected on the biochemical composition of the follicular fluid

and indirectly influence oocyte quality (Ali *et al*., 2008).

Oocyte maturation and follicular development need glucose which can be utilized via oocytes, cumulus cell complex (COCs) and follicular somatic cells via major pathways of glucose oxidation (glycolysis, citric acid cycle (TCA), oxidative phosphorylation and pentose phosphate pathway (HMP or PPP) (Leese and Lenton 1990). Phosphoribosyl phosphate is one of PPP products in oocyte cytoplasm which being used by oocyte for purine synthesis (Sutton-McDowall *et al*., 2010). These purines are involved in regulation of nuclear maturation and cytoplasmic homeostasis of the oocytes. The PPP gives NADPH+H which is responsible for glutathione (GSH) and glutathione peroxidase (GPx) as an antioxidant for oocyte protection and cell viability (Sutton-McDowall *et al*., 2010).

Glucose oxidation pathway is considered one of the stress factor on oocyte where the reactive oxygen substrate O- (superoxide anion) was released from electron pathway (Fattman *et al*. 2003). The superoxide dismutase (SOD) helps to remove the superoxide anion via dismutation reaction, producing H_2O_2 and molecular oxygen which well known that cells are living under aerobic conditions. SOD is considered one of the most important antioxidant parameter for oocyte competence and maturation development (Nozik-Grayck *et al*. 2005). Therefore, the aim of this work is to assay the glucose level and SOD activity in follicular fluid of different sized ovarian follicles and correlate the glucose level and SOD activity in follicular fluid with that of blood serum during the different phases of estrous cycle in the buffalo.

MATERIALS AND METHODS

Follicular fluid was collected from different sized ovarian follicles (small sized follicles of 2-4mm and medium sized follicles of 5-8mm) during different phases of estrous cycle (follicular phase and luteal phase) from 40 ovaries of 20 cycling non-pregnant buffaloes slaughtered in a local slaughterhouse (Elmonieb abattoir, Giza Governorate, Egypt). Also, blood samples were collected from these buffaloes during slaughtering without anticoagulants and grouped to two groups according to estrous cycle of each buffalo (follicular phase and luteal phase) according to the morphological appearance of the ovaries (Mondal *et al*., 2004 and Jaglan *et al.,* 2010).

Immediately after slaughtering, both ovaries of each buffalo were collected in plastic bags containing 0.9 % NaCl as normal saline solution at temperature ranged from 30°C to 37°C and transported in a tank to be inspected in the laboratory.

In the laboratory, ovaries were washed once with 70% ethanol and at least three times with normal saline solution (NSS) supplemented with 100 IU/m1 penicillin and 100µg/m1 streptomycin at 35ºC (Leibfried-Rutledge *et al*., 1987). The stages of estrous cycle either follicular or luteal were identified according to morphological features of the corpus luteum as previously established by Mondal *et al*., (2004) and Jaglan *et al*., (2010). Follicular diameter was measured using a caliper and follicles were grouped into two size categories: small (2-4 mm) and medium (5-8 mm) according to Dominguez (1995).

The aspirated follicular fluids were collected in 15 ml sterile Falcon tubes (Fa, Con, USA) from different sized follicles and centrifuged at 3000 rpm for 10 minutes. Supernatant (follicular fluid samples) was divided into aliquots to avoid repeated freezing

and thawing and kept at -20 °C till used for biochemical analysis.

Blood samples were transported in tank to laboratory for serum separation after blood centrifugation at 3000 rpm for 10 minutes. Collected serum samples were divided into aliquots to avoid repeated freezing and thawing and kept at -20°C till used for biochemical analysis.

Glucose level and SOD activity of the follicular fluid and blood serum were assayed chemically by an enzymatic method utilizing kits supplied from Bio Diagnostic Research Office (Giza, Egypt) according to Trinder (1969) and Nishikimi *et al.,* (1972), respectively.

Data were expressed as means ± standard errors (SE). The significant differences were tested by analysis of variance (ANOVA) followed by post hoc test. Statistical analyses were performed using SPSS.

RESULTS

The means ± SE of the follicular fluid (FF) glucose level (mg/dl) and Superoxide dismutase (SOD) activity (Table 1) were significantly ($P < 0.05$) higher in medium sized ovarian follicles (85.89 ± 1.59 and 53.66 ± 0.76 respectively) as compared with small sized follicles (72.09 ± 0.84 and 49.30 ± 0.41 respectively).

Table (1): Glucose level (mg/dl) and Superoxide dismutase (SOD) activity (U/ml) in follicular fluids (Mean ± SE) of different sized ovarian follicles in the buffalo.

Follicular size	Glucose level (mg/dl)	(SOD) activity (U/ml)
Small size (2-4 mm.)	72.09 ± 0.84^{b}	49.30 ± 0.41^{a}
Medium size (5-8 mm.)	85.89 ± 1.59^{a}	53.66 ± 0.76^{b}

Means in the some column with different letter superscripts are different significantly ($P<0.05$).

Table (2) shows the effect of estrous cycle phases on glucose level (mg/dl) and superoxide dismutase (SOD) activity in the follicular fluid of different size ovarian follicles. Both glucose level and superoxide dismutase (SOD) activity means ± SE were significantly ($P < 0.05$) high during the follicular phase either in medium size or small size follicles when compared with the luteal phase.

Table (2): Glucose level (mg/dl) and Superoxide dismutase (SOD) activity (U/ml) in follicular fluids (Mean ± SE) of different sized ovarian follicles during the luteal and follicular phases in the buffalo.

Estrous phase	Follicular size	Glucose level (mg/dl)	(SOD) activity (U/ml)
Luteal phase	Small size follicles	69.42 ± 0.32^{d}	48.18 ± 0.22^{c}
	Medium size follicles	80.55 ± 0.76^{b}	51.45 ± 0.62^{b}
Follicular phase	Small size follicles	76.49 ± 1.51^{c}	50.95 ± 1.10^{b}
	Medium size follicles	94.98 ± 0.08^{a}	58.36 ± 0.19^{a}

Means in the some column with different letter superscripts are different significantly ($P<0.05$).

Table (3) demonstrates the effect of estrous cycle phases on glucose level (mg/dl) in blood serum. Non-significant difference was detected in serum glucose level (mg/dl) between follicular (92.15 ± 1.54) and luteal phases (89.37 ± 1.06). Data in the same table show significant and positive correlations in glucose level between blood serum and follicular fluid during both follicular (r=0.94) and luteal (r=0.98) phases.

Table (3): Correlation coefficient between glucose level (mg/dl) in blood serum and follicular fluid (FF) during the luteal and follicular phases of estrous cycle in the buffalo.

Estrous cycle	Glucose level (mg/dl) in blood Serum	Glucose level (mg/dl) in FF	Correlation coefficient (r)
Luteal phase	89.37 ± 1.06^{a}	74.99 ± 1.49^{b}	0.981
Follicular phase	92.15 ± 1.54^{a}	84.41 ± 3.82^{a}	0.938

Means in the some column with different letter superscripts are different significantly (P<0.05).
Correlation coefficient (r) between the means in the same row.

Superoxide dismutase activity (Table 4) was significantly high (P<0.05) during the follicular phase in both blood serum and FF as compared with the luteal phase. Significant, weak and positive correlations were noted in SOD activity between blood serum and follicular fluid during both follicular and luteal phases.

Table (4): Correlation coefficient between superoxide dismutase activity (U/ml) in blood serum and follicular fluid (FF) during the luteal and follicular phases of the estrous cycle in the buffalo.

Estrous cycle	(SOD) activity (U/ml) in blood serum	(SOD) activity (U/ml) in FF	Correlation coefficient (r)
Luteal phase	38.46 ± 0.43^{b}	49.81 ± 0.53^{b}	0.510
Follicular phase	45.47 ± 0.50^{a}	54.12 ± 1.61^{a}	0.277

Values with different letter superscripts are different significantly (P<0.05).
Correlation coefficient (r) between the means in the same row.

DISCUSSION

Results of the current study showed a significant increase in the mean values of glucose level in buffalo follicular fluid of medium sized ovarian follicles (5-8 mm diameter) when compared with small sized follicles (2-4 mm diameter). Such finding is in agreement with reports of Nandi *et al*. (2008), Tabatabaei and Mamoei (2011) and Khan *et al*., (2011) in the buffalo. Similarly, in the camel, El-Bahra *et al*. (2015) found a high glucose level in medium size follicles when compared with small sized follicles which Tabatabaei and Mamoei (2011) and El-Bahra *et al*. (2015) suggested that the high level of follicular fluid glucose in medium sized follicles might be due to the biochemical metabolites of follicular fluids, which are essential for maturation and even fertilization of the oocyte.

The significant increase in the follicular fluid glucose level during the follicular phase versus the luteal phase agrees with results of Shabankareh *et al*. (2013) in the cow. Moreover, Shabankareh *et al.* (2013) suggested that the high follicular fluid glucose level during follicular phase might be due to development of new blood vessels during formation of the corpus luteum which results in greater blood flow than in ovarian tissue. Therefore, follicular growth in absence corpus luteum (CL^-ovaries) seemed to be higher than that in present corpus luteum (CL^+ ovaries) as mentioned by Kaczmarek *et al.,* (2005). On other hand, Acar *et al*. (2013) found a high follicular fluid glucose level during the luteal phase when compared with the follicular phase in Anatolian water buffalo.

Current study presented no significant differences in blood serum glucose level during the follicular phase as compared with the luteal phase. Such finding agrees with results of Acar *et al*. (2013) in the buffalo who suggested that this result might be due to differences in animal nutritional factors. The significant positive correlation coefficients between glucose levels in blood serum and follicular fluid in the resent study are in accordance with Tabatabaei and Mamoei (2011) in the buffalo and El-Bahr *et al*. (2015) in the camel. The superoxide dismutase activity (SOD) in the follicular fluid increased significantly in medium sized follicles versus small follicles, such result conflicts with that of El-Shahat and Kandil (2012) and Hozyen *et al*. (2014) in the buffalo and Combelles *et al*. (2011) in the bovine. Follicular fluid SOD activity showed a significant increase during the follicular phase versus the luteal phase. Similar finding was reported by El-Shahat and Kandil (2012) in the buffalo which suggested that SOD may remove the superoxide anion in a dismutation reaction, producing H_2O_2 and molecular oxygen. It is well known that cells living under aerobic conditions constantly face the oxygen (O_2) paradox. O_2 is required to support life, but its metabolites, such as superoxide anions ($O2^{-\cdot}$), hydroxyl radicals ($OH^{\cdot}$), and hydrogen peroxide (H_2O_2), collectively termed ROS, can modify cell function, and endanger cell survival, or both. These oxidants are suggested to originate mainly from steroidogenesis occurring in granulosa cells as suggested by Cassano *et al*. (1999). However, Hozyen *et al*. (2014), presented a significant increase in SOD (U/ml) activity in the same species during the luteal phase when compared with the follicular phase.

The significant increase in blood serum SOD activity during the follicular phase compared with luteal phase agreed with reports of Abo-El maaty and El-Shahat (2012) in the mare. Finally, our results showed a weak positive correlation coefficient between SOD activity blood serum and follicular fluid.

In conclusion, significant increases in follicular fluid glucose level and SOD activity in medium sized follicles were noticed during the follicular phase of the estrous cycle as compared with small follicles and luteal phase. This seems important to oocyte maturation and increased number and activity of the follicular cells towards the mature Graafian follicle, a prerequisite to ovulation.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of Academy of Science and Technology through the project in title"Assessment the viability of cryopreserved buffalo embryo and semen" and this word was done in Embryo and Genetic Resource Conservation Bank in National Research Centre, financially supported by STDF (CB grant ID 2339).

REFERENCES

Abo-El maaty, A.M. and El-Shahat, K. H. (2012). Hormonal and biochemical serum assay in relation to the estrous cycle and follicular growth in Arabian mare Asian Pacific Journal of Reproduction, **1**(2): 105-110.

Acar, D.B, Birdane, M.K., Dogan, N. and Gurler, H. (2013). Effect of the stage of estrous cycle on follicular population, oocyte yield and quality, and biochemical composition of serum and follicular fluid in Anatolian water buffalo. Animal Reproduction Science, 145: 128– 345.

Ali, S., Ahmad, N., Akhtar, N., Zia-ur-Rahman and Noakes, D.E. (2008). Metabolite contents of blood serum and fluid from small and large sized follicles in dromedary camels during the peak and the low breeding seasons. Animal Reproduction Science, 108: 446–456.

Cassano, E., Tosto, L., Balestrieri, M., Zicarelli, L. and Abrescia P. (1999). Antioxidant defense in the follicular fluid of water buffalo. Cell Physiology and Biochemistry. 9:106 –16.

Combelles, C.M., Emily, A.H., Louis, J. P., David, C.W., and Qiaqia, W.U (2011). Profiling of SOD isoenzymes in compartments of the developing bovine antral follicle. Reproduction, **139**: 871–881.

Das, G. K. and Khan, F. A. (2010): Summer anestrous in buffalo. Reproduction Domestic Animal, 45: 483-494.

Dominguez, M. M. (1995). Effect of body condition, reproductive status and breed on follicular population and oocyte quality in cows. Theriogenology, 25: 800-805.

El-Bahra, S.M., Ghoneimc, I.M. and Waheed, M.M. (2015). Biochemical and hormonal analysis of follicular fluid and serum of female dromedary camels (*Camelus dromedaries*) with different sized ovarian follicles. Animal Reproduction Science, 159: 98–103.

El-Shahat, K. H. and Kandil, M. (2012). Antioxidant capacity of follicular fluid in relation to follicular size and stage of estrous cycle in buffaloes. Theriogenology, **77**: 1513-1518.

Fattman, C.L., Schaefer, L.M. and Oury, T.D. (2003). Extracellular superoxide dismutase in biology and medicine. Free Radical Biological Medicine, 35: 236–256.

Fortune, J.E. (1994): Ovarian follicular growth and development in mammals. Biology of Reproduction. 50: 225–232.

Hozyen, H.F., Hodallah, H., Ahmed, B., Essawy B, and Shalaby, S.I.A. (2014). Seasonal changes in some oxidant and antioxidant parameters during folliculogenesis in Egyptian buffalo. Animal Reproduction Science, 151**:** 131–136.

Jaglan, P., Das, G. K., Kumar, B. V., Kumar, R., Khan, F. A. and Meur, S. K. (2010). Cyclical changes in collagen concentration in relation to growth and development of buffalo corpus luteum. Veterinarian Research Communcation, 34: 511-518.

Kaczmarek, M.M., Schams, D. and Ziecik, A.J. (2005). Role of vascular endothelial growth factor in ovarian physiology. Reproduction Biology. 5(2): 111–136.

Khan F. A, Das, Pande, Mir, and Shankar (2011). Changes in biochemical composition of follicular fluid during reproductive a cyclicity in water buffalo (*Bubalus bubalis*). Animal Reproduction Science, 127: 38-42.

Leese J.H. and Lenton E.A. (1990). Glucose and lactate in human follicular fluid: concentrations and interrelationships. Human Reproduction, 5: 915–919.

Leibfried-Rutledge, M. L., Critser, E. S., Eyestone, W. H., Northey, D. L. and First, N. L. (1987). Development potential of

bovine oocytes matured *in vitro* or *in vivo*. Biology Reproduction. 36: 376-383.

Mondal, S.; Kumar, V.; Reddy, I. J. and Khub, S. (2004). Progesterone and nucleic acid content of buffalo corpus luteum in relation to stages of estrus cycle. Indian Journal of Animal Science, 74: 710-712.

Nandi, S., Girish, Manjunatha, and Ramesh, Gupta (2008). Follicular fluid concentrations of glucose, lactate and pyruvate in buffalo and sheep, and their effects on cultured oocytes, granulosa and cumulus cells. Theriogenology**, 69**: 186–196.

Nishikimi, M.,Roa, N.A., and Yogi, K. (1972). Measurement of superoxide dismutase. Biochemical Biophysics Research. Communication., 46: 849-854.

Nozik-Grayck E., Suliman, H.B. and Piantadosi, C.A. (2005). Extracellular superoxide dismutase. Internal Journal of Biochemistry and Cell Biology, 37: 2466–2471.

Shabankareh H. K., Kor, N.M. and Hajarian, H. (2013). The influence of the corpus luteum on metabolites composition of follicular fluid from different sized follicles and their relationship to serum concentrations in dairy cows. Animal Reproduction Science, 140: 109– 114.

Soliman, I. and Bassiony, H. (2011). Role of buffalo in international trade. Agriculture Science Technology, B1: 799-809.

Sutton-McDowall, M.L., Gilchrist, R.B., and Thompson, J., (2010). The pivotal role of glucose metabolism in determining oocyte developmental competence. Reproduction, 139: 685–695.

Tabatabaei, S. and Mamoei, M. (2011). Biochemical composition of blood plasma and follicular fluid in relation to follicular size in buffalo. Comp Clinical Pathology, **20**:441–445.

Trinder P. (1969). Enzymatic colorimetric glucose determination. Annals of Clinical Biochemistry. 6: 24.

الملخص العربي

التحليل البيوكيميائي لمستوى الجلوكوز ونشاط السوبر أوكسيد ديسميوتيز في سائل الحويصلات ومصل الدم للجاموس

حسام نصر توفيق[1] و اميمة محمد قنديل[1] ونبيل عبدالمنعم حميدة[2] واحمد صبري صلاح عبدون[1] وهشام حسن الخضراوي[1] وسامي احمد عبدالعزيز[3]

[1]قسم التكاثر في الحيوان والتلقيح الاصطناعي - شعبة البحوث البيطرية- المركز القومي للبحوث -القاهرة

[2]قسم التناسل والتوليد و التلقيح الاصطناعي- كلية الطب البيطري -جامعة القاهرة

[3]قسم الكيمياء الحيوية و كيمياء التغذية- كلية الطب البيطري -جامعة القاهرة

ان تناقص الكفاءة التناسليه هي واحدة من المشاكل الحرجه في الجاموس ولذلك فان الدراسات الحديثه تعمل على استيضاح الاختلافات في تركيب سائل الحويصلات ومصل الدم خلال هذه الحاله. لذلك فان هذه الدراسه تهدف الى تقييم التغيرات في مستوى الجلوكوز ونشاط السوبراوكسيد ديسميوتيز في سائل الحويصلات للاحجام المختلفه للحويصلات وتقييم هذه المعايير في سائل الحويصلات ومصل الدم خلال المراحل المختلفة من دورة الشبق في الجاموس. تم تجميع عينات الدم والمبايض من ٢٠ جاموسه خلال مراحل الشبق (اثناء مرحله التبويض او اثناء وجود الجسم الاصفر) و سحب سائل الحويصلات من الحويصلات صغيرة الحجم (٢-٤ مم) و الحويصلات متوسطه الحجم (٥-٨ مم) كلا على حدة. كان مستوى الجلوكوز ونشاط السوير اوكسيد ديسميوتيز اعلى معنويا ($P < 0.005$)في الحويصلات متوسطه الحجم (٨٥.٨٩±١.٥٩ممج/ديسل) و(٥٣.٦٦±٠.٧٦وحدة/مل) على الترتيب عن الحويصلات صغيرة الحجم (٧٢.٠٩±٠.٨٤مج/ديسل) و (٤٩.٣٠±٠.٤١وحده/مل) على الترتيب. بالاضافه الى ذلك كان مستوى الجلوكوز في سائل الحويصلات ونشاط السوبر اوكسيد ديسميوتيز اعلى معنويا ($P < 0.005$) اثناء فترة التبويض عن فترة تواجد الجسم الاصفر سواء في الحجم المتوسط او الصغير من الحويصلات. ايضا كان نشاط السوبر اوكسيد ديسميوتيز (وحدة / مل) في مصل الدم اعلى معنويا ($P < 0.005$) اثناء فترة التبويض (0.50 ± 45.47) عن فترة الجسم الاصفر ± 38.46 (0.43.الاستنتاج:مستوى الجلكوز ونشاط السوبر اوكسيد ديسمسوتيز إزداد زياده معنويه فى سائل حويصلات جراف متوسطه الحجم ومصل الدم اثناء فتره الشبق عن فتره تكوين الجسم الاصفر.