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# Metabolites Comparison between Serum and Follicular Fluid of Small, Medium and Large Follicles of Camels with Active Ovaries

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## ABSTRACT

**Background:** Camel follicular fluid is a complex biofluid that provides the microenvironment for oocyte development and maturation. The aims of current study were to compare serum metabolites with follicular metabolites of small, medium and large follicles of she-camels.

**Methods:** Thirty pairs of active ovaries were chosen from non-pregnant she-camels aged 9-16 years old in addition to 10 blood samples after slaughetr. Ovarian follicles were classified into small (< 0.5 cm), medium (0.5-8.0 cm) and large follicles (0.9-2.0 cm). Follicular fluid was aspirated using a syringe with 18-g needle. Biochemical analyses were carried out of serum and follicular fluid samples using biochemistry analyzer (Skyla VB1) and digital analyzer (ZEALSON).

**Result:** The results showed that serum had higher total protein concentration compared to follicular fluid across all follicle sizes versus urea and creatinine values. Glucose values were the highest in serum group if compared to follicular fluid of all follicle sizes. In addition, glucose values were increased progressively of small FF, medium FF and large FF groups, respectively. The serum group had the highest HDL and triglycerides values compared to follicular fluid of all follicle sizes' groups. Higher AST values were found in small FF, medium FF and large FF groups compared to the serum group whereas the highest ALT value was found in small FF group.

**Key words:** Biochemistry, Follicles, Follicular fluid, Metabolites, Serum.

## INTRODUCTION

Folliculogenesis is ovarian follicles' development by which the primordial follicles in the ovarian cortex develop further into pre-ovulatory follicles, which are capable of ovulation (Feng *et al.,* 2017; Yang *et al.,* 2020; Mohammed *et al.,* 2024 a,b). This complex process is essential for female fertility and is regulated by a delicate interplay of hormones (Hood *et al.,* 2023; Wang *et al.,* 2025). The stages of ovarian follicle development includes primordial follicle, primary follicle, secondary follicle, antral follicle, pre-ovulatory follicle (Cox and Takov, 2023). Primordial follicle consists of a primary oocyte surrounded by a single layer of flattened granulosa cells. Primary follicle is triggered to develop from the primordial pool, oocyte grows and the surrounding granulosa cells become cuboidal and start to proliferate (Orozco-Galindo *et al.,* 2025). Secondary follicle is characterized by multiple layers of granulosa cells and small fluid-filled spaces (Wang and Yang, 2025). In antral follicle, the small fluid-filled spaces coalesce to form a large cavity called the antrum, filled with follicular fluid and the oocyte is located eccentrically within the follicle, surrounded by a layer of granulosa cells called the cumulus oophorus (Morikawa *et al.,* 2022). The follicle continues to grow and becomes increasingly dependent on hormonal stimulation (Jinno, 2025). Pre-ovulatory follicle is the largest ovarian follicles with large antrum and primary oocyte completed first meiotic division to become secondary oocyte (Holesh *et al.,* 2023).

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Follicular fluid is a complex biofluid that provides the microenvironment for oocyte development, oocyte maturation and further embryonic development (Mohammed *et al.,* 2005; Ali *et al.,* 2007; Zia-Ur-Rahman *et al.,* 2008; Yang *et al.,* 2023). Its composition is similar to blood plasma but not identical and it contains various substances crucial for follicle growth, oocyte maturation and ovulation (Pan *et al.,* 2024). Follicular fluid contains nutrients including glucose, amino acids, lipids and various

metabolites, which are essential for the growth and development of the oocyte (Mohammed *et al.,* 2019a,b; Shahzad *et al.,* 2024).

Research continues to unravel the intricate composition of follicular fluid and the precise roles of its various components, holding promise for improving our understanding of fertility and enhancing assisted reproductive technologies (Mohammed and Kassab 2014; Mohammed *et al.,* 2011, 2019 a,b; Pan *et al.,* 2024). Because the limited developmental competence of camel oocytes *in vitro* compared to those of ruminant species, further studies are still required through exploring follicular fluid composition and improving culture conditions using follicular fluid. Therefore, the aims of the current study were to compare the camel serum metabolites with those of follicular fluid obtained from small, medium and large follicles in active status.

## MATERIALS AND METHODS

Ethical approval was not required for this study because blood samples and ovaries were recovered at the time of slaughtering from she-dromedaries. This study was carried out from January to May 2025 at Al-Ahsaa region. The samples and experimental analyses were processed in the laboratory of animal and fish department of the agriculture and food sciences college at king faisal university.

### Ovarian and blood samples collection

Thirty pairs of ovaries were obtained from non-pregnant she-camels aged 9-16 years old in addition to 10 blood samples after slaughter. Ovarian tissues and blood samples were stored in a cooler and transported to the laboratory within one hour of slaughtering. Ovarian follicles were classified into small (< 0.5 cm), medium (0.5-8.0 cm) and large follicles (0.9-2.0 cm). Follicular fluid was aspirated using a syringe with 18-g needle. Blood samples were centrifuged for 10.0 min at 2000 g and the collected serum and follicular fluid were kept at -20°C until further analysis.

### Biochemical analyses

Biochemical analyses were carried out on serum and follicular fluid samples. The obtained serum and follicular fluid samples were analyzed using biochemistry analyzer (Skyla VB1). The resulting biochemistry profiles include total protein (g/dl), urea (mg/dl), creatinine (mg/dl), glucose (mg/dl), aspartate Aminotransferase (U/l), alanine Aminotransferase (U/l), lactate dehydrogenase (U/l), iron (µg/dl). values. In addition, high-density lipoprotein (mg/dl) and triglycerides (mg/dl) values were recorded using digital analyzer (ZEALSON).

### Statistical analysis

Biochemistry values of serum and follicular fluid of small, medium and large follicles were statistically analyzed using General Linear Model procedure of one way ANOVA (SAS 2008) according to the following model:

$$Yij = \mu + Ti + eij$$

Where

µ = Mean.

Ti = Effects of serum and follicular fluid of small, medium and large.

Eij = Standard error.

Duncan's multiple range test (1955) was used to compare between means of control and follicular fluid of small, medium and large groups.

## RESULTS AND DISCUSSION

The results of comparing serum metabolites with those of follicular fluid obtained from small, medium and large ovarian follicles of she-camels are presented in Table 1.

There were significant differences between serum and FF groups in total protein values ($p<0.0001$). The total protein values were significantly decreased with increasing follicle' size. The serum group had the highest total protein value, followed by values of small FF, medium FF and large FF groups, respectively. Urea and creatinine parameters showed the highest levels in the small FF group compared

**Table 1:** Metabolites comparison between serum and follicular fluid of small, medium and large follicles of camels with active ovaries.

| Parameters | Serum | Small FF | Medium FF | Large FF | SE | P value |
|---|---|---|---|---|---|---|
| Total protein, g/dl | 6.31$^{a}$ | 5.45$^{b}$ | 4.96$^{c}$ | 4.2$^{d}$ | 0.24 | <0.0001 |
| Urea, mg/dl | 17.16$^{d}$ | 56.0$^{a}$ | 44.6$^{b}$ | 34.5$^{c}$ | 1.41 | <0.0001 |
| Creatinine, mg/dl | 1.18$^{c}$ | 2.40$^{a}$ | 1.56$^{b}$ | 1.25$^{c}$ | 0.30 | <0.0001 |
| Glucose, mg/dl | 112.5$^{a}$ | 49.0$^{d}$ | 69.66$^{c}$ | 97.83$^{b}$ | 6.98 | <0.0001 |
| HDL, mg/dl | 57.50$^{a}$ | 51.5$^{b}$ | 48.0$^{c}$ | 44.0$^{d}$ | 1.14 | 0.001 |
| Triglycerides, mg/dl | 44.50$^{a}$ | 15.80$^{b}$ | 15.50$^{c}$ | 15.30$^{d}$ | 0.13 | <0.0001 |
| Aspartate aminotransferase, U/l | 60.20$^{b}$ | 81.83$^{a}$ | 79.66$^{a}$ | 76.5$^{a}$ | 10.76 | <0.0001 |
| Alanine aminotransferase, U/l | 43.3$^{b}$ | 62.83$^{a}$ | 44.66$^{b}$ | 35.0$^{b}$ | 3.07 | <0.0001 |
| Lactate dehydrogenase, U/l | 553.3$^{a}$ | 542.0$^{a}$ | 390.6$^{b}$ | 374.0$^{b}$ | 9.66 | <0.0001 |
| Iron, µg/dl | 213.0 | 204.0 | 199.3 | 210.5 | 17.56 | 0.06 |

$^{a,b,}$ Values between groups with different superscripts significantly differ at $P<0.05$.

SE: Standard error, Small FF: Follicular fluid from <0.5 cm follicle, Medium FF: Follicular fluid from 0.9-2.0 follicle, Large FF: Follicular fluid from 0.5-8.0 cm follicle.

to the lowest levels in serum group. Urea and creatinine levels then progressively decreased with increasing follicle size but they were still higher than serum group values. The serum group had the highest glucose level compared to lowest level in small FF group, which then progressively increased in the medium FF and large FF groups. Both HDL and triglycerides values were highest in the serum group and FF groups showed a significant decrease as follicles' size increased. AST levels were significantly higher in the small FF, medium FF and large FF groups compared to the serum group. In addition, ALT levels were highest in the small FF group compared to those of serum, medium FF and large FF groups. Furthermore, serum and FF small groups had the highest LDH levels compared to those of medium FF and large groups. No significant differences among serum and FF groups in iron values.

The follicular fluid biochemical metabolites of ovarian follicles are essential for oocytes' maturation and further fertilization processes. Hence, the changes of these metabolites may affect oocytes' growth and quality. Results of the present experiments are shown in Table 1 to compare the metabolites of serum versus follicular fluid collected from small, medium and large camel follicles. Collectively, the obtained results indicated differences between serum versus follicular fluid metabolites. Follicular fluid is a complex mixture derived from both transudation of serum across the blood-follicle barrier and secretions from follicular cells (Abdulrahman Alrabiah *et al.,* 2022). It contains proteins, amino acids, sugars, enzymes, hormones, growth factors and other molecules crucial for oocyte development (Mo *et al.,* 2023; Pytel *et al.,* 2024; Mohammed and Alshaibani 2025). In addition, the metabolic properties of blood-follicle barriers changed significantly during follicle growth and development.

**Total protein**

The serum group had the highest total protein values ($p<0.0001$), followed by values of small FF, medium FF and large FF groups, respectively. Consequently, the total protein values were significantly decreased with increasing follicle' size. The total protein values in serum and follicular fluid are related but distinct, reflecting different physiological compartments and functions. In camels, the normal range for total protein in serum is typically 6.0 to 8.3 g/dL (Mohammed and Mahmoud, 2011; Dowelmadina *et al.,* 2012). Serum total protein levels are used to assess general health status and can be affected by various conditions, including liver disease, kidney disease, malnutrition, dehydration and inflammatory conditions (Abdoslam *et al.,* 2018). The blood-follicle barrier regulates the passage of molecules from serum into the follicular fluid. This barrier is selective, meaning that not all serum molecules pass through equally. Smaller proteins tend to have higher concentrations in follicular fluid relative to serum compared to larger proteins (Paes *et al.,* 2020). A decrease in total protein concentration as follicles grow larger might be due to utilization by follicular cells or changes in barrier permeability (Pan *et al.,* 2024).

**Urea and creatinine**

Urea and creatinine parameters showed the highest levels in the small FF group compared to the lowest levels in serum group. Urea and creatinine levels then progressively decreased with increasing follicle size but they were still higher than serum group values as indicated in other studies (Nandi *et al.,* 2007; Mohammed and Mahmoud 2011; Aller *et al.,* 2013; Nandi *et al.,* 2016). Urea is a small, water-soluble molecule that is the primary nitrogenous waste product in mammals (Weerakoon *et al.,* 2023). Because of its small size, urea can readily diffuse across biological membranes, including the blood-follicle barrier. The differences could be attributed to dilution effect and metabolic activity of ovarian follicles (Baker and Wolfe, 2020). Larger follicles contain a greater volume of follicular fluid. Furthermore, smaller follicles might have a relatively less efficient mechanism for the clearance or exchange of these waste products compared to larger, more developed follicles with potentially better vascularization or transport systems (Zhu *et al.,* 2023). Urea concentrations were higher in fluid aspirated from de-veloping follicles than in serum, most likely due to active transport or local urea synthesis by follicular cells (Nandi *et al.,* 2007; Aller *et al.,* 2013). The values of creatinine in serum and follicular fluid are related due to the exchange of substances between the blood and the follicular environment, but their concentrations were increased in follicular fluid due to follicle metabolic activity and specific fluid dynamics within the follicle (Bekkouche *et al.,* 2022).

**Glucose**

The current finding recorded that serum group had the highest glucose level compared to lowest level in small FF group, which then progressively increased in the medium FF and large FF groups. Serum and follicular fluid glucose levels are related, as glucose is a small molecule that can cross the blood-follicle barrier. However, the concentrations in each ovarian follicle sizes were differed and influenced by various factors. Glucose in follicular fluid originates from the blood plasma *via* transport across the blood-follicle barrier. It is also utilized by the cells within the follicle (oocyte, granulosa cells and theca cells) for energy and metabolic processes. Generally, the glucose concentration in follicular fluid is lower than that in serum. Studies across different species, including humans, buffaloes, cows, sheep and she-camels have reported follicular fluid glucose levels ranging from 30% to 80% of the corresponding serum glucose levels (Leroy *et al.,* 2004; Arshad *et al.,* 2005; El-Bahr *et al.,* 2015). Glucose enters the follicular cells *via* glucose transporter (GLUT) proteins. GLUT1 and GLUT4 are among the transporters found in ovarian tissues (Nishimoto *et al.,* 2006). Some studies have observed that glucose levels in follicular fluid increase with increasing follicle size in species like buffaloes and camels, while other studies have not found a significant correlation or have reported variable results (El-Bahr *et al.,* 2015). The

increased volume of follicular fluid in large follicle may be attributed to a decrease in glucose values involved in metabolic activities. This result might be attributed to decrease in metabolic processes in large follicles**.**

**High-density lipoprotein and triglycerides**

Both HDL and triglycerides values were highest in the serum group and FF groups showed a significant decrease as follicles' size increased. This finding could suggest a potential relationship between follicle development and lipid metabolism. Different stages of follicle development have varying metabolic demands that influence HDL and triglycerides levels. Factors within the follicular fluid or local production by follicles of different sizes could affect HDL and triglycerides concentrations in the surrounding environment or systemically (Arias *et al.,* 2022). As follicles mature and grow, there might be a greater utilization or lower transport of HDL and triglycerides into the follicular fluid. The changing metabolic needs or the composition of the follicular fluid during different stages of follicle development could influence HDL and triglyceride levels.

**Aspartate and alanine aminotransferases and lactate dehydrogenase**

Aspartate aminotransferase levels were significantly higher in the small FF, medium FF and large FF groups compared to the serum group. In addition, ALT levels were highest in the small FF group compared to those of serum, medium FF and large FF groups. Furthermore, serum and FF small groups had the highest LDH levels compared to those of medium FF and large groups. Similar AST values were found in serum and follicular fluid of dromedary camel breeds (Bekkouche *et al.,* 2022). The role of AST in follicular fluid is not fully understood, but it is involved in amino acid metabolism, specifically the reversible transfer of an α-amino group between aspartate and glutamate (Holeček, 2023). The higher concentrations of AST and ALT in follicles may suggest that aspartic and glutamine might be important during the stages of ovarian follicular development. The serum and FF small groups had the highest LDH levels compared to medium FF and large groups. Lactate dehydrogenase is an enzyme played a crucial role in anaerobic glucose metabolism and converting pyruvate to lactate. LDH is present in ovarian follicular fluid, originating from serum transudation and the metabolic activity of follicular cells and the oocyte. LDH in follicular fluid plays a role in the metabolic environment of the developing oocyte and may serve as an indicator of follicular health. Iron value is comparable in both serum and follicular fluid groups.

## CONCLUSION

In conclusion, integrated analysis of both serum and follicular fluid matrices provides a more comprehensive understanding of fertility and this might be helpful for improving camel oocyte maturation and further embryo development *in vitro*.

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**Disclaimers**

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the official stance of their affiliated institution.

**Informed consent**

Ethical approval of scientific research deanship committee of king faisal university (ETHICS3145).

**Conflict of interest**

The authors have no conflicts of interest to disclose.

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