

## **EFFECTS OF FOLLICLES SIZE ON SOME BLOOD PARAMETERS IN SHE CAMELS SUFFERING FROM LONG CALVING INTERVAL**

By

**Howayda S. Belal<sup>\*</sup>; Elmetwaly H.A<sup>\*\*</sup> and Mohamed Z. M<sup>\*\*\*</sup>**

<sup>\*</sup>Biology Of Reproduction Department, Animal Reproduction Research Institute (ARRI), <sup>\*\*</sup>Camel Research Department, Animal Production Research Institute (APRI) and <sup>\*\*\*</sup>Diagnostic imaging and Endoscopic unit (DIEU), Animal Reproduction Research Institute (ARRI)

### **ABSTRACT**

The present study aimed to evaluate the effect of follicular size on some biochemical and hormonal profiles in serum of non pregnant dromedary she camels. During breeding season a total of 15 apparently healthy dromedaries she camels suffering from long calving interval extended to more than two years. Ultrasound examination of these animals were classified the animals into two groups according to follicular size, group 1 (n=10) with small sized follicle (2-4mm), group 2 (n= 5) with large sized follicles (10-20 mm). The blood was collected separately from each she camels with small and large follicles to estimate the changes in metabolic (glucose; cholesterol), hormonal (estradiol, progesterone, thyroid hormones) and ionic (ionic calcium; selenium). The result of this study revealed significant ( $p < 0.05$ ) increase in glucose, cholesterol, ionic calcium ( $Ca^{+2}$ ) and selenium with increasing follicle size. Camels with small follicles had lower ( $p < 0.05$ ) serum progesterone,  $17\beta$  estradiol and triiodothyronine (T3) than those with large follicles. Follicular size had non significant effect on serum T4 levels.

### **Keywords:**

She Camel-Follicular Sizes-Serum-Biochemical- Hormones-Ultrasonographic

### **INTRODUCTION**

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 replaces 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 (**Padalino et al. 2016**).

The ovarian cells produce soluble substance like steroids hormone, growth factors (**Fortune et al.,2004**) inhibition factors (**Aruna kumari 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 an important role in the metabolic activities of the ovarian cells.

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**). Ultrasound is being used in she-camel for detecting ovarian follicular sizes (**Skidmore et al., 1995; Tibary and Anouassi, 1996**).

The present study aimed to evaluated the effect of follicular size on some biochemical and hormonal profiles in serum of non pregnant dromedary she camels

## **MATERIAL AND METHODES**

### **Animals:**

The present study was conducted during the breeding season (from October - November) in Animal Production Research Institute (APRI), camel farm at Matrouh governorate and some special camel farms in Matrouh. A total of 15 apparently healthy with history of long calving interval over 2 years and free from parasitic infestation were used. She camels age was between 5 and 11 years old and weighing about 500 kg. According to ultrasound examination, she camels were classified into two groups. Group 1 (n=10) with small sized follicle (2-4mm) and group 2 (n= 5) with large sized follicles (10-20mm). The camels were housed in an open yard with shelter.

### **Feeding:**

She camels were fed diet composed mainly of commercial concentrates mixture (12% crude protein and 70% TDN; 4.5kg/ head/day) in addition to a good quality roughage materials that were alfalfa hay in summer and Egyptian green alfalfa in winter (16kg/head/day). All animals had a free access to drinking water.

### **Blood sampling:**

Blood samples were collected on plain vacutainer tubes. Serum was harvested after centrifugation of tubes plain vacutainer at 3000 rpm for 10 minutes, and then stored at -20°C until analysis.

### **Estimation of some biochemical constituents in serum:**

Blood samples were analyzed calorimetrically using commercial kits for: glucose, cholesterol and ionic calcium according to **Trinder, (1969); Richmond, (1973) and Gindler and king,**

(1972) respectively using Biodiagnostic and research reagents. The serum Se concentrations were measured using a graphite furnace atomic absorption spectrophotometer with Zeeman correction (Z-5000; Hitachi High-Technologies, Tokyo, Japan).

**Estimation of some hormonal constituents in serum:**

Progesterone (P4) (Tietz, 1995), estradiol (E17β) (Wisdom, 1976), total triiodothyronine (Braverman,1996) and total thyroxin (Muzzaffari and Gharib,1998) were assayed using ELISA micro wells kits (Monobind Inc. Lakeforest, CA92630.USA).

**Statistical analysis:**

Data were presented as mean ± SEM. Independent-samples T test was carried out for the obtained data using SPSS,(2007) program version 16.0 and P ≤ 0.05 was considered as statistically significant.

**RESULTS**

Results revealed significant (p<0.05) increase in the concentration of serum glucose and serum cholesterol in large follicles than in small ones. Also, significant (p<0.05) increase was recorded in the concentration of serum Ca<sup>+2</sup> and selenium in relation to the size of follicles. The concentration of estradiol -17 β, progesterone and triiodothyronine (T3) hormones were significantly (p<0.05) increased in large follicles compared to small ones of non pregnant she camels. However, follicular size had non significant effect on serum T4 levels.

**Table (1):** Concentration of some biochemical and hormones in serum of non pregnant dromedary she camels with small and large size follicles during breeding season mean values (± SE).

Parameters	Small follicles (2 - 4 mm)	Large follicles (10 - 20 mm)
Glucose(mg/dl)	43.64±3.2 <sup>b</sup>	71.32±6.1 <sup>a</sup>
Cholesterol(mg/dl)	139.8±1.2 <sup>b</sup>	172.6±4.3 <sup>a</sup>
Calcium (ca <sup>+2</sup> )(mg/dl)	12.25±0.09 <sup>b</sup>	23.45±1.0 <sup>a</sup>
Selenium(mg/dl)	3.34±1.04 <sup>b</sup>	6.17±1.37 <sup>a</sup>
Progesterone(ng/ml)	0.37±0.07 <sup>b</sup>	0.97±0.06 <sup>a</sup>
Estradiol 17β(pg/ml)	27.42±5.6 <sup>b</sup>	39.03±3.7 <sup>a</sup>
Thyroxine(µg/dl)	7.4±0.3 <sup>a</sup>	6.29±1.1 <sup>a</sup>
Tri-iodothyronine(ng/ml)	11.52±0.14 <sup>b</sup>	18.06±2.04 <sup>a</sup>

Mean values with different letters within the same row for each biochemical and hormones differed significantly (p<0.05).

## DISSCUSSION

The present study revealed that, serum glucose concentrations were significantly ( $p < 0.05$ ) higher in large follicles than in small ones. Results of the current study are compatible with previous studies in camels (**El-Shahat et al., 2013; Padalino et al., 2016; Hassan et al., 2018; Shehab-El-Din et al., 2019**). Glucose metabolism is less intensive in large follicles compared to small ones (**Ying et al., 2011**). In contrast to these results, **Rahman et al., (2008)** observed higher glucose levels in small follicles than in large ones in dromedary camels. However, non-significant differences in the serum concentrations of glucose among the different follicle sizes similar findings were recorded by **Ghoneim et al., (2013)** and **El-Bahr et al., (2015)**. The present study reported a significant ( $p < 0.05$ ) increase in the concentration of cholesterol with increase in the follicle size. The result of the current study is agreed with that previously recorded by **Albomohsen et al., (2011)** and **Hassan et al., (2018)**. The granular cells need cholesterol during its growth and multiplication. Therefore it's withdrawn from follicular fluid led to decrease its concentration in the small sized follicles (**Endresen et al., 1990**). Nonetheless, when the size of follicles enlarged, its cell multiplication is decreased and leads to release cholesterol into the follicular fluid that used in the formation of sex steroids (**Su et al., 2008**). The results of the current study are incompatible with previous reports in she camels that done by **Rahman et al., (2008)** who found that, the lower level of cholesterol in the large follicles indicates the biotransformation of cholesterol to sex steroids. However, non significant difference in the serum cholesterol has been reported by **Ali et al., (2008)** and **El-Bahr et al., (2015)** in she camels.

The level of calcium ion concentration is significantly ( $P < 0.05$ ) increased with increase the follicular size. The results of the current study are agreed with previous studies in camels (**Hassan et al., 2018**). 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 are involved in the formations of estrogen; this hormone is increased during follicular development and consequently requires large quantities of calcium ions that withdraw from blood inside the follicular fluid, then raising its calcium concentration (**Nandi et al., 2007**).

Growth of granulosa cells is considered an important feature during the developmental process of follicles; i.e., the folliculogenesis. The proliferation of small primary follicles (with fewer granulosa cells) to maturing pre-ovulatory follicles (with many strata of cells) is

the characteristic event in folliculogenesis (**Basini and Tamanini, 2000**).Selenium regulates the growth of the granulosa cells and  $17\beta$ -estradiol bio-synthesis in adult ovaries (**Qazi et al., 2018**).Also,Se levels are increased in large healthy follicles and might perform a vital antioxidant function during later growth and the proliferation of follicles(**Ceko et al., 2015**). Regarding hormonal profiles, the result showed that in non - pregnant groups, there was a positive correlation between estradiol  $17\beta$  concentrations and follicular size. Similar findings were recorded in female dromedary camels (**Ali et al.,2011;El-Shahat et al., 2013; 2019**). Estradiol  $17\beta$  alone has little effect on the granular cells in maturing follicles, but its effect is important in initiating LH receptor expression and responsiveness (**Segaloff et al., 1990**) and prevention of atresia (**Billing et al.,1993**). Since all camels included in the present study were non pregnant,serum progesterone levels were expected to be low than 1ng/ml.Serum progesterone concentration was significantly ( $p<0.05$ ) higher in camels have large follicles than that with small follicles. These results are similar with those reported by **Rahman et al., (2008) and Ali et al., (2011)**. **Tibary and Anouassi (1997)** found that, serum progesterone levels were below 1.0 ng/ml in non pregnant camels in the absence of ovulation. Camels having large follicles on their ovaries had significantly higher serum T3 contents than those with small ovarian follicles ( $p<0.05$ ). However, the follicular size had non significant effect on serum T4 contents (Table 1).This finding agrees with previous studies in female dromedary camels (**Ali et al., 2011**). According to **Spicer et al., (2001)** T3 and T4 may have a major positive impact on LH production which would result in estrogen production by follicles that was contrary to those recorded by **Ghoneim et al.,(2013) and Rahman et al., (2008)**.

## REFERENCE

- Albomohsen H.,Mamouei S.,Tabatabaei S.and Fayazi J. (2011):**Metabolite composition variations of follicular fluid and blood serum in Iranian dromedary camels during the peak breeding season. J. Anim. Vet., (3): 327-331.
- Ali S., Ahmad N., Akhtar N., Rahman Z. U. and Noakes D.E. (2008):** Metabolite contents of blood serum and fluid from small and large sized follicles in dromedary camels during the peak and the low breeding seasons. Anim. Reprod. Sci., 108, 446 - 456.
- Ali S., Ahmad N., Akhtar N., Rahman Z.U. and Ahmad M. (2011):** Hormonal profiles in the serum and follicular fluid of female camel (*Camelus dromedarius*) during the peak and the low breeding season. Pak. Vet. J., 31:331-335.

- Arunakumari G., Vagdevi R., Rao B.S., Naik B.R., Naidu K.S., Suresh K.R.V. and Rao V.H. (2007):** Effect of hormones and growth factors on in vitro development of sheep preantral follicles. *Small. Rumin. Res.*, 70: 93-100
- Basini G. and Tamanini C. (2000):** Selenium stimulates estradiol production in bovine granulosa cells: possible involvement of nitric oxide. *Domes. Anim. Endocrinol.*, 18:1-17.
- Billing H., Furuta I. and Hsueh A.J. (1993):** Estrogens inhibit and androgens enhance ovarian granulosa cell apoptosis. *Endocrinology*, 133:2204-2212.
- Braverman, L.E. (1996):** Evaluation of thyroid status in patients with thyrotoxicosis. *Clin. Chem.*, 42, 174-178.
- Ceko M.J., Hummitzsch K., Hatzirodos N., Bonner W.M., Aitken J.B., Russell D.L., Lane M., Rodgers R.J. and Harris H.H.(2015):** X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. *Metallomics*, 7:71-82.
- El-Bahr S.M., Ghoneimc I.M. and Waheed M.M. (2015):** Biochemical and hormonal analysis of follicular fluid and serum of female dromedary camels (*Camelus dromedarius*) with different sized ovarian follicles. *Anim. Reprod. Sci.*, 1-6.
- El-Harairy M.A., Zeidan A.E.B., Afify A.A., Amer H.A. and Amer A.M. (2010):** Ovarian activity, biochemical changes and histological status of the dromedary she camel as affected by the different seasons of the year. *Nat. Sci.*, 8:54-60.
- El-Shahat K.H., Abo-El-Maaty A.M. and Moawad A.R. (2013):** Follicular fluid composition in relation to follicular in pregnant and non-pregnant dromedary camels(*Camelus dromedarius*). *Anim. Reprod.*, 10, 16-23.
- El-Shahat K.H., Ali S., Mohamed F. and Abo-El Maaty A.M. (2019):** Dynamics of follicular fluid composition in relation to follicular size and corpus luteum in dromedary camels. *J. Biol. Sci.*, 12 (3):423 - 429.
- Endresen M.J., Haug E., Abyholm T. and Henriksen T. (1990):** The source of expression in granulosa cells. *J. Anim. Sci.*, 89, 1769 -1786.
- Fortune J.E., Rivera G.M. and Yang M.Y. (2004):** Follicular development: the role of the follicular microenvironment in selection of the dominant follicle. *Anim. Reprod. Sci.*, 82/83:109 -126.
- Ghoneim I.M.,Waheed M.M.,El-Bahr S.M.,Al-heider A.and Al-Eknah M.M.(2013):** Comparison of some biochemical and hormonal constituents of oversized follicles and preovulatory follicles in camels (*Camelus dromedarius*). *Theriogenology*, 79, 647 - 652.
- Gindler M. and King J.D. (1972):** *Am.J.Clin.Path.*58, 376.
- Hassan M. S., Al-Nuaimi A. J., Al-Yasari M. A. and Jameel Y. J. (2018):** Study the effects of follicular size on some biochemical follicular fluid composition in she camel (*Camelus Dromedarius*). *Advances in Animal and Veterinary Sciences*, (6), 341-346.

- Iwata H., Hashimoto S., Ohota M., Kimura K., Shibano K. and Miyake M. (2004):** Effects of follicles size and electrolytes and glucose in maturation medium on nuclear maturation and developmental competence of bovine oocytes. *Reprod*, 127:159-164.
- Muzzaffari E.L. and Gharib H. (1998):** Thyroxin suppressive therapy in patients with nodular thyroid disease. *Ann. Intern. Med.*, 128, 386-394.
- Nandi S., Girish Kumar V., Manjunatha B.M., Ramesh H.S. and Gupta P.S.P. (2008):** Follicular fluid concentrations of glucose lactate and pyruvate in buffalo and sheep, and their effects on cultured oocytes, granulosa and cumulus cells. *Theriogenology*. 69:186-196
- Nandi S., Girish Kumar V., Manjunatha B.M. and Gupta P.S.P. (2007):** Biochemical composition of ovine follicular fluid in relation to follicle size. *Journal compilation, Japan's Society of Developmental Biologist. Growth Differ.* 49: 61- 66.
- Padalino B., Rateb S.A., Ibrahim N.B., Manaco D., Lacalandea G.M. and El-Bahrawy K.A. (2016):** Behavioral indicators to detect ovarian phase in dromedary she-camel. *Theriogenology*. (85):1644 -1651.
- Qazi I.H., Angel C., Yang H., Pan B., Zoidis E., Zeng C., Hongbing Han H., and Guang-Bin Zhou G. (2018):** Selenium, selenoproteins, and female reproduction: A Review. *Molecules*, Dec; 23(12): 3053.
- Rahman, Z.U., Bukhari, S.A., Ahmad, N., Akhtar, N., Ijaz, A., Yousaf, M.S. and Haq, I.U. (2008):** Dynamics of follicular fluid in one-humped camel (*Camelus dromedarius*). *Reprod. Dom. Anim.*, 43, 664 - 671.
- Richmond W. (1973):** *Clin. Chem.*, 19, 1350.
- Sharma R.K. and Vasta R. (1998):** Biochemical changes in trace elements in antral follicles of goats. *Indian. J. Anim. Sci.*, 68 (4): 330 - 331.
- Shehab-El-Deen M.A.M.M., Al-Dobaib S.N. and Al-Sobayil K.A. (2019):** Biochemical compositions in the follicular fluid of different follicle groups and their relationship with the blood concentrations in dromedary camels. *International Journal of Biology*, Vol. 11, No. 4; 112-122.
- Segaloff D.L., Wang H.Y. and Richards J.S. (1990):** Hormonal regulation of luteinizing hormone/chorionic gonadotropin receptor mRNA in rat ovarian cells during follicular development and luteinization. *Mol. Endocrinol.*, 4:1856 - 1865.
- Skidmore J.A., Billah M. and Allen W.R. (1995):** The ovarian follicular wave pattern in the mated and non-mated dromedary camel (*Camelus dromedarius*). *J. Reprod. Fertil. Suppl.*, 49, 545-548.
- SPSS. (2007):** Statistical Package for Social Science. SPSS Inc., Chic, IL, USA Copyright © for Windows 2007; version 16.0.

- Spicer L.J., Alonoso J. and Chamberlain C.S. (2001):** Effects of thyroid hormones on bovine granulosa and thecal cell function in vitro: Dependence on insulin and gonadotrophins. *J. Dairy Sci.*, 84:1069-1076.
- Su Y.Q., Sugiura K., Wigglesworth K., O'Brien M.J., Affourtit J.P., Pangas S.A., Matzuk M.M. and Eppig J.J. (2008):** Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP-15 and GDF-9 control cholesterol biosynthesis in cumulus. *Development*, 135:111- 121.
- Tibary A. and Anouassi A. (1996):** Ultrasonographic changes of the reproductive-tract in the female camel (*Camelus-Dromedarius*) during the follicular cycle and pregnancy. *J. Camel Practice and Res.*, 3, 71-90.
- Tibary A. and Anouassi A. (1997):** Theriogenology in Camelidae. *Anatomy, Physiology, Pathology and Artificial Breeding*. Abu Dhabi, UAE: Abu Dhabi Printing and Publishing. 131-142.
- Tietz N.W. (1995):** *Clinical guide to laboratory Test*, 3rd Edition W.B. Saunders, Co., Philadelphia, 216-217.
- Trinder P. (1969):** *Ann. Clin. Biochem*, 6, 24.
- Wisdom, G. B. (1976):** Enzyme-immunoassay. *Clin. Chem.* 22, 1243 -1255.
- Ying S., Wang Z., Wang C., Nie H., He D., Jia R., Wu Y., Zhou Z., Yan Y., Zhang Y. and Wang F. (2011):** Effect of different levels of short-term feed intake on folliculogenesis and follicular fluid and plasma concentrations of lactate dehydrogenase, glucose, and hormones in Hu sheep during the luteal phase. *Reprod.* 1. 142: 699-710.