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Study the effects of follicular size on some biochemical follicular fluid composition in She camel (*Camelus dromedarius*)

Mayada S. Hassan [1], Ali J. Al-Nuaimi [1], Ali M. Al-Yasari [2], Yasser J. Jameel [1]

[1] College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq
[2] College of Veterinary Medicine, Al-Muthana University, Muthanna, Iraq

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***Corresponding author:**
Mayada S. Hassan
College of Veterinary Medicine, University of Kerbala, Kerbala, Iraq

Abstract

This study was designed to estimate the biochemical composition of ovary folAlicular fluid in relation to its size variations in local Iraqi She camels (*Camelus dromedarius*). Hundred ovary were collected from 50 adult she camel, 4 to 10 years old that slaughtered at Al Najaf abattoir during the breeding season of camelids. The ovaries were transferred immediately to the laboratory in a cold box. Later on, the follicular fluid was collected separately from small and large size follicles, (3-9 mm) and (10-19 mm) respectively. The fluid was kept at -4 0C for further analysis. The follicular fluid samples were analyzed to estimate the metabolic composition (cholesterol, glucose and total protein), and the ionic compositions (calcium, sodium and potassium). The results of this study revealed significant (P< 0.05) increase in the glucose and cholesterol concentration, while there was significant decrease in the total protein in large size follicles. Meanwhile, significant increase was seen in the concentration of Na+ and Ca+2 in relation to the size of the follicles. However, significant decrease was occurred in the concentrations of K with increasing follicle size. In conclusion, the results of this study revealed a significant variation in the concentration of the follicular fluid metabolic and the ionic compositions with the variations of its size.

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Introduction

Camel is the unique animal that can live for several weeks without water. Camels are providing milk, hid and meat though a harsh and severe conditions. Moreover, it is used in racing and competition. There are two species of camel included in the genus *Camelus*. The first species is *Camelus dromedaries*, the dromedary or one-humped camel, the world population of which is estimated to be 15,368,000, with approximately 80% in Africa and 20% in Asia. The second species is *C. Bactrians,* the bactrian or two-humped camel, of which there are 1.7 million in their natural habitat in Asia (Al Salihi, 2016; Wardeh, 2004). The breeding of the local camelids is seasonal that start at autumn and increase drastically until the end of winter, meanwhile, it decreases significantly at spring and summer (El-Harairy *et al.*, 2010). The follicular wave is a term replace the estrous cycle. It reflexes the physiological, structural and behavior changes that occur during identified period between one ovulation and another because camels are induced ovulation. Besides, the ovulatory activities are only limited during the follicular changes (Padalino *et al.* 2016). The formation of follicular fluid is starting inside the ovary follicle earlier during its development (Bodhaganahalli *et al.*, 2015). It produces from local substances produce locally, and part of this fluid is filtrated from blood serum that related with the metabolic activities of follicular cell (Gerard *et al.*, 2002). Therefore, the compositions of follicular fluid are alike but not identical to blood plasma (Nishimoto *et al.*, 2009). The ovary cells produce soluble substance like steroids hormone, growth factors (Fortune *et al.*, 2004) inhibition factors (Arunakumari *et al.* 2007), ionic and fat substances (Nandi *et al.*, 2008), as well as some of minerals and salts (Sharma and Vasta,1998). All these substances play important role in the metabolic activities of the ovary cells. Consequently, the functional status of the follicles and the follicular fluid has an important vital role on the ovary cells that referred to the functional status of the follicle (Abdoon, 2001). The follicular fluid has biological activities, it is providing the internal environment for growing of ova and granular cells and protect the ova from the external condition. It is a good media and contains fat, steroids, amino acid and different protein and minerals. This substance provide an environment that provide fat, steroids and amino acids and different protein that provide a good environment for maturation of ova and effect on the conception (El-Shahat *et al.*,2013). The follicular fluid has the ability to keep the Meiosis of the egg in silent stage and protect the released egg from analysis during fertilization (Chang *et al.*,2005), and raising the attractiveness, movement and hat reaction of the sperm (Somfai *et al.*,2012). Follicular fluid is also played a big role in auto-organization (Autocrine) and (Paracrine) of follicular cells, moreover it regulates the maturity of the cytoplasm and nucleus (Cytoplasm) of the egg and ovulation (Campbell, 2009). Knowledge of the follicular fluid components can give information about the needs of the growth and maturity of the follicles and eggs, moreover it is used as a guide to configure an active complement culture medium for maturity and identify the requirements of egg development (Zeidan *et al.*, 2011). The study of follicular fluid in she camels is benefited the improving of in vitro maturation of the egg (IVM) (El-Hassanein *et al.*,2010). The metabolic activities and characteristic of follicular cell wall during its growth and development are changeable, and variations in its biochemical compositions and size are expected (Ali *et al.*, 2011). Consequently, this study was designed to estimate the concentrations of metabolic and ionic constituents including cholesterol, glucose, total protein and Calcium, Sodium and Potassium of the follicular fluid and its relation to the follicular size of she camels.

Materials and Methods

1. Collection of follicular fluid

The study was conducted in the laboratories in the Faculty of Veterinary Medicine/ University of Kerbala during the period extended from 1/10/2017 until 31/12/2017. Hundred ovaries were collected from 50 adult, (4-10) years old she camels that slaughtered at Al Najaf province abattoir during the breeding season. All these animals were in good healthy with a normal genital tract according to post-slaughter examination. The ovaries collected and placed in a plastic bag containing the normal phosphate buffered saline (PBS) (0.9%). Then, the bag was placed in a cool box and immediately transferred to the laboratory within two hours. In the laboratory, all ovaries were washed twice with PBS and placed on the filter sheets to absorb the excess water (Nandi *et al.*,2007). Subsequently, the follicles were removed from each other. The follicles of each ovary were measured by the Vernier calipers (Nichi-Japan) and were classified according to these measurements into two categories, the small and large groups with (3-9 mm) and (10-19mm) in diameter respectively. The follicular fluids were collected separately from each animal in each group and placed in sterilized plastic tube and kept at -4 ^{0}C for further analysis.

2. Biochemical analysis of follicular fluid

The samples of the follicular fluid were analyzed to measure the concentration of the metabolic and ionic components in both groups. A commercial kit from RANDOX-kit-England was used to estimate the concentration of glucose and total protein using spectrophotometer-PD303-Germany the optical method that read at 546 nanometers wavelength. A commercial kit, Cromatest-kit-Spain was used to estimate the cholesterol concentration using optical spectrometer and 500 nanometers wavelength. The Biomaghreb-kit-Tunisia was used to determine the ions concentration using the optical spectrometer that read at 500 nm, 550 nm and 578 nm wave length for sodium, calcium and potassium ions respectively.

3. Statistical analysis

Complete randomized design was used to investigate the effect of the follicular size on the metabolic and ionic components concentration level. The mean differences between the averages using a multiplicity test (Duncan, 1955) to compare the differences between the averages. Statistical analysis of data was done according to SAS program (SAS, 2004).

Results and discussion

A significant increase ($P < 0.05$) in the concentration of cholesterol of the follicular fluid was appeared with increase in the follicular size (Table. 1). Its concentrations in the small and large follicles were 5.22 ± 0.40 mg / dL and in 7.54 ± 0.03 mg / dL respectively. The follicular cholesterol is derived from two sources, the acetate in the follicular granular cells and from the lipo-proteins of the blood plasma (Nandi *et al.*,

2007). Cholesterol is considered as the primary substance for the building of the lipid hormones, besides the follicular fluid contains only high-density lipoproteins (HDL). Therefore, the follicular granular cells are depended on the cholesterol derived from these plasma-derived fats by crossing the basement membrane of its cells (Mishra *et al.*, 2003). The low-density lipoproteins(LDL) molecules was lack of in the follicular fluid because its own a large size molecule that can't cross the blood vessel- follicular wall barriers (Clarke *et al.*, 2006). The granular cells need cholesterol during its growth and multiplication. Therefore, it is withdrawn from follicular fluid that led to decrease its concentration in the small size follicle. Nonetheless, when the size of follicle enlarged, its cells multiplication is decreased and lead to release cholesterol into the follicular fluid that use in the formation of lipid hormones (Su *et al.*, 2008). The results of the current study are agreed with previous studies in she camel, buffalo and sheep that done by Albomohsen *et al*., (2011); Arshad *et al*., (2005)and Nandi *et al*.(2007)respectively. Meanwhile, the results of the current study are incompatible with previous reports in she camel, buffalo and goats that done by Rahman *et al*., (2008) and AbdEllah *et al*., (2010)and Deshpande and Pathak (2010)respectively.
A significant increase ($P<0.05$) was appeared in the concentration of the follicular glucose in relation with increasing of follicular size. Its concentrations were 43.64 ± 4.76 mg / dl and 71.32 ± 10.08 mg / dL in small follicles and large follicles respectively. Glucose plays an important role for the ovarian metabolism because it acts as an important energy source for the ovary via anaerobic metabolism pathway that leads to formation of lactate (Boland *et al.*,1994 and Rabiee *et al.*,1999). In small follicle, the significant increase in the glucose concentration may be due to lack of its metabolism and consumption by the few numbers of granular cells in compare to large follicles (Nandi *et al.*,2007 and Leroy *et al.*,2004). However, other researcher found that high permeability of blood vessel- follicle wall barriers during the follicular growth led to filtrate more glucose from blood plasma into follicular fluid (Ying *et al.*, 2011 and Nishimoto *et al.*, 2006). Moreover, Nishimoto *et al.* (2006) described the importance of glucose concentration in the growth media necessary for in vitro development and maturity of eggs. These observations are indicating to the harmful effects of decreasing and increasing glucose concentration on the growth and maturity of the egg and lead to incomplete maturation cell's nucleus. The results of the current study are compatible with previous study in camels (Padalino *et al.*, 2016) and disagreed with (Rahman *et al.*, 2008), who mentioned that the level of glucose was relatively high in the small follicles in compare to the large follicles in *camelus dromedaries* she camel. This variation may be occurred due species differences in different countries and even in the same country (Khanna *et al.*, 2004). The results of this study are also in agreement with results in another species of animals as buffalo (Arshad *et al.*, 2005), cattle (Leroy *et al.*, 2004), sheep (Nandi *et al.*, 2007) and goats (Herrick *et al.*, 2006).
A significant decrease ($P < 0.05$) in the total protein concentration with the increase in the size of the follicle is also appeared in Table.1. Its concentrations in follicular fluid was 6.14 ± 0.19 g / dL in small follicle, while its concentration decreased to 4.63 ± 0.13 g / dL in large follicle. The follicle needs a protein at the beginning of its formation to build up the multiple layers of granular cells and the cells surrounding the egg. Therefore, this process makes the follicle needs a lot of protein that will draw

from the blood serum and excreted in the follicle and led to increase its concentrations in the small follicles (Chang *et al.*, 2005). The lipoprotein is secreted from follicular granular cells and are involved in the new follicular formation and its blood vessels, and linear division of egg before ovulation. Therefore, it will increase at the beginning of the formation of the small follicle, thus increase in its follicular fluid (Hunter *et al.*, 2004). However, the decreasing of protein concentration in the large size follicle was the increasing in the production of lipid hormones, that need binding proteins, therefore its consume is decrease in large follicles (Kiker *et al.*, 2005). Moreover, the results of the current study are in agreement with previous studies in camels (Rahman *et al.*, 2008 and Albomohsen *et al.*,2011), nevertheless it is incompatible with (Bodhaganahalli *et al.*,2015) in camels. Meanwhile, these results are agreed with the results reported in buffalo (Thangavel and Nayeem, (2004), cows (Leroy *et al.*,2004) and goats (Singh *et al.*,1999) differ with (Arshad *et al.*,2005), but are disagreed with (Nandi *et al.*,2007) in sheep and buffalo (Arshad *et al.*, 2005).

Table. (1) : shows the concentration of metabolic components in follicular fluid of small and large follicles of the local camels

Composition (Metabolites)	**Small follicle (3-9 mm)**	**Large follicle (10-19 mm)**
Cholesterol (mg/dl)	5.22 ± 0.40 (B)	7.54 ± 0.03 (A)
Glucose (mg/dl)	43.64 ± 4.76(C)	71.32 ± 10.08 (A)
Total protein (g/dl)	6.14 ± 0.19 (A)	4.63 ± 0.13 (B)

Values with different letters within the same row are significantly different (P <0.05)

The level of calcium ion concentration is significantly (P <0.05) affected by the follicular size. Its concentration was increased with the increase of follicular size. The calcium concentrations were 2.25 ± 0.96 mmol / L. and 3.45 ± 1.09mmol / L. in the follicular fluid of the small and large follicles respectively. Calcium plays an important role in the production of lipid hormones of the developing follicles and it regulates the secretion of breeding hormones necessary for ovaries and ovulation (Iwata *et al.*, 2004). Moreover, calcium ions is involved in the formations of estrogen. The level of this hormone is increased during follicular development and consequently, require large quantities of calcium ions that withdraw from blood inside the follicular fluid, then raising its calcium concentration (Nandi *et al.*, 2007). The results of the current study are agreed with previous studies in camels (AlFattah *et al.*, 2012), buffalo (Kaur *et al.*, 1997), sheep (Nandi *et al.*, 2007) and goats (Sava, *et al.*,2005), while it is incompatible with (Arsha *et al.*, 2005) in sheep.

The concentration of sodium ion was affected significantly (P <0.05) with variations of the follicular size. Its concentrations was 93.33 ± 4.75 mmol / L in small follicle size. Meanwhile, it was increased with increasing of follicular size that reached 145.96 ± 4.26 mmol / L. Sodium ion has a relation with vitality of the follicle and its activities in the production of estrogen that has the ability in retained sodium inside the cells (Nandi *et al.*, 2007). The size of follicle was increased with its growth continuity because the movement of water from blood into follicular fluid. However, this process requires osmosis process across cell wall that increase with the elevation of sodium ions in the large follicle (Sharma *et al.*,1995). The results of the present study are agreed with previous studies in camels (AlFattah *et al.*, 2012), cattle (Iwata *et al.*, 2004) , buffalo (Kaur *et al.*,1997) , goats (Bordoloi *et al.*,2001) and sheep (Nandi *et al.*,2007). While, these results are incompatible with (Rabiee *et al.*,1999) in cattle and (Arshad *et al.*,2005) in buffalo.

The concentration of potassium ions was significantly reduced (P <0.05) with the increase of follicular size. Its concentration was 12.96 ± 0.68 mmol / L in the fluid of small follicular size. However, its concentration was significantly decreased in to 6.12 ± 0.57 mmol / L in the fluid of large follicular size. The decreasing of the potassium ion concentration is related with the follicle development that lead to increase glucose consumption. This process leads to move potassium ions from extracellular spaces to intracellular space and thus reduces its concentration in the follicular fluid when follicular size enlarges. The concentration of potassium ions in the follicular fluid revealed high significance in compare to its concentration in the serum accompanied with missing a correlation between them indicated that Potassium ion may be excreted locally in the follicular fluid (Leroy *et al.*, 2004 and AlFattah *et al.*, 2012). These results are in agreement with (AlFattah *et al.*, 2012) in camels and (Arshad *et al.*, 2005) in buffalo and (Leroy *et al.*, 2004) in cattle and (Nandi *et al.*, 2007) in sheep.

Table (2): Shows the concentration of ionic components small and large follicular fluid of local camels

Composition (Ions mmol/L)	**Small follicle (3-9 mm)**	**Large follicle (10-19 mm)**
Calcium	2.25 ± 0.96 (B)	3.45 ± 1.09 (A)
Sodium	93.33 ± 4.75 (C)	145.96 ± 4.26 (A)
Potassium	12.96 ± 0.68 (A)	6.12 ± 0.57 (B)

Values with different letters within the same row are significantly different (P <0.05)

In conclusion, this study approved the variations in the concentration of metabolic and ionic components of follicular fluids in relation to the follicular size and its development stage. The results of this study can be considered in the formulation of egg culture media use in the in vitro fertilization.

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