SOME BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN FOLLICULAR FLUID OF SHE- CAMEL DURING FOLLICULAR GROWTH

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ABSTRACT

Ovarian samples were collected from 30 non- pregnant she-camels. The ovarian follicles were classified according to size and morphology into small, medium and large follicles. Follicular fluid samples were analyzed for total protein, glucose, urea, uric acid, total bilirubin, electrolytes sodium and potassium. Total lipids, cholesterol and insulin hormone were estimated. The results revealed that, there was no significant variation in the levels urea, uric acid and total bilirubin. There was a significant increased in the level of total protein, glucose, insulin hormone, total lipids and sodium in the follicular fluid as follicles increases in their size. In contrast, there was a significant decreased in the follicular fluid levels of both cholesterol and potassium in the large size follicles. It is concluded that the biochemical and physiological changes that occurs during follicular development could be of crucial importance for follicular growth, oocyte maturation and affect ovulation and further fertilization of oocyte.

INTRODUCTION

The ovarian activity of the she camel shows follicular wave. Each follicular wave can be divided into 4 distinct phases: follicular recruitment, follicular growth and establishment of dominance, follicular maturity and follicular regression (**Tibary and Memon 1999**).

Follicular fluid plays a major role in the biochemical, physiological and metabolic aspects of oocyte maturation, growth of follicles and ovulation (**Hafez, 1993**). Many modern studies were reported specific constituents of follicular fluid in buffalo cow during estrous cycle (**Eissa, 1996**), and in she camel during follicular wave (**Abo Heakal and El Sadawy 1999**). Moreover, some biochemical metabolic disorders in the follicular fluids of cystic and atretic ovarian follicles were reported in non pregnant she camels (**Amer et al, 1997**). Composition of follicular fluid reflect changes in the secretion of the theca interna and granulosa cells. Also follicular fluid originates

from the peripheral plasma by transudation across the follicle basement lamina and accumulates in the antrum **(Hafez, 1993)**.

The aim of the present investigation was to determine biochemical alterations in levels of total protein, glucose, urea, uric acid, total lipids, bilirubin, cholesterol, sodium and potassium ions in follicular fluid. Moreover, estimation of insulin hormone in different follicular size in she camel.

MATERIAL AND METHODS

Ovaries contains different follicles were collected from 30 non pregnant she camels immediately after slaughter at Al-Qassim slaughterhouse. The ovarian follicles were classified aeeording to size and morphology into small (less than 5 mm diameter), medium (5-10mm) and large follicle (10-25mm) according to **Ismail, 1982 and McNatty et al., 1984)**. Follicular fluids were aspirated from eaeh type of follieles and then centrifuged at 2500-3000 rpm for 15 min. to remove cellular debris. The follicular fluid samples collected from ovaries carrying small follicles were pooled. The samples of follicular fluid were kept in deep freezer at —20°c till bioehemical and hormonal assay were earried out.

Biochemical analysis and hormonal assay :

Follicular fluid samples were analyzed for total proteins, glucose, urea, urie acid and total bilirubin using commercial kits supplied by Biomerieux (France). Sodium and potassium levels were analyzed using digital flame analyzer. Total lipids and cholesterol were measured using commercial kits (Nubenco and Axion Diagnostic, USA respectively). Insulin hormone level was measured by radioimmunoassay kit (RIA) according to **Wilson and Miles (1977)**.

Statistical analysis:

Statistical analysis of the data was performed using student t test.

The results were expressed as mean +SE ($p < 0.01$).

Results

The results of the present investigation are shown in table (1). It revealed that, there was no signifieant variation in the levels of urea, uric aeid and total bilirubin at different follicular sizes. There was a significant increase ($p<0.01$) in total protein as the size of the follicle increase. The glucose concentration was greater in the large follicle than medium and small one. Insulin level was significantly increased in the large size follicle as compared with small and medium follicle.

Also the sodium level in the follicular fluid of large follicle was significantly higher than that of small and medium one.

Both level of cholesterol and potassium were significant decreased as follicular size increase in the large follicle.

Table (1): Levels of total proteins, glucose, insulin, total lipids, cholesterol, bilirubin, uric acid, urea, sodium and potassium in follicular fluid of she camel at different follicular stages

Parameter	Small follicle	Medium follicle	Large follicle
Total proteins (g/dL)	4.88±0.31	8.48±0.60 *	9.4±0.66 *
Glucose (mg/dL)	95.6±9.6	221.3± 12.8*	242.7± 7.2*
Insulin (μU/ml)	68.8 ±8.4	88.0±7.76*	159.5± 8.7*
Total lipids (mg/dL)	489.8± 22.4	524.2± 45.0*	582.6 ±10.3*
Cholesterol (mg/dL)	164.0 13.0	100.0 12.0*	88.3 6.2*
Bilirubin (mg/dL)	4.8± 0.6	4.3± 0.9	4.6 ±0.8
Uric acid (mg/L)	83.6 ±9.4	86.6 ±7.9	85.48± 8.4
Urea (g/L)	0.494 ±0.01	0.605± 0.05	0.566± 0.07
Sodium (mmol/L)	148.1± 3.5	159.8± 3.1*	162.1 ±5.1*
Potassium (mmol/L)	6.48± 0.21	5.0 ±0.56*	4.8± 0.52*

* Mean ± standard error (P< 0.01)

DISCUSSION

Follicular development and ovulatory processes in mammals involve biochemical changes as a result of substantial modifications in cellular metabolism. In camel, there is a follicular wave and induced ovulation (**Tibary and Memon 1999**). Little is known about ovarian physiological and biochemical changes during follicular growth. In the present investigation, there was a significant increase in total protein as the size of the follicle increases. In contrast with findings of (**Mabrouk et al 1989 ;Amer et al 1997**) in camel, there no change in the total protein in the follicular fluid. The granulose and theea cells are responsible for protein synthesis (**Mc Donald 1977**). Therefore the inereases in total protein reflect the growth and development of the follieles. The glucose concentration was greater in the large follicle than medium and small follicle. **Amer et al (1997)** reported that glucose level was moderate in large size follicles. Follicular glucose represent about 80% of the total carbohydrate content present in cow **Pascu et al (1968)**. **Sutton et al (2003)** reported that metabolites included in media of in -vitro oocyte maturation such as glucose, pyruvate, oxygen and amino acid shown to have differential influences on oocyte maturation. The cholesterol level was significantly decreased as follicular size inerease, in contrast to the total lipids increases as follicular size increase. Cholesterol is an important precursor for ovarian steroid hormones biosynthesis by granulosa cells (**Selvaraju and Rajasundaram 2001**). Oestradiol level showed a significant increase in follicular fluids of the large follicle more than in small follicle (**Elwishy et al 1984 ; Mabrouk et al 1989; Salem et al 1997**). Therefore, the decrease in cholesterol level in follicular fluid can be attributed to increase estrogen biosynthesis by granulosa cells. The estrogens are formed by the aromatisation of androgens via aromatase enzyme **Murray et al (1999). Abo Heakal and EL Sadawy (1999)** reported that testosterone was decreased in follicular fluid of the large size follicles than medium ones.

In the present investigation, there was no significant variation in the levels of urea, uric acid and total bilirubin. These may be due to no changes in their blood levels during follieular growth. Among the insulin hormone level in the follieular fluid, there was significantly increased in the large size follicles as compared with small and medium ones. These finding was in agreement with result of **Abo Heakal and El Sadawy (1999)**, that insulin was increased in blood and follicular fluid of the large size follicles. The changes in insulin level may affect the development of granulosa cells and subsequent steroidogenesis. Many studies with granulosa eells have demonstrated that insulin has a dose dependent stimulatory effect on granulosa eells proliferation (**Saunmande, 1991; Langhout et al, 1991; Peluso et al, 1995**). Moreover administration of insulin increased IGF1 in follicular fluid of medium follicles and reduced the number of atretic follicles **Matamoros et al (1991)**. In addition, both insulin and IGF1 stimulated oestradiol secretion in a dose dependent manner (**Adashi and Rohan, 1992**). Furthermore, insulin and IGF1

stimulate proliferation and steroidogenesis of bovine granulosa cells **(Gutierrez et al 1997)**.

Changes in sodium and potassium concentration reflect homeostatic dynamism operating in follicles at different stages of developments, maturation and maintenance of metabolism. In the present study sodium level in ovarian follicular fluid varied significantly between different follicles. The mean sodium level in follicular fluid of large follicle was significantly higher than that of small and medium follicle. The increase in sodium level in follicular fluid along with the increase in size of the follicle may be attributed to the increase in estrogen level. Oestradiol concentration in the follicular fluids increases with the inereased follieular growth in cattle **(Stagniller and England 1982)**, and in she eamel **(Salem et al 1997)**. Moreover, **Bordoloi et al (2001)** reported an increase in sodium concentration in follicular fluid of goat ovary along with the increase follicular size. The potassium level in follicular fluid was significantly deereased as follieular size increased from small to medium and medium to large. These finding is in agreement with the observation of **Bordoloi et al (2001)**. It is concluded that the biochemical and physiological changes that occurs during follieular development could be of crucial importance for follicular growth, ooeyte maturation and affect ovulation and further fertilization of oocyte.

REFERENCES

Abo Heakal N. and El Sadawy H. A. (1999) : Some hormones, minerals and fatty acids constituents in plasma and follicular fluid of she camel in relation to ovarian follicles. Alex. J. Vet. Science Vol. 15, 4:709-719.

Adashi E. Y. and Rohan R. M. (1992) : Intraovarian regulation peptidergic signaling systems. Trends in Endoerinology and metabolism 3 :243-248.

Amer H. A., Salem H. A. H. and Serur B. H. (1997) : Some biochemical metabolic disorders in follicular fluids of cystic and atretic follieles as compared with healthy follieles of Saudi non pregnant camels. J. of Camel Practice and Research 4, 1:71-76.

Bordoloi P. K., Sarmah B. C., Dutta D. J. and Deka B. C. (2001) : Electrolyte concentration in follicular fluid of goat. Indian Vet. J. 78:260-261.

Elssa H. M. (1996) : Concentrations of steroids and biochemical constituents in follicular fluid of buffalo cows during different stages of the oestrous cycle. Br. Vet. J. 152,5 :373-81.

Elwishy A. B., Hemeida N. A., Elsayed A. A., Seida A. M., Galab and Reimers T. J. (1984) : Oestrogen and progesterone levels in the blood of pregnant and non pregnant camels (camelus dromedaries) . J. Mri. 5:39-49.

Gutierrez C. G., Ralph J. H., Wilmut I. And Webb R. (1997) : Follicle growth and antrum formation of bovine preantral follicles in long term in vitro culture. J. of Reproduction and Fertility. Abstract series 19:18(abstr.).

Hafez E. S. E. (1993) : Folliculogenesis , egg maturation and ovulation : In reproduction in farm animals 6 th Ed. E.S.E. Hafez(ed.) Philadelphia, Lea and Febiger.

Ismail A. A. (1982) : Physiological studies on some reproductive states of she camel. Ph D Thesis represented to Faculty of Vet. Med. Zagazig university . Egypt.

Langhout D. J., Spicer L. J. and Geisert R. D. (1991) : Development of a culture system for bovine granulosa eells: effects of growth hormone, estradiol and gonadotropins on cell proliferation, steroidogenesis and protein synthesis. J. of Animal Science 69:3321-3334.

Matomoros I. A., Cox N. M. and Moore A. B. (1991) : Effect of exogenous insulin and body condition on metabolic hormones and gonadotropin -induced follicular development in pre pubertal gilts. J. Anima. Sel. 69,5:2081-91.

Mc Donald L. E. (1977) : Veterinary Endocrinology and Reproduction. 2nd Ed. Lea and Febiger, USA.

Mc Natty K. D. P., Heath K. M., Henderon S., Lun P. R., Hurst L. M. Ellis G. W., Mont-

goner L., Morrison and Thurely D. C. (1984) : Some aspects of theca and granulosa cell function during follicular development in bovine ovary. J. of Reproduction and Fertility.72: 39.

Mobrouk E. A., Salem H. A. H., Ibrahim S. S., El-Hindi H. and Farahat A. A.(1989) : Oestradiol and progesterone levels in follicular fluid and serum of non pregnant one humped she camel in relation to follicular development. Alex. J. of Vet. Science. 5:45.

Murray R. P., Granner D. K., Mayes P. A. and Rodwell V. W. (1999) : Harper's Biochemistry 25 Ed. A lange Medical Book. Prentice Hall International Editions (UK) Limited London.

Pascu T. Dema M., Mihai D., Lumca H. and Salageana G. (1988) : Valeurs du ea Mg et Du glucose a differents moments du cycleestral dans le Liquide follieular et la sang de memes vaches. VI Congress International deReproduction Animale et d'Ismenination Artificielle. Paris 1:785-8.

Peluso J., Luciano A. M., Pappalardo A. and White B. A. (1995) : Cellular and moleeular mechanisms that mediate insulin-dependent rat granulosa cell mitosis. Biology of Reproduction 52:124-130.

Salem H. A. H., Serur B. H. and Amer H. A. (1997) : Oestradiol, progesterone and thyroxine in follicular fluids of normal, cystic and atretic follicles of non pregnant camels in Saudi Arabia. J.of eamel Practice and Research. Vol. 4,1 :81-83.

Saumande J. (1991) : Culture of bovine granulosa cells in a chemically defined serum free medium the effect of insulin and fibronectin on the response to FSH. J. of Steroid Biochemistry and Molecular Biology 38: 189-196.

Selvaraju S. and Rajasundaram R. C. (2001) : Effeet of GnRH and eCG on serum total cholesterol (STC) profile in norgestomet primed post- partum anoestrus cows. Indian Vet. J. 78: 313-315.

Stagniller R. B. and England B. G. (1982) : Folliculogenesis in bovine. Theriogenology 17:43.

Sutton M. L., Gilchrist R. B. and Thompson J. G. (2003) : Effects of in Vivo and in Vitro environments on the metabolism of the culture oocyte complex and its influenee on oocyte developmental capacity. Human Reproduction Update. 9,1:35-48.

Tibary A. and Memon M. A. (1999) : Reproductive physiology in the female south American Camelidae. J. of Camel Practice and Research. Vol. 6,2:217-233.

Wilson M. A. and Miles L. S. M. (1977) : Radioimmunoassay of insulin. In Hand book of Radioimmunoassay, G.E. Abraham (ed.) M.Dkker. Inc, New York. P 275.

الملخص العربى

بعض التغيرات الفسيولوچية والحيوية فى السائل الجريبى للنوق أثناء نمو الجريبات على المبيض

المشتركون فى البحث

على عبدالله القرعاوى

قسم الطب البيطرى - كلية الزراعة والطب البيطرى

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استخدم فى هذه الدراسة ثلاثون من النوق غير العشار وتم جمع المبايض منها بعد الذبح مباشرة، قسمت هذه المبايض حسب حجم وشكل حويصلات المبيض (الجريبات) إلى ثلاث مجموعات وهى جريبات صغيرة، متوسطة، وكبيرة، تم تجميع السائل الجريبى من الجريبات وقياس مستوى البروتينات الكلية، الجلوكوز، اليوريا، حمض اليوريك، صبغة الصفراء (البيليروبين)، وأملاح الصوديوم والبوتاسيوم، كذلك تم قياس مستوى الدهون الكلية، الكوليستيرول، وهرمون الإنسولين، أظهرت النتائج عدم وجود تغيرات ذو دلالة إحصائية فى مستوى كل من اليوريا، حمض اليوريك، وصبغة الصفراء فى المراحل المختلفة لنمو الجريبات، فى المقابل، دلت النتائج على وجود تغيرات معنوية فى مستوى كل من البروتينات الكلية، الجلوكوز، هرمون الإنسولين، الدهون الكلية، وعنصر الصوديوم وكان ذلك واضحاً فى جريبات جراف. على العكس، حدث نقص معنوى فى مستوى كل من الكوليستيرول، والبوتاسيوم فى جريبات جراف عنه فى المراحل الأخرى، وخلاصة الدراسة تدل على أن قد يكون لهذه التغيرات الفسيولوچية والحيوية أهمية خاصة تؤثر فى نمو الجريبات وتزيد من كفاءة نمو البويضات وبالتالى عملية الإباضة ومعدل خصوبة النوق.