

## Metabolite Composition Variations of Follicular Fluid and Blood Serum in Iranian Dromedary Camels During the Peak Breeding Season

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**Abstract:** Metabolic changes in blood serum may be reflected in the biochemical composition of follicular fluid and can be indirectly influenced oocyte quality. The purpose of this study was to investigation metabolite composition variations of follicular fluid and blood serum in Iranian dromedary camels during the peak breeding season (October-march). Following slaughter, blood samples were collected from 50 female camels and follicular fluid aspirated from small (5-9 mm) and large (10-20 mm) follicles were analyzed for various metabolite concentrations, using the commercial kits.

**Key words:** Follicular fluid, Iranian dromedary camel, breeding season, metabolite, biochemical environment, globulin

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### INTRODUCTION

Changes in the biochemical constituents of blood are important indicators of physiological state of an animal (Perveen and Usmani, 1993). Metabolic changes in the blood serum may be reflected in the biochemical composition of follicular fluid and can indirectly influence oocyte quality. O'Callaghan and Boland (1999) have suggested that the decline in 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 it has already been shown that changes in concentrations of gonadotropins, steroids and growth factors in follicular fluid of dairy cows were linked with alterations in oocyte quality (Wehrman *et al.*, 1993; Izadyar *et al.*, 1997; Driancourt and Thuel, 1998), it is not unlikely that metabolites which are present in the follicular fluid can influence oocyte quality. Moreover, several *in vitro* studies showed that metabolites, such as glucose (Hashimoto *et al.*, 2000), urea (De-Wit *et al.*, 2001) and  $\beta$ -hydroxy butyrate (Gomez, 1997) may influence the competence of bovine oocytes to mature and after fertilization, to grow to the blastocyst stage. Within the ovarian follicle, the developing oocyte is surrounded by the follicular fluid. Besides meeting nutritional requirements of the growing oocyte, follicular fluid also maintains a proper environment for its growth and maturation. Follicular fluid, a complex extra-cellular fluid is in part a transudate of serum, as surrounding cell layers

permit the free diffusion of proteins of up to 500 kDa (Payer, 1975; Gerard *et al.*, 2002). This fluid is also composed of locally produced substances within the follicle which are related to the metabolic activity of follicular cells (Gerard *et al.*, 2002). This fluid also contains molecules implicated in follicular cell proliferation and differentiation (Adashi, 1994). The knowledge of the biochemical composition of follicular fluid can also provide useful information about the requirements for cell and oocyte growth and maturation.

Moreover, such information may be used as a provisional guide for formulating suitable culture media for *in vitro* cell culture and oocyte maturation in a particular species (Gerard *et al.*, 2002). The follicular fluid forms the biochemical environment of the oocyte before ovulation (Gosden *et al.*, 1988; Jozwik *et al.*, 2001).

It is an avascular compartment within the mammalian ovary, separated from the peri follicular stroma by the follicular wall that constitutes a blood-follicle barrier (Okuda *et al.*, 1982). Follicular fluid is in part an exudate of serum and is in addition 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 (Wise, 1987; Gosden *et al.*, 1988). Therefore, a different biochemical composition of the follicular fluid in different-sized follicles can be expected. Before focusing on possible effects of metabolic changes on follicle and oocyte

quality, it is necessary to determine physiological concentrations of the most common metabolites in follicular fluid from different-sized follicles and to investigate to what extent the serum and follicular fluid levels are correlated. Therefore, the aims of this study were to determine the chemical composition of follicular fluid in tow different follicle sizes and blood serum in female dromedary Iranian camels.

**MATERIALS AND METHODS**

Ovaries from 50 female adult camels (*Camelus dromedarius*) in good health and with normal reproductive tracts upon macroscopically examination after slaughter were collected in breeding season used for this study. No information regarding the nutritional or reproductive status of these camels was available. About 10 mL jugular bloods was collected from each animal, serum was separated and stored at -20°C until analyzed for metabolite contents. Immediately after collection, ovaries were wrapped in plastic sheets, placed in an ice box and taken to the laboratory where the diameter of graafian follicles on each ovary was measured using a Vernier Calipers and follicles were categorized as small (5-9 mm) and large (10- 20 mm). Follicular fluid was aspirated from small and large follicles using a sterile syringe and 22 G needle and was stored at -20°C for subsequent biochemical analysis. For each animal and follicle class, a different needle and syringe were used. In certain cases, the amount of fluid collected from small follicles was too small to carry out analysis for all biochemical constituents. In such cases, fluid collected from follicles of the same category from the same ovary of the same animal was pooled (Gerard *et al.*, 2002; Leroy *et al.*, 2004).

**Biochemical analysis of the serum and follicular fluid:** In each sample, the concentrations of glucose, cholesterol, total proteins, albumin, globulin, triglycerides were measured. The determination of metabolite levels in follicular fluid and blood serum was done using wet chemistry techniques on chemistry auto analyzer (Model BT-3000). Standard commercial kits were used for analysis and procedures were adopted as recommended by the manufacturer of kits.

**Statistical analysis:** Results are expressed as (Means±SE) the overall mean concentration±SE of each metabolite was calculated for follicular fluid and for blood serum in all camels. The concentrations of each factor in the follicular fluid were compared between the two follicle classes. A comparison was made for the levels in the follicular fluid of each follicle class and those of serum. Concentrations in the tow different follicle classes were compared and a

paired samples t-test was performed to compare concentrations found in the blood serum and the follicular fluid (SPSS 16.0) a value of p<0.05 was considered statistically significant.

**RESULTS AND DISCUSSION**

The concentration of metabolites in the follicular fluid from small, large follicles and blood serum is shown in Table 1 and triglyceride concentrations decreased significantly as follicle size increased (p<0.05). Conversely, the concentrations of glucose, albumin and total protein increased none significantly as follicle size increased. The overall mean serum glucose concentration in the female dromedary was 120.4±8.21 mg dL<sup>-1</sup>. This value is higher than the concentrations reported in the literature for other species, e.g., 39.67±0.61 mg dL<sup>-1</sup> in postpartum buffaloes (Quayam *et al.*, 1988) and 82±3 mg dL<sup>-1</sup> in cows (Simpson *et al.*, 1994). The camel is a beast of the desert where the climatic conditions are very severe and harsh. The high serum glucose level might be important for the survival of this species in such a harsh environment. Besides species differences, nutritional plan or physiological status of the animal can also influence serum glucose levels. The overall mean glucose content in the follicular fluid was 70.70±7.26 mg dL<sup>-1</sup> in small and 88±13.43 mg dL<sup>-1</sup> in large follicles. The data also show that glucose concentrations in follicular fluid were lower than measured in serum (p<0.05). In fact, the glucose concentration in follicular fluid was 58.7% small and large 73.08% of that found in blood serum.

The glucose concentration increases when the follicle diameter increases which confirms the results of Landoua *et al.* (2000) for dairy cows and Chang *et al.* (1976) for sows. This could mean that glucose metabolism is less intensive in large follicles compared with small ones, resulting in a lower consumption of glucose from follicular fluid and in a reduced secretion of lactate into the follicular fluid. Increasing amount of follicular fluid is a second explanation for the increase in glucose and the decrease in lactate, since in large follicles a relatively

Table 1: Means (±S.E.) for concentrations of cholesterol, globulin, albumin, total protein, triglyceride and glucose in follicular fluid of each follicle class and in serum of 50 iranian dromedary camels

Items	Small follicle (5-9 mm)	Large follicle (10-20 mm)	Blood serum
Cholesterol (mg dL <sup>-1</sup> )	7.0±0.880 <sup>b</sup>	6.30±0.70 <sup>b</sup>	38.30±4.50 <sup>a</sup>
Globulin (g dL <sup>-1</sup> )	1.94±0.26 <sup>b</sup>	2.43±0.14 <sup>b</sup>	3.28±0.27 <sup>a</sup>
Albumin (g dL <sup>-1</sup> )	4.28±0.13 <sup>a</sup>	4.18±0.11 <sup>b</sup>	3.88±0.14 <sup>a</sup>
Total protein (g dL <sup>-1</sup> )	6.22±0.25 <sup>b</sup>	6.61±0.13 <sup>ab</sup>	7.16±0.33 <sup>a</sup>
Glucose (mg dL <sup>-1</sup> )	70.70±7.26 <sup>b</sup>	88.0±13.43 <sup>b</sup>	120.4±8.21 <sup>a</sup>
Triglycerides (mg dL <sup>-1</sup> )	31.30±3.97 <sup>a</sup>	17.80±4.23 <sup>b</sup>	34.30±5.25 <sup>a</sup>

<sup>a,b</sup>:Data with different superscripts within a row differ significantly between follicle classes and blood serum, statistical level of significance: p<0.05

smaller number of granulosa cells consumes glucose from and secretes lactate into a relatively larger amount of follicular fluid (McNatty *et al.*, 1979; Gosden *et al.*, 1988). A further reason for this observation could be the increased permeability of the blood-follicle barrier during follicular growth (Okuda *et al.*, 1982; Bagavandos *et al.*, 1983).

Collins *et al.* (1997) and Gerard *et al.* (2002) observed that in mares the follicular fluid glucose contents decreased as the dominant follicle (20-25 mm in diameter) developed to a pre-ovulatory follicle (33-35 mm in diameter).

On the other hand in dairy cows, Leroy *et al.* (2004) recorded an increase in the follicular fluid glucose contents as the follicles increased in size (Table 1). Cholesterol plays a significant role in the physiology of the ovary as it is the precursor of steroid hormones secreted by this organ. The overall mean serum cholesterol level in female camels was  $38.30 \pm 4.5$  mg dL<sup>-1</sup>. The reference value of serum cholesterol in camels published by the Central Veterinary Research Laboratory, Dubai, UAE (Anonymous, 1997) was 30-62 mg dL<sup>-1</sup> which is comparable to that observed in the present study. In the present study, cholesterol concentrations in FF of large ( $6.30 \pm 0.70$  mg dL<sup>-1</sup>) follicles were lower than that of small ( $7.0 \pm 0.88$  mg dL<sup>-1</sup>) 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% of level found in serum. Similar findings were observed in the present study, where serum cholesterol level was higher ( $p < 0.05$ ) than that found in small or large follicles. The overall mean serum total proteins concentration was  $7.16 \pm 0.33$  g dL<sup>-1</sup>.

According to Dalvi *et al.* (1998), the blood total protein contents in Indian camels, averaged  $7.42 \pm 0.54$  g%. In buffaloes, plasma total proteins concentration was  $7.68 \pm 0.34$  g dL<sup>-1</sup> (Arshad *et al.*, 2005). Besides species and breed difference, variation in climatic conditions may be attributed for these minor discrepancies. In camels, the overall mean serum albumin and globulin concentrations were  $3.88 \pm 0.14$  and  $3.28 \pm 0.27$  g dL<sup>-1</sup>, respectively. The reference serum albumin level for camels was  $3.75 \pm 0.75$  g dL<sup>-1</sup>, the range was 3.0-4.5 g dL<sup>-1</sup> (Anonymous, 1997) which supports the findings in the present study. However, Dalvi *et al.* (1998) recorded the mean serum albumin and globulin levels of  $2.9 \pm 0.33$  and  $4.53 \pm 0.49$  g%,

respectively in Indian camels kept in a hot humid climate. 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 follicles ( $p < 0.05$ ). The overall mean follicular fluid albumin concentration in small and large follicles was  $4.28 \pm 0.13$ ,  $4.18 \pm 0.11$  g dL<sup>-1</sup>, respectively.

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 serum contained significantly lower albumin level than large follicles ( $p < 0.05$ ). This higher albumin level in FF than in serum 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. Globulin has a significant importance in the body due to its immunity producing activity.

The mean globulin concentration in small sized follicles was  $1.94 \pm 0.26$  g dL<sup>-1</sup>, while for large sized follicles, this value was  $2.43 \pm 0.14$  g dL<sup>-1</sup>, the difference between two follicular classes was non significant. A significant difference was observed between levels of globulin in follicular fluid and serum. This shows that the level of albumin was higher while that of globulin was lower in follicular fluid than the blood. The globulin present in follicular fluid, though in small quantity, might be necessary for protecting the follicle from external environments (Arshad *et al.*, 2005).

The overall mean serum triglycerides content in the female camels was  $34.30 \pm 5.25$  mg dL<sup>-1</sup> which is comparable to range (22-42 mg dL<sup>-1</sup>) proposed for the camels in UAE (Anonymous, 1997).

The serum concentration of triglycerides was significantly higher than the level measured in small ( $31.30 \pm 3.97$  mg dL<sup>-1</sup>) and large ( $17.80 \pm 4.23$  mg dL<sup>-1</sup>) follicles ( $p < 0.05$ ). Triglyceride levels in small follicles lower no- significant than in serum and significantly higher in large follicles ( $p < 0.05$ ). These data favor the idea that follicular triglyceride levels are mainly a result of local metabolic processes. 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.*, 1993). 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.

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).

### CONCLUSION

Results showed that accompanied by increased in follicular fluid concentrations of glucose, total protein, globulin from small to large follicles the concentration of albumin, triglycerides and cholesterol decreased. The concentration of all variables except albumin in blood serum was higher than follicular fluid and for cholesterol, globulin and glucose was significant ( $p < 0.05$ ). The results from the present study suggested that the oocyte and the granulosa cells of ovary follicles of female dromedary camels grow and mature in a biochemical environment that changes from small to large follicles.

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