Ovarian Follicular Fluid Constituents in Relation to Stage of Estrus Cycle and Size of the Follicle in Buffalo

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Abstract

The goal of the present study was to evaluate the difference in constituent of the ovarian follicular fluid in different stages of the estrus cycle and in medium and large sized follicle and also to evaluate the relation between serum and follicular fluid constituents in cyclic buffalos. A total of 34 clinically healthy buffalo (*Bubals bubals*), aged 7-10 years, were sent for slaughter in Moesha Abattoir, Assiut province in winter 2009. Blood samples and the whole genital tract of each animal were collected. The stage of the cycle (proestrus n= 8, estrus n= 7, metestrus n= 7 and diestrus n= 12) was determined post mortem. Biochemical analysis of serum and follicular fluid was performed through measuring total protein, albumin, chloride, potassium, phosphorus, magnesium, glucose, cholesterol, triglyceride, urea, creatinine levels and lactate dehydrogenase (LDH) activity. Results of the present study revealed that during the estrus cycle, only follicular triglyceride, urea, creatinine and phosphorus level showed significant changes. A positive correlation was found between follicular albumin, phosphorus levels and follicular diameter. Total protein, albumin, globulins, glucose, chloride and creatinine were significantly higher in the serum than that in the follicular fluid. Follicular triglyceride level and potassium level were significantly higher than serum level. Follicular LDH activity was higher in large sized follicle than small sized one. Further studies are required to elucidate the relation between concentration of urea and creatinine in the follicular fluid and oocyte viability.

Key Words: estrus, buffalo, follicular fluid, serum.

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 (Edwards, 1974; Bagavandoss et al., 1983; Wise, 1987; Gosden et al., 1988). Therefore, a different biochemical composition of the follicular fluid in different-sized follicles can be expected. The present study aimed to measure to the difference in constituent of the ovarian follicular fluid in different stages of the estrus cycle and in medium and large sized follicle and also to evaluate the relation between serum and follicular fluid constituents.

Materials and Methods

Animals and samples

A total of 34 clinically healthy buffalo-cows (Bubals bubals), aged 7-10 years, were sent for slaughter in Moesha Abattoir, Assiut province in winter 2009. The animals were rectally examined to confirm non pregnancy. Blood samples were collected from jugular vein immediately before slaughtering in plain vacutainer tube. The whole genital tract of each animal was collected and put in plastic bags taking the same number of the blood sample. All samples placed in isolated box on ice bags and transported within 20-30 minutes to the laboratory.

The stage of the cycle (proestrus n= 8, estrus n= 7, metestrus n= 7 and diestrus n= 12) was determined post mortem. The genitalia were placed in clean water

Table 1. Serum biochemical parameters in follicular fluid during the estrus cycle in buffalo cows

Parameter	Proestrus(n=8)	Estrus(n=7)	Metestrus(n=7)	Diestrus(n=12)
Total protein (g/dl)	4.91 ± 1.1a	4.79 ± 0.45a	4.42 ± 0.7a	4.78 ± 0.50a
Albumin (g/dl)	2.33 ± 0.62a	2.40 ± 0.35a	1.97 ± 0.67a	2.28 ± 0.53a
Globulin (g/dl)	2.57 ± 0.85a	2.39 ± 0.46a	2.44 ± 0.98a	2.49 ± 0.38a
A/G ratio	0.91 ± 0.32 a	1.04 ± 0.31a	1.01 ± 0.72a	0.94 ± 0.32 a
Glucose (mmol/l)	1.57 ± 0.62a	1.09 ± 0.49a	1.19 ± 0.32a	1.33 ± 0.89a
Cholesterol (mg/dl)	40.99 ± 12.45a	42.17 ± 13.43a	37.57 ± 10.31a	39.41 ± 5.07a
Triglyceride (mg/dl)	19.36 ± 6.77a	22.93 ± 6.14a	41.55 ± 10.93c**	37.96 ± 13.72c**
Urea (mmol/l)	6.03 ± 3.22a*	3.72 ± 0.56b	6.42 ± 0.89a, c**	4.78 ± 1.16a, b, c
Creatinine (mg/dl)	0.56 ± 0.12a	0.51 ± 0.28a	0.74 ± 0.32a, c*	0.49 ± 0.18a, b
LDH (U/l)	323.10 ± 87.44a	314.22 ± 157.67a	274.18 ± 87.57a	216.85 ± 126.70a
Chloride (mmol/l)	90.74 ± 6.86a	96.88 ± 2.77a	91.43 ± 9.51a	92.33 ± 9.14a
Potassium (mmol/l	5.68 ± 1.11a	6.02 ± 0.79a	6.48 ± 0.78a	5.88 ± 0.69a
Magnesium (mmol/l)	1.53 ± 0.698a	1.67 ± 0.69a	1.73 ± 0.56a	2.13 ± 0.91a
Phosphorus (mmol/l)	2.24 ± 0.50a	2.04 ± 0.32a, b	1.41 ± 0.51b, c*	1.71 ± 0.69b, c*

Data expressed as Mean ± SD, In each row value with different letter are significant

bath for measuring the diameter of the follicles and corpora lutea using real-time B-mode ultrasonographic device (5/7.5 MHz linear array transducer,Hitachi, EUB-405B, Japan) (Pierson and Ginther 1987). We categorized the follicles according to its diameter into two groups, first, was the large (more than 8 mm) and second was the medium sized (6-8 mm). After measuring the diameters of both follicles and corpora lutea, the contents of the follicles were aspired using sterile syringe and placed in Eppindorof tubes. Each CL was dissected gently from the ovarian matrix and then was weighted using balance (Setra BL. 410S, Setra Systems, Inc. USA).

Serum was separated from the blood samples according to Coles (1986). Serum and follicular fluid were kept at -20C till analysis. Analysis was performed within two weeks from collection. The following parameters were measured in serum and follicular fluid; total protein, albumin, chloride, potassium, phosphorus, magnesium, glucose, cholesterol, triglyceride, urea, creatinine levels and lactate dehydrogenase (LDH) activity.

Biochemical analysis

Biochemical analysis of follicular and serum constituents were performed using commercial test kits supplied by Spectrum Diagnostics (Cairo, Egypt) and by means of Digital VIS/Ultraviolet Spectrophotometer (Cecil instruments, Cambridge, England, Series No. 52.232).

Statistical analysis

Data were expressed as Mean ± SD, Statistical analysis was conducted using SPSS 13.0 for windows (SPSS, Chicago, USA).The difference in the biochemical parameters in follicular fluid during the stages of the estrus cycle were compared using one way ANOVA followed by least significant difference (LSD) post-hoc analysis ($p<0.05$).

Results

During the Estrus cycle

The level of the triglyceride showed a significant increase in the follicular fluid at the metestrus ($p<0.01$) and Diestrus ($p<0.01$) than at the proestrus and estrus stages. There was a significant increase in the follicular urea level during the proestrus ($p<0.05$) and metestrus stage ($p<0.01$) than the estrus stage. Follicular creatinine level significantly increased during the metestrus stage ($p<0.05$) than the other stages of the estrus cycle. There were significant decrease in the phosphorus level in the metestrus ($p<0.05$) and diestrus stage ($p<0.05$) than the proestrus stage (Table 1).

Size of the follicle

The activity of LDH showed a significant increase in the large sized follicle ($p<0.01$) (Table 3).

Biochemical parameters in follicular fluid and serum

The levels of total protein ($p<0.01$), albumin ($p<0.01$), globulins ($p<0.01$), glucose ($p<0.01$), chloride ($p<0.01$) and creatinine ($p<0.05$) were significantly higher in the serum than that in the follicular fluid. Follicular triglyceride level ($p<0.05$) and potassium level ($p<0.01$) were significantly higher than serum level.

Correlation between biochemical parameters and diameter of the follicle

Follicular albumin (r=0.386*) and phosphorus (r=0.424**) levels showed a significant positive correlation with follicular diameter. Follicular triglyceride level showed a significant negative correlation with the diameter of the follicle (r = - 0.463**)

Discussion

In the present study, there were no differences in concentration of follicular total protein, albumin and globulins during stages of the estrus cycle. There was no significant increase in follicular proteins in large

Table 2. Follicular diameter and corpus leutum weight and size during the estrus cycle in buffalo cows

Parameter	Proestrus(n=8)	Estrus(n=7)	Metestrus(n=7)	Diestrus(n=12)
Follicle diameter (mm)	11.10 ± 0.89a	13.30 ± 1.94b**	7.08 ± 1.32c**	9.06 ± 1.77d**
Corpus leutum weight (g)	2.12 ± 0.54a	-	1.03 ± 0.78b**	1.87 ± 0.42a
Corpus leutum size (mm)	16.26 ± 1.49a	-	12.47 ± 3.77b**	16.37 ± 1.74a

Data expressed as Mean ± SD, In each row value with different letter are significant

Table 3. Serum biochemical parameters in medium (6-8 mm) and large (more than 8 mm) sized follicle

Parameter	Medium size follicle (n=12)	Large size follicle (n=22)
Total protein (g/dl)	4.34 ± 0.71	4.84 ± 0.70
Albumin (g/dl)	2.03 ± 0.60	2.36 ± 0.44
Globulin (g/dl)	2.31 ± 0.49	2.47 ± 0.59
A/G ratio	0.96 ± 0.54	0.98 ± 0.28
Glucose (mmol/l)	0.97 ± 0.34	1.22 ± 0.50
Cholesterol (mg/dl)	37.39 ± 7.68	41.84 ±11.01
Triglyceride (mg/dl)	34.88 ± 12.07	26. 95 ± 13.37
Urea (mmol/l)	5.43 ± 1.34	4.94 ± 2.29
Creatinine (mg/dl)	0.66 ± 0.28	0.51 ± 0.18
LDH (U/l)	190.88 ± 92.58	459.89 ± 136.64**
Chloride (mmol/l)	91.75 ± 8.73	93.43 ± 8.63
Potassium (mmol/l	6.21 ± 0.73	6.00 ± 1.00
Magnesium (mmol/l)	1.88 ± 0.71	1.77 ± 0.80
Phosphorus (mmol/l)	1.81 ± 0.39	1.99 ± 0.53

Data expressed as Mean ± SD, In each row value with different letter are significant

follicle than in small one (Table 3), Similar findings have been reported in dairy cows by Leroy et al. (2004) and in buffaloe by Arshad et al. (2005). Follicular albumin showed a significant positive correlation (r = 0.386*) with the follicular size (Fig. 1), albumin 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 (Arshad et al., 2005). Serum total protein, albumin and globulin were significantly higher than follicular levels (Table 4). Follicular total protein was about 75% of that present in serum, the same reported by Leroy et al. (2004), albumin and globulin were about 72% and 78% respectively of that present in serum. The high correlation between total protein content in follicular fluid and in serum suggests that a substantial part of the protein content in follicular fluid originates from serum (Edwards, 1974; Wise, 1987).

The present study indicated that there was no difference in follicular glucose level during the estrus cycle and also between small and large sized follicle (Table 1& 3). Serum glucose level was significantly higher than follicular levels, 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.

Follicular cholesterol levels didn't differ during the stage of the estrus cycle. Arshad et al. (2005) and Leroy et al. (2004) stated that a cholesterol level in large follicle was significantly higher than in small follicle. In the present study, the increase in cholesterol levels in large follicles wasn't significant which agree with that reported by Arshad et al. (2005). Cholesterol, present in follicular fluid, is bound to the high-density lipoprotein fraction (HDL) because the only other cholesterol-containing lipoprotein fraction, the low-density lipoprotein fraction (LDL), is too large to pass the blood–follicle barrier (Puppione, 1977; Grummer and Carroll, 1988; Wehrman et al., 1991; Bauchart, 1993). The higher total cholesterol concentration in large follicles can be explained by the increased permeability of the follicular wall in that follicle class, permitting the entrance of the larger HDL fraction (Bagavandoss et al., 1983; Wehrman et al., 1991). Serum total cholesterol level was significantly higher than follicular level, and was about 45% of the concentration found in blood, Previous studies reported that follicular cholesterol concentration about 41% (Arshad et al., 2005) and 42% (Leroy et al., 2004), which is lower than the percent established in the present study.

In the present study, follicular triglyceride level significantly increased at the metestrus and diestrus stage of the cycle (Table 1), at the same time follicular triglyceride level were significantly higher in small follicles than in large one. In addition, a significant negative correlation (r = - 0.463*) was found between follicular triglyceride level and follicular diameter (Fig. 3), triglyceride level in the follicular fluid was lower in the

Table-4. Serum biochemical parameters in follicular fluid and serum of buffalo cows

Parameter	Follicular fluid	Serum
Total protein (g/dl)	4.73 ± 0.73	6.25 ± 0.88**
Albumin (g/dl)	2.25 ± 0.56	3.09 ± 0.67**
Globulin (g/dl)	2.48 ± 0.65	3.17 ± 0.98**
Glucose (mmol/l)	1.30 ± 0.66	3.43 ± 1.03**
Cholesterol (mg/dl)	39.83 ± 9.51	85.43 ± 22.91**
Triglyceride (mg/dl)	31.75 ± 13.93	23.02 ± 16.25*
Blood urea (mmol/l)	5.28 ± 1.98	5.97 ± 1.71
Creatinine (mg/dl)	0.56 ± 0.23	0.72 ± 0.26*
LDH (U/l)	278.08 ± 108.44	310.42 ± 105.92
Chloride (mmol/l)	92.45 ± 7.93	105.21 ± 8.79**
Potassium (mmol/l	5.98 ± 0.85**	4.88 ± 1.06
Magnesium (mmol/l)	1.82 ± 0.76	1.64 ± 1.02
Phosphorus (mmol/l)	1.79 ± 0.63	1.37 ± 0.34

Data expressed as Mean ± SD, In each row value with different letter are significant

serum than in the follicular fluid. The same was reported previously by Leroy et al. (2004). These data favour the idea that follicular triglyceride levels are mainly a result of local metabolic processes (Leroy et al. 2004). A relatively stable concentration of triglycerides is maintained in the bovine ovarian follicle, regardless of increases in serum due to physiological status or diet (Wehrman et al., 1991). Triglycerides probably do not pass through the follicular membrane since they are transported primarily by the very low-density lipoprotein fraction (VLDL), which is too large to pass through this barrier (Grummer and Carroll, 1988). In follicular fluid, triglycerides may serve as an alternative energy source since cells cultured in vitro can absorb and consume triglycerides out of the medium. Also, oocytes and embryos show lipid accumulation when cultured in triglyceride containing media (Kim et al., 2001, Abe et al., 2002).

Data of the current study revealed significant increase in follicular urea in proestrus and metestrus stage of the cycle and insignificant changes in follicular fluid from medium and large sized follicle, also there was insignificant change in serum and follicular urea level, our results disagree with the results reported by Leroy et al. (2004), who reported a significant changes in urea level in small, medium and large sized follicle. Reports about the effect of elevated urea levels on fertility are contradictory, although all authors agree that the possible adverse effect of diet-induced elevated urea levels must act at the level of the oocyte (Sinclair et al., 2000; Dawuda et al., 2002). Follicular creatinine level showed a significant increase at the metestrus stage than the diestrus, no significant changes was reported in follicular creatinine level in medium and large sized follicle, serum creatinine was significantly higher than follicular level, which indicated that follicular creatinine formed as a result of inward movement from blood.

Follicular LDH activity was higher at the proestrus and estrus relative to metestrus and diestrus (Table 1), This may attributed to the significant increase in the size of the follicle during the proestrus and estrus stages (Table 2) and supported by the significant increase in follicular LDH activity large sized follicle than small sized one, the same was reported by Adiga et al. (2002) in human follicular fluid. The higher serum LDH activity indicated that there was an inward movement for the LDH from serum to the follicular fluid.

No significant changes were reported in follicular sodium, potassium and chloride and magnesium levels during the estrus cycle and between the sizes of the follicles. Follicular phosphorus level was higher at the estrus and metestrus than at the proestrus and diestrus stage. The significant higher concentration of follicular potassium relative to serum level and the absence of correlation indicating that potassium may be released locally in the follicular fluid, the same was reported by Leroy et al. (2004). Chloride level was significantly higher in serum than in the follicular fluid, indicating that follicular chloride may be transported by inward movement from blood. In conclusion, during the estrus cycle, only follicular triglyceride, urea, creatinine and phosphorus level showed significant changes. A positive correlation was found between follicular albumin and phosphorus levels and follicular diameter. Total protein, albumin, globulins, glucose, chloride and creatinine were significantly higher in the serum than that in the follicular fluid. Follicular triglyceride level and potassium level were significantly higher than serum level. Follicular LDH activity was higher in large sized follicle than small sized one. Further studies are required to elucidate the relation between concentration of urea and creatinine in the follicular fluid and oocyt viability.

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