

Fine Structures of the Oocyte in Relation to Serum, Follicular Fluid Steroid Hormones and IGF-I in the Ovulatory-Sized Follicles in One-Humped Camel (*Camelus dromedarius*)

Mojtaba Kafi¹, Seyed Fakhroddin Mesbah², Najmeh Davoodian^{1*}, and Ali Kadiivar³

1. Department of Animal Reproduction, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

2. Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

3. Research Institute of Animal Embryo Technology, Shahrekord University, Shahrekord, Iran

Abstract

Background: The following study was carried out to determine the ultrastructural features of the oocyte of the ovulatory-sized follicles in relation to concentrations of steroids and IGF-I in the Follicular Fluid (FF) and serum in the dromedary camel.

Methods: Camel follicles with a clear and healthy appearance were categorized into three classes: follicles 10 to 13.9, 14-17.9 and 18-30 mm diameter. The FF and serum samples were assayed for estradiol-17 β , progesterone and IGF-I. Recovered Cumulus-Oocyte Complexes (COCs) were prepared for transmission electron microscopy.

Results: The mean ($\pm$ SD) FF concentrations of progesterone and IGF-I was significantly ($p < 0.05$) higher in follicles 18 to 30 mm diameter compared to other groups of follicles. There was no difference in the mean ($\pm$ SD) serum estradiol-17 β , progesterone and IGF-I concentrations between camels with different ovulatory-sized follicles ($p > 0.05$). Oocytes from follicles 18 to 30 mm diameter (group 3) showed more advanced signs of maturation including the disappearance of the nuclear envelope, increased number of microvilli in erect position, the increase in number and size of vesicles and more even distribution of the mitochondria throughout the ooplasm.

Conclusion: The final stages of oocyte maturation in dromedary camel is associated with increasing progesterone and IGF-I concentrations and constant high estradiol concentration in the follicular fluid which are paralleled with well-defined ultrastructural changes in oocytes.

Avicenna J Med Biotech 2014; 6(1): 57-61

Keywords: Camel, IGF-I, Oocyte, Steroids, Ultrastructure

* **Corresponding author:**
Najmeh Davoodian, Ph.D.,
School of Veterinary Medicine,
Shiraz University, Shiraz, Iran
E-mail:
najmeh179@gmail.com
Received: 22 June 2013
Accepted: 15 Sep 2013

Introduction

The camel, a popular and economically important species performs well under arid and semi-arid climate conditions, still remains one of the most neglected species in the field of scientific research. The reproductive efficiency of camels under natural conditions is being reported to be low¹. Various attempts have been made to improve the reproductive efficiency and genetic potential of dromedary camel². Techniques such as

in vitro production³ and cloning⁴ in the dromedary camel have been successfully carried out with a low success rate. Therefore, there is a serious need to perform more basic research regarding the biology of follicular development and oocyte maturation *in vivo*. In addition, knowing the endocrinology and morphological aspects of the final stage of preovulatory follicle and oocyte maturation are the key elements for designing op-

timum *in vitro* conditions to develop oocyte and embryo culture systems.

Our study is the first to show the relationship between ultrastructure of the oocyte to hormone concentrations in the FF in *Camelus dromedarius* (*C. dromedarius*). The ultrastructural changes of the immature oocytes⁵ and oocytes from different follicle sizes⁶ were described in the dromedary caml. Kafi *et al*⁷ also described the ultrastructural features of the oocytes during *in vitro* maturation in the dromedary camel. Therefore, the following study was carried out to determine the ultrastructural features of the oocyte of the ovulatory-sized follicles associated with the concentrations of steroids and IGF-I in the FF and serum in the dromedary camel.

Materials and Methods

Ovaries were recovered from sexually mature non-pregnant camels (*C. dromedarius*)⁶. During slaughtering, jugular blood samples were collected, centrifuged at 1400 for 30 min and stored in -20°C until analysis.

The ovulatory-sized follicles with a healthy and clear appearance were divided into three classes: 1) follicles 10-13.9 mm diameter, 2) follicles 14-17.9 mm diameter and 3) follicles 18-30 mm diameter. The follicles were aspirated and the cumulus-oocyte complexes were isolated under stereomicroscope. COCs were prepared for transmission electron microscopy as previously described⁶. The FF samples were centrifuged at 3000×g for 15 min, decanted and stored in small aliquots at -20°C for hormone assay.

Estradiol 17-β, progesterone and IGF-I concentrations in the FF and serum were determined using a validated commercial radioimmunoassay kit (Immunotech kit, France). The differences in mean (±SD) hormone concentrations were statistically compared using a one-way ANOVA. Duncan's multiple range test was applied to find the significant differences in multiple mean comparisons

using the SPSS software. Correlations between serum and FF hormone concentrations were assessed by correlation analysis. The level of significance was set at $p < 0.05$.

Results

The class size and the mean (±SD) follicular fluid concentrations of estradiol-17β, progesterone and IGF-I in different size follicles and the serum values of the hormones assayed are shown in table 1. The mean (±SD) follicular fluid concentrations of estradiol-17β, progesterone and IGF-I were significantly different ($p < 0.05$) in follicles 10-13.9 mm diameter than follicles with 18-30 mm diameter. Further, the mean (±SD) FF concentrations of progesterone and IGF-I were significantly different ($p < 0.05$) in follicles 14-17.9 mm diameter than that of follicles 18-30 mm diameter. There was no significant difference in the mean (±SD) follicular fluid concentrations of estradiol-17β between the follicles 14-17.9 and 18-30 mm diameter.

The Estrogen:Progesterone (E/P) ratio in the follicular fluid was significantly higher in follicles 10-13.9 mm diameter (59.07 ± 14.12) than in the FF in the follicles 14-17.9 mm diameter (18.90 ± 5.19) and 18-30 mm diameter (4.07 ± 1.34) compared with 10 to 13.9 mm diameter. E/P ratio was not significantly different between follicles 14-17.9 mm diameter and follicles 18-30 mm diameter (Table 1).

The concentrations of estradiol-17β, progesterone and IGF-I in the serum were not correlated to those of the FF ($r = 0.23$ for estradiol-17β, $r = -0.03$ for progesterone and $r = 0.33$ for IGF-I; $p > 0.05$), respectively. Also there were no differences between serum concentrations of estradiol-17β, progesterone and IGF-I of camels with different ovulatory-sized follicles ($p > 0.05$).

Oocyte ultrastructure evaluation

Group 1- follicles 10-13.9 mm: The oocytes were surrounded by relatively expanded granulosa cells. Zona pellucida was homogenous containing nu-

Table1. The mean±SD follicular fluid and serum concentrations of estradiol-17β, progesterone and IGF-I in ovulatory-sized follicles in the dromedary camel

Groups	Follicle diameter (mm)	No. of oocytes	Estradiol-17β concentration (ng/ml)		Progesterone concentration (ng/ml)		IGF-I concentration (ng/ml)		FF Estrogen/ Progesterone ratio
			FF	serum	FF	serum	FF	serum	
1	10-13.9	7	217.6±25.5 ^a	50.9±52.4	3.7±0.7 ^c	0.54±0.7	140.1±52.7 ^e	153.2±97.6	59.07±14.12 ^g
2	14-17.9	4	248.5±14.9	52.9±56.7	13.6±3.4 ^c	0.86±0.8	195.7±35.1 ^e	208.2±20.1	18.9±5.19 ^h
3	18-30	6	254.6±21.0 ^b	97.4±77.5	64.8±20.2 ^d	0.61±0.4	256.4±35.1 ^f	250.8±89.4	4.07±1.34 ⁱ

Values in the same column with different superscripts are significantly different

a,b) significant at $p < 0.07$; c,d) significant at $p < 0.05$; e,f) significant at $p < 0.01$; g,h,i) significant at $p < 0.001$

merous projections of cumulus cells. The nucleus was located eccentrically in half of the oocytes. The Perivitelline Space (PVS) was small with limited numbers of microvilli. Large numbers of different size vesicles were distributed through the oo-plasm. Clusters of mitochondria and Golgi complex were observed with peripheral distribution (Figure 1). Few numbers of lipid droplets were observed. Vesicles, mitochondria and lipid droplets were intermixed and located close to both vesicular and tubular endoplasmic reticulum in peripheral area.

Group 2- follicles 14-17.9 mm

Most ultrastructural features, in general, were similar to last group except that the PVS was slightly enlarged, the numbers of microvilli increased and the nucleus was peripherally located in all the oocytes (Figure 2).

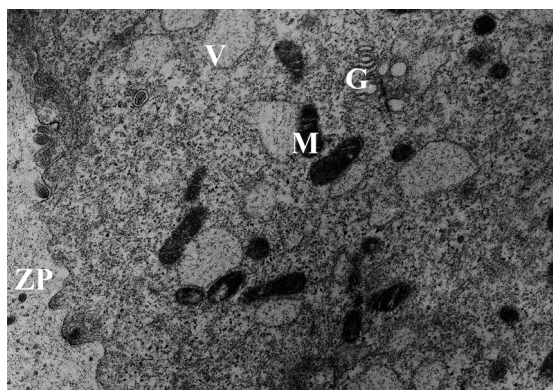


Figure 1. Electron micrograph showing details of an oocyte from group 1 follicles. Zona Pellucida (ZP), Mitochondria (M), Vesicles (V) and Golgi complex (G), $\times 11500$

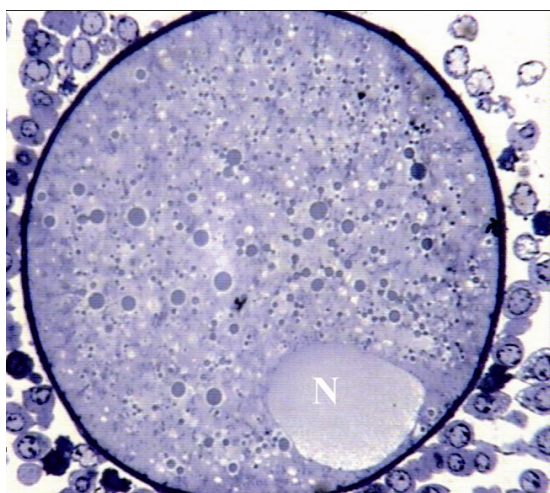


Figure 2. Light micrograph of an oocyte from group 2 which contains a peripherally located nucleus (N), $\times 720$

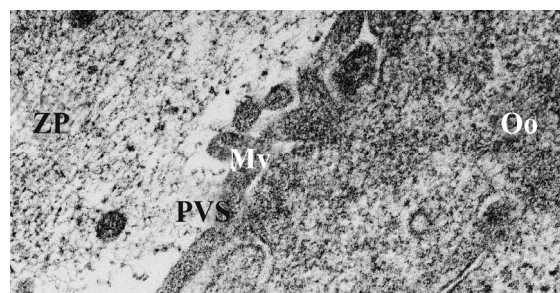


Figure 3. Electron micrograph of an oocyte from group 3. Microvilli (Mv), Perivitelline Space (PVS), Ooplasm (Oo), Zona Pellucida (ZP), $\times 21000$

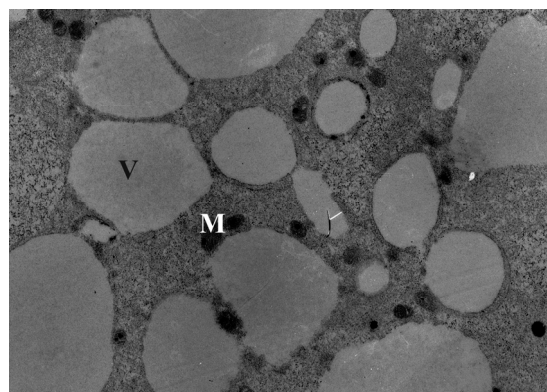


Figure 4. Electron micrograph of an oocyte from group 3, Mitochondria (M), Large vesicles (V), $\times 6600$

Group 3- follicles 18-30 mm

All the oocytes showed no granulosa cells around. The number of microvilli was progressively increased (Figure 3). Cortical granules were also noted in the close proximity to the oolemma. The mitochondria were distributed through the ooplasm and the number and size of vesicles were observed to increase and distributed generally (Figure 4). The nuclear envelope was no longer apparent. The presence of the first polar body in the PVS was noted in one of the oocytes.

Discussion

The results of the present study showed that as the diameter of the ovulatory-sized follicle increases, the mean concentrations of estradiol-17 β , progesterone and IGF-I in the FF concomitantly increases in the female dromedary camel. Similarly, Rahman⁸ reported that concentrations of estradiol-17 β and progesterone in the follicular fluid increased with the growth of the follicles in the female dromedary camel. In the equine, Spicer *et al*⁹ showed that estradiol-17 β and IGF-I concentrations in the FF was greater in large ovulatory than in medium size follicles. In cattle, IGF-I

stimulates estradiol synthesis which is important for final maturation of the preovulatory follicle and the oocyte¹⁰. The concomitant increase of FF IGF-I and estradiol-17 β , simultaneously with the increase in ovulatory-sized follicles in the present study suggests the presence of a similar relationship between IGF-I and estradiol-17 β in the female dromedary camel as it occurs in the mare and cattle. Although a clear increasing pattern in serum concentrations of estradiol-17 β and IGF-I was observed in camels with different ovulatory-sized follicles in the present study, this was not statistically significant due to the presence of the large standard deviations. The existence of a wide variation in blood steroid concentrations has been reported in the dromedary camel¹¹.

Our results showed that changes in the fine structure of the dromedary camel oocytes collected from preovulatory follicles are, in general, similar to that in cattle¹² and equine¹³. Furthermore, the results of the present study revealed that oocytes from group 3 follicles showed more advanced signs of maturation as defined in the *in vivo* matured oocytes in cattle¹² and equine¹³, and *in vitro* matured camel oocytes⁷. The mean FF concentrations of estradiol and IGF-I were the highest in ovulatory-sized follicles (group 3) compared to the other follicle class size groups in the present study. Teissier¹⁴ in the human, Grondahl¹³ in the equine, Sirotkin¹⁵ in the porcine and Zhao¹⁶ in the rat reported that elevated estradiol in the follicular fluid indicate a more advanced stage of oocyte maturation.

We did not observe a massive formation of cortical granules in close proximity to the oolemma as the diameter of follicles increased. This was not similar to the well-defined dynamics of cortical granule formation in our previous observations during *in vitro* maturation in the dromedary camel oocyte¹⁷. The reason for this different observation could be *in vivo* vs. *in vitro* environment, and or there may be more than one organelle involved in cortical granule formation¹⁸. In the present study, in one oocyte from group 3, the first polar body had been fully extruded. This can be ascribed to the occurrence of an infertile mating and then surging in luteinizing hormone in days before slaughtering the animal. It has been shown that E/P ratio greater than 1 is a sign of healthy follicles and oocytes in cattle¹⁹. Although E/P ratio decreased as the size of follicles increased in our study, however the ratio remained greater than 1. Considering the normal ultrastructure of the oo-

cytes and E/P ratio in the FF greater than 1 observed in the present study, it can be inferred that E/P ratio greater than 1 is an indicative for the healthiness of follicles in the dromedary camel.

Conclusion

In conclusion, the present study is the first to show that, the final stages of oocyte maturation *in vivo* is associated with increasing progesterone, estradiol-17 β and IGF-I concentrations which are paralleled with ultrastructural changes leading to the final stages of oocyte maturation in the dromedary camel. Further, follicle diameter influences concentrations of progesterone, estradiol-17 β and IGF-I in the FF in the dromedary camel.

Acknowledgement

The authors would like to thank Professor M. Saeb for allowing us to perform hormone assay in his laboratory and Mr. A. Safavi for his technical assistance. The work was financially supported by Shiraz University.

References

1. Skidmore JA. Reproduction in dromedary camels: an update. *Anim Reprod* 2005;2(3):161-171.
2. Skidmore JA, Billah M, Allen WR. Investigation of factors affecting pregnancy rate after embryo transfer in the dromedary camel. *Reprod Fert Devel* 2002;14:109-116.
3. Badr MR, Abdel-Malak MG. In vitro fertilization and embryo production in dromedary camel using epididymal spermatozoa. *Global Vet* 2010;4:271-276.
4. Wani NA, Wernery U, Hassan FAH, Wernery R, Skidmore JA. Production of the first cloned camel by somatic cell nuclear transfer. *Biol Reprod* 2010;82(2):373-379.
5. Nili H, Mesbah F, Kafi M, Nasr Esfahani MH. Light and transmission electron microscopy of immature camelus dromedarius oocyte. *Anat Histo Embryol* 2004;33(4):196-199.
6. Davoodian N, Mesbah F, Kafi M. Oocyte ultrastructural characteristics in camel (*Camelus dromedarius*) primordial to large antral follicles. *Anat Histo Embryol* 2011;40(2):120-127.
7. Kafi M, Mesbah F, Nili H, Khalili A. Chronological and ultrastructural changes in camel (*Camelus dromedarius*) oocytes during in vitro maturation. *Theriogenology* 2005; 63(9):2458-2470.
8. Rahman ZU, Bukhari SA, Ahmad N, Akhtar N, Ijaz A, Yousaf MS, et al. Dynamics of follicular fluid in one-humped camel (*Camelus dromedarius*). *Reprod Dom Anim* 2008;43(6):664-671.
9. Spicer LJ, Santiago CA, Davidson TR, Bridges TS, Chamberlain CS. Follicular fluid concentrations of free

- insulin-like growth factor (IGF)-I during follicular development in mares. *Domest Anim Endocrinol* 2005;29(4): 573-581.
10. Spicer LJ. Proteolytic degradation of Insulin-like growth factor binding proteins by ovarian follicles: A control mechanism for selection of dominant follicles. *Biol Reprod* 2004;70(5):1223-1230.
 11. Skidmore JA. Reproductive physiology in female old world camelids. *Anim Reprod Sci* 2011;124(3-4):148-154.
 12. Hyttel P, Callesen H, Greve T. Ultrastructural features of preovulatory oocyte maturation in superovulated cattle. *J Reprod Fertil* 1986;76:615-625.
 13. Grondahl C, Hyttel P, Grondahl ML, Eriksen T, Gotfredsen P, Greve T. Structural and endocrine aspects of equine oocytes maturation in vivo. *Mol Reprod Devel* 1995;42:94-105.
 14. Teissier MP, Chable H, Paulhac S, Aubard Y. Comparison of follicle steroidogenesis from normal and polycystic ovaries in women undergoing IVF: relationship between steroid concentrations, follicle size, oocyte quality and fecundability. *Hum Reprod* 2000;15(12):2471-2477.
 15. Sirotkin AV, Dukesova J, Makarevich AV, Kubek A, Bulla J. Evidence that growth factors IGF-I, IGF-II and EGF can stimulate nuclear maturation of porcine oocytes via intracellular protein kinase A. *Reprod Nutr Dev* 2000;40(6):559-569.
 16. Zhao J, Taverne MA, Van der Weijden GC, Bevers MM, Van Den Hurk R. Insulin-like growth factor-1 (IGF-I) stimulates the development of cultured rat pre-antral follicles. *Mol Reprod Dev* 2001;58(3):287-296.
 17. Revelli A, Delle Piane L, Casano S, Molinari E, Massobrio M, Rinaudo P. Follicular fluid content and oocyte quality: from single biochemical markers to metabolomics: A review. *Reprod Biol Endocrinol* 2009;7:40-52.
 18. Fair T, Hulshof SCJ, Hyttel P, Greve T, Boland M. Oocyte ultrastructure in bovine primordial to early tertiary follicles. *Anat Embryol* 1997;195:327-336.
 19. Grado-Ahuir JA, Aad PY, Spicer LJ. New insights into the pathogenesis of cystic follicles in cattle: Microarray analysis of gene expression in granulosa cells. *J Anim Sci* 2011;89(6):1769-1786.