Anim. Reprod., v.10, n.1, p.16-23, Jan./Mar. 2013

Follicular fluid composition in relation to follicular size in pregnant and non-pregnant dromedary camels (*Camelus dromedaries*)

K.H. El-Shahat[1], A.M. Abo-El Maaty[2], A.R. Moawad[1,3,4]

[1]Department of Theriogenology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.
[2]Department of Animal Reproduction and AI, National Research Centre, Egypt.
[3]Department of Obstetrics and Gynecology, McGill University, Montreal, Canada.

Abstract

The aim of this study was to evaluate the effects of the reproductive status of female dromedary camels (pregnant *vs.* non-pregnant) on the chemical composition, hormonal profile and antioxidant capacity of follicular fluid collected from different sized ovarian follicles during the breeding season. One hundred ovaries were collected at slaughterhouse from fifty female dromedary camels. The ovaries were collected in pairs from each animal and allocated into two groups according to the reproductive status of the females; 25 pairs were obtained from pregnant females and 25 pairs were obtained from non-pregnant animals. The follicles on each ovary were categorized according to their diameter into three categories; small (1-3 mm), medium (4-9 mm) and large (10-20 mm). Follicular fluid (FF) aspirated from each follicle category from each pair of ovaries was analyzed. The results showed that the average number of follicles per ovary was greater ($P < 0.05$) in the ovaries obtained from non-pregnant females compared to those collected from pregnant ones (6.4 ± 1.2 *vs*. 3.6 ± 0.9, respectively). Progesterone concentrations were significantly higher in the follicular fluid collected from all follicle categories in pregnant animals than those obtained from non-pregnant animals. Glucose concentrations were higher ($P < 0.05$) in the follicular fluid collected from large follicles in the non-pregnant group (64.9 ± 6.1 mg/dl) than those obtained from the same follicle category in the pregnant ovaries (45.4 ± 4.0 mg/dl). Concentrations of malondialdehyde (MDA) were higher ($P < 0.05$) in the FF collected from small, medium and large follicles in pregnant ovaries than non-pregnant ones. In conclusion, these data indicate that FF composition differs according to the reproductive status of the female. In pregnant camels, the presence of the corpus luteum on the ovaries could play an important role not only in the process of follicle growth and development, but also in the concentrations of biochemical metabolites and hormonal profiles in the FF of dromedary camels.

Keywords: camel, corpus luteum, follicular fluid, ovary, follicular size, pregnancy.

Introduction

The formation of follicular fluid (FF) inside the ovarian follicle begins in the early stage of follicle development. Various reports suggest that follicular fluid proteins are derived from two sources: blood and surrounding somatic cell layers (granulosa and theca cells). Previous studies showed that the "blood-follicle barrier" is permeable for proteins below 500 kDa (Gosden *et al*., 1988) and most proteins and other components can easily bypass through the basal lamina to enter the follicular antrum or escape towards circulating blood. Indeed, ovarian cells secrete a number of soluble substances such as steroids, growth factors and other peptidergic factors into the follicular fluid (Fortune *et al*., 2004). All these factors have proven to play an important role in the metabolic activity of ovarian cells, and subsequently reveal the physiological status of the follicle. Moreover, numerous studies showed that these substances are crucial for oocyte maturation and fertilization, granulosa cell proliferation and differentiation and eventual ovulation and luteinization (Richards, 1994). However, the role played by many of these factors in ovarian function is still unknown. FF has a variety of oocyte-related functions including the maintenance of meiotic arrest (McNatty *et al*., 1979), protection against proteolysis, extrusion during ovulation (Espey and Lipner, 1994), enhancement of spermatozoa attraction, motility and acrosome reaction (Dell'Aquila *et al*., 1997; Rodriguez *et al*., 2001; Wang *et al*., 2001) and buffering against adverse hematic influences (Gosden *et al*., 1988). Therefore, the study of follicular fluid components may contribute to an understanding of the mechanisms involved in follicle differentiation and development. Moreover, the beneficial effects of FF on *in vitro* maturation (IVM), fertilization and subsequent embryo development have been investigated in many species. For instance, a previous study reported that supplementation of IVM medium with FF enhances the redistribution of active mitochondria and subsequently increases pathenogenetic development in pig oocytes (Brevini *et al*., 2005). Furthermore, a recent study in cattle showed that supplementation of IVM medium with bovine FF promotes sperm penetration both by the

[4]Corresponding author: adelreda902@hotmail.com
Phone: +1(514)836-6342; Fax: +1(514)843-1457
Received: May 31, 2012
Accepted: November 9, 2012

improvement of cumulus expansion and by enhancing ATP levels in oocytes (Somfai *et al*., 2012). Therefore, the study of FF compositions in dromedary camels could be beneficial in the improvement of IVM systems in camelids. Some progress has been made towards the characterization of FF in many mammalian species. Glucose, pyruvate, lactate, histidine, phenylalanine and asparagine concentrations have been detected in human FF (Leese and Lenton, 1990; Jimena *et al*., 1993; Gull *et al*., 1999). Similarly, Guérin *et al*. (1995) determined amino acid profiles in the FF of many domestic species, albeit without consideration for follicle size or ovarian status. Moreover, alanine, glutamate and glutamine profiles have been reported in equine FF (Gerard *et al*., 2002). In addition, we previously reported that the concentrations of different antioxidants in buffalo FF vary according to the follicle size and stage of the estrous cycle (El-Shahat and Kandil, 2012). In dromedary camels, previous studies reported that the concentrations of different metabolites and hormonal profiles in the FF varied according to follicular size and breeding season (Ali *et al*., 2008; Rahman *et al*., 2008; Ali *et al*., 2011). For example, Rahman *et al*. (2008) showed that the concentrations of glucose, cholesterol, triglycerides and total proteins were lower in large than in small follicles. However, they reported higher concentrations of progesterone and estradiol 17-β in the FF obtained from large follicles than those collected from small ones. Another study showed that the estradiol concentration in the FF of dromedary camels was significantly higher during the low breeding season (May to October) compared to the peak breeding season (November to April; Ali *et al*., 2011). However, no previous studies have evaluated the effect of reproductive status on the compositions and concentrations of different metabolites in the follicular fluid of dromedary camels. Therefore, this study was designed to evaluate the effects of the reproductive status (pregnant *vs*. non-pregnant) of female dromedary camels (*Camelus dromedaries*) on chemical composition, hormonal profile and antioxidant capacity of the follicular fluid collected from different sized ovarian follicles during the breeding season.

Materials and Methods

Follicular fluid collection and processing

A total of one hundred ovaries were collected from mature female camels (5 to 10 yr old) slaughtered at a local slaughterhouse during the period between January to March 2012 (the breeding season). As previously reported, the camel is known to be an induced ovulator (Skidmore *et al*., 1995) and the corpus luteum (CL) can only be seen during pregnancy (El-Wishy, 1992). The ovaries were allocated according to the reproductive status of the females into two groups; ovaries obtained from pregnant females (25 pairs; pregnant group) and ovaries obtained from non-pregnant animals (25 pairs, non-pregnant group). The ovaries from each animal were collected in pairs and placed in plastic bags and transported to the laboratory within 2 h post-slaughter in normal physiological saline (0.9% NaCl) at 30°C. In the laboratory, the ovaries were washed twice in warm 0.9% NaCl saline and then cleaned of any extraneous tissues. After that, the diameters of the ovarian follicles for each female were measured using calipers. Based on this, the follicles were allocated into three categories; small (1-3 mm), medium (4-9 mm) and large (10-20 mm). Abnormalities such as cystic (>20 mm, Tibary and Anouassi, 1997), hemorrhagic and atretic follicles, as recognized macroscopically (Kruip and Dieleman, 1982), were not used in the study. Follicular contents from each category for each animal were separately aspirated using an individual 18-gauge needle attached to 10 ml syringe. The follicular fluid was then transferred to a 10 ml conical tube and allowed to settle for 15 min. After that, the fluid was centrifuged at 3000 rpm for 10 min. Following centrifugation, the supernatant was collected, fractioned into small aliquots and stored at -20°C for further investigation.

Biochemical analysis of follicular fluid

Follicular fluid samples were analyzed for different biochemical metabolites and the analysis for the concentrations of each parameter in different groups was repeated at least three times. Hormones such as progesterone, estradiol 17-β, testosterone and thyroxin were analyzed using commercially available ELISA kits according to the methods described by Dobeli (1980), Tietz (1995) and Chopra *et al*. (1971). Briefly, the intra-assay and inter-assay coefficient of variations were kept at 7.5 and 8%, 10.2 and 11.5%, 4.5 and 6.3% and 3 and 3.7% for progesterone, estradiol 17-β, testosterone and thyroxin, respectively. The sensitivity of assays for progesterone, estradiol 17-β, testosterone and thyroxin was 0.05 ng/ml, 5.9 pg/ml, 0.038 ng/ml and 0.4 µg/dl, respectively. Total proteins, albumin, glucose, cholesterol and triglycerides as well as malondialdehyde (MDA) and total antioxidant capacity (TAC) were evaluated spectrophotometrically using commercially available kits based on the methods described by Gornal *et al*. (1949), Trinder (1969), Doumas *et al*. (1971), Flegg (1973), Fassati and Prencipe (1982), Ohkawa *et al.* (1979) and Koracevic *et al*. (2001), respectively. Globulin was detected by subtracting albumin concentrations from total protein concentrations.

Statistical analyses

All values were expressed as means ± SEM. The data were analyzed using SPSS® Statistical Software (SPSS® 11.01 for Windows, 2007). Simple one-way ANOVA was done to identify the effect of reproductive status on all follicle categories and between them. The Duncan's Multiple Range test was used to separate between significant means. The difference between means within and between the same follicle classes was detected by Student *t*-test. All results were

considered to be statistically significant at $P < 0.05$.

Results

Effect of reproductive status on follicular population

The average number of follicles per ovary was lower ($P < 0.05$) in pregnant than non-pregnant camels (3.6 ± 0.9 *vs*. 6.4 ± 1.2, respectively). Moreover, the numbers of small and large follicles were lower ($P < 0.05$) in the ovaries collected from pregnant animals compared to those obtained from non-pregnant females (2.0 ± 0.3 *vs*. 4.0 ± 0.8, and 0.8 ± 0.1 *vs*. 1.4 ± 0.2, respectively). However, the mean number of medium size follicles was not affected by the reproductive status of the females (Table 1).

Table 1. Effect of reproductive status on follicular populations in dromedary camels.

Source of ovaries	Follicle categories			Average number of follicles/ovary
	Small (1-3 mm)	Medium (4-9 mm)	Large (10-20 mm)	
Pregnant group	2.0 ± 0.3^{a} (100)	0.8 ± 0.2^{a} (40)	0.8 ± 0.1^{a} (40)	3.6 ± 0.9^{a} (180)
Non-pregnant group	4.0 ± 0.8^{b} (200)	1.0 ± 0.3^{a} (50)	1.4 ± 0.2^{b} (70)	6.4 ± 1.2^{b} (320)

[a,b]Within columns, means without a common superscript were different ($P < 0.05$). The numbers within parenthesis represent the number of follicles in each category.

Effect of reproductive status on FF hormonal profiles

The concentrations of estradiol 17-β, testosterone and thyroxin were lower ($P < 0.05$) in the follicular fluid obtained from large follicles in pregnant animals than those collected from the non-pregnant group. On the other hand, this trend was reversed for progesterone. In the pregnant group, the concentrations of progesterone were higher ($P < 0.05$) in large than small follicles. In non-pregnant animals, estradiol 17-β concentrations were higher ($P < 0.05$) in the FF collected from large follicles than those obtained from small follicles. However, the concentrations of other hormones were not affected by follicle category in this group (Table 2).

Table 2. Effect of reproductive status on hormonal profiles in the follicular fluid of dromedary camels.

Hormone	Follicular size	Source of ovaries	
		Pregnant group	Non-pregnant group
Progesterone (ng/ml)	Small	$30.6 \pm 1.7^{a,c}$	$15.2 \pm 2.2^{b,c}$
	Medium	$45.6 \pm 2.0^{a,d}$	$13.7 \pm 0.6^{b,c}$
	Large	$50.7 \pm 1.8^{a,d}$	$12.3 \pm 0.8^{b,c}$
Estradiol 17-β (pg/ml)	Small	$10.4 \pm 1.2^{a,c}$	$10.9 \pm 1.6^{a,c}$
	Medium	$13.8 \pm 1.4^{a,c}$	$35.4 \pm 2.2^{b,d}$
	Large	$15.4 \pm 1.6^{a,c}$	$40.3 \pm 3.7^{b,d}$
Testosterone (ng/ml)	Small	$1.7 \pm 0.2^{a,c}$	$1.4 \pm 0.1^{a,c}$
	Medium	$1.4 \pm 0.3^{a,c}$	$1.4 \pm 0.1^{a,c}$
	Large	$0.4 \pm 0.2^{a,d}$	$1.2 \pm 0.1^{b,c}$
Thyroxin (µg/dl)	Small	$7.9 \pm 0.6^{a,c}$	$7.4 \pm 0.3^{a,c}$
	Medium	$6.3 \pm 0.8^{a,c,d}$	$7.0 \pm 0.8^{a,c}$
	Large	$4.7 \pm 0.4^{a,d}$	$7.9 \pm 1.1^{b,c}$

[a,b] Within rows, means without a common superscript were different ($P < 0.05$). [c,d]Within columns, means without a common superscript were different ($P < 0.05$).

Effect of reproductive status on FF biochemical metabolites level

The effect of reproductive status on the concentrations of different metabolites in the FF obtained from different size ovarian follicles is presented in Table 3. The only significant effect between the two groups was observed in glucose concentrations in the FF collected from large follicles (45.4 ± 4.0 *vs.* 64.9 ± 6.1 mg/dl in the pregnant and non-pregnant groups, respectively). However, the rest of the metabolites did not significantly differ between the two groups in all follicle classes. Within the same group, the concentrations of cholesterol and triglycerides were higher ($P < 0.05$) in small follicles than large ones in pregnant animals. However, this trend was not the same in the non-pregnant group (Table 3).

Table 3. Effect of reproductive status on biochemical metabolites in the follicular fluid of dromedary camels.

Metabolites	Follicular size	Source of ovaries	
		Pregnant group	Non-pregnant group
Total protein (g/dl)	Small	$4.0 \pm 0.3^{a,c}$	$3.7 \pm 0.1^{a,c}$
	Medium	$3.6 \pm 0.2^{a,c}$	$3.6 \pm 0.1^{a,c}$
	Large	$3.4 \pm 0.1^{a,c}$	$3.9 \pm 0.2^{a,c}$
Albumin (g/dl)	Small	$2.5 \pm 0.3^{a,c}$	$3.0 \pm 0.3^{a,c}$
	Medium	$2.5 \pm 0.3^{a,c}$	$3.0 \pm 0.3^{a,c}$
	Large	$2.2 \pm 0.1^{a,c}$	$3.2 \pm 0.1^{a,c}$
Globulin (g/dl)	Small	$1.4 \pm 0.3^{a,c}$	$0.7 \pm 0.0^{a,c}$
	Medium	$1.1 \pm 0.2^{a,c}$	$0.6 \pm 0.2^{a,c}$
	Large	$1.3 \pm 0.0^{a,c}$	$0.7 \pm 0.0^{a,c}$
Glucose (mg/dl)	Small	$32.4 \pm 1.7^{a,c}$	$36.2 \pm 3.2^{a,c}$
	Medium	$36.8 \pm 2.6^{a,c,d}$	$33.2 \pm 3.8^{a,c}$
	Large	$45.4 \pm 4.0^{a,d}$	$64.9 \pm 6.1^{b,d}$
Cholesterol (mg/dl)	Small	$149.1 \pm 8.6^{a,c}$	$142.6 \pm 1.3^{a,c}$
	Medium	$139.5 \pm 3.1^{a,c,d}$	$140.4 \pm 1.1^{a,c}$
	Large	$131.5 \pm 8.4^{a,d}$	$139.8 \pm 1.2^{a,c}$
Triglyceride (mg/dl)	Small	$59.9 \pm 5.5^{a,c}$	$54.9 \pm 2.6^{a,c}$
	Medium	$53.7 \pm 2.5^{a,c,d}$	$51.0 \pm 3.1^{a,c}$
	Large	$47.7 \pm 0.6^{a,d}$	$52.8 \pm 1.3^{a,c}$

[a,b]Within rows, means without a common superscript were different ($P < 0.05$). [c,d]Within columns, means without a common superscript were different ($P < 0.05$).

Effect of reproductive status on FF MDA and TAC assay

MDA concentrations were higher ($P < 0.05$) in the FF obtained from all follicle categories in pregnant animals compared to non-pregnant animals. In pregnant animals, the concentration of MDA was higher ($P < 0.05$) in the large follicles than in medium and small follicles. However, the opposite was observed in non-pregnant females. TAC concentration was higher ($P < 0.05$) in the FF obtained from large follicles in non-pregnant group compared to their counterpart in the pregnant group (Table 4).

Table 4. Effect of reproductive status on the concentrations of malondialdehyde (MDA) and total antioxidant capacity (TAC) in the follicular fluid of dromedary camels.

Parameter	Follicular size	Source of ovaries	
		Pregnant group	Non-pregnant group
MDA (nmol/ml)	Small	$50.7 \pm 12.6^{a,c}$	$35.5 \pm 5.5^{b,c}$
	Medium	$54.6 \pm 5.9^{a,c}$	$32.0 \pm 6.9^{b,c}$
	Large	$78.4 \pm 3.9^{a,d}$	$21.0 \pm 2.1^{b,d}$
TAC (mM/l)	Small	$9.1 \pm 3.4^{a,c}$	$15.8 \pm 11.0^{b,c}$
	Medium	$18.2 \pm 4.9^{a,d}$	$19.1 \pm 7.2^{a,c}$
	Large	$11.7 \pm 3.1^{a,c,d}$	$29.6 \pm 9.5^{b,d}$

[a,b] Within rows, means without a common superscript were different ($P < 0.05$). [c,d] Within columns, means without a common superscript were different ($P < 0.05$).

Discussion

Follicular fluid is a crucial environment for the growth and maturation of both ovarian somatic and germ cells. It contains substances involved in cell differentiation, gamete quality and rupturing of the follicular wall. The present study evaluated the variations in the concentrations of different metabolites in the follicular fluid collected from different sized ovarian follicles in pregnant and non-pregnant female camels slaughtered during the breeding season. The results indicated that the follicular activity as indicated by the average number of follicles per ovary was significantly higher in the ovaries obtained from non-gravid organs than those collected from gravid ones. These findings suggest that the reproductive status (pregnant *vs.* non-pregnant) of the animals has a great impact on follicular growth and development. Similar observations were previously reported among different species such as camels (Abdoon, 2001; Ghoneim, 2001), cattle (Pierson and Ginther, 1987) and buffalo (Amer *et al*., 2008). Furthermore, in dromedary camels previous studies showed that the numbers of collected oocytes, quality and frequencies of *in vitro* maturation were significantly affected by the reproductive status of the females, as lower values were reported in the pregnant group than the non-pregnant one (Moawad, 2005). The lower follicular activity reported in the pregnant group could be attributed to the higher levels of follicular atresia caused by the presence of the CL (Hafez, 2006).

Regarding hormonal profiles, the results showed that in the non-pregnant group there was a positive correlation between estradiol 17-β concentrations and follicle size. This phenomenon was the opposite for progesterone. In pregnant females, testosterone concentration in the FF was significantly lower in the large follicles than in medium and small ones. It has been well documented that the main source for the synthesis and release of sex steroid hormones in females is the follicular cells. Estradiol 17-β alone has little effect on the granulosa cells in maturing follicles, but its effect is important in initiating LH receptor expression and responsiveness (Segaloff *et al*., 1990), antrum formation (Wang and Greenwald, 1993), gap-junction configuration (Burg-Hardt and Anderson, 1981) and prevention of atresia (Billing *et al*., 1993). Progesterone has been reported to play a role in the process of follicle rupture and ovulation in humans (Zalanyi, 2001). The higher concentration of progesterone reported in the present study the large compared to small follicles in pregnant animals suggests that luteinization of granulosa cells can occur in dromedary camels. This phenomenon was previously reported in mares and cattle (Collins *et al*., 1997). Similarly, previous findings showed that progesterone concentration was significantly higher in camel FF collected from large follicles than that obtained from small follicles (Rahman *et al*., 2008; Ali *et al*., 2011), although in these studies, only non-pregnant females were used. Follicular size has been shown to affect its estrogen contents in many species. In cattle, estrogen concentrations increased as the size of the follicle also increased (Henderson *et al*., 1982). In mares, the concentration of estradiol 17-β was significantly higher in the FF collected from late dominant follicles than that obtained from early dominant follicles, and these levels decreased between the late dominant and preovulatory follicle stages (Gerard *et al*., 2002). In camels, Ali *et al*. (2011) reported higher estradiol contents in large follicles than in small follicles. These observations confirm our findings in the present study. The low levels of testosterone reported here in the FF obtained from large follicles in pregnant group could be attributed to an increase in granulosa cell numbers and/or aromatase activity (Ismail *et al*., 1988). Thyroid hormones are imperative for normal ovarian function and follicular growth as well as for general metabolic functions in the body. Herein, the concentration of thyroxin hormones was significantly higher in small follicles compared to large ones in the pregnant group, suggesting its role in granulosa cell differentiation and follicular development. However, the concentration of this hormone did not significantly differ among follicle classes in the non-pregnant group. Similarly, Rahman *et al.* (2008) and Ali *et al*. (2011) reported no significant effects of both follicular size and season on the concentrations of these hormones in the FF of non-pregnant dromedary camels.

Concerning biochemical metabolites, the results showed that the FF levels of total proteins, albumin and globulin were not affected by the reproductive status of the animal or by follicular size, indicating that the follicular contents of these metabolites do not change with the increase in the follicular growth. On the contrary, Rahman *et al*. (2008) reported lower concentrations of the above mentioned metabolites in large follicles than in small ones in camels. The discrepancy between these results may be due to the differences in the size of the selected ovarian follicles used in each study. In the present study, we considered 1 to 3 mm follicles as small ones; however, in Rahman and his colleagues' study, 2-6 mm follicles were classified as small ones. Glucose plays an important role in ovarian metabolism because it is considered the major energy source for the ovary. The present study revealed that glucose concentrations were significantly higher in the large follicles than in small ones, irrespective of the reproductive status. One explanation for this observation could be that glucose metabolism is less intensive in large follicles compared to small ones, resulting in a lower depletion of the glucose contents of the follicles. Another explanation may be an increase in the amount of FF in the large follicles, which accounts for lower numbers of granulosa cells, and subsequently less consumption of

glucose (Gosden *et al*., 1988). In contrast to these results, Rahman *et al*. (2008) observed higher glucose levels in small follicles than in large ones in dromedary camels. They suggested that small follicles have the ability to filter and reserve the high blood concentrations of glucose for utilization in their development to mature Graafian follicles. The variations between the results may be ascribed to the differences in the size of the selected ovarian follicles used in each study as mentioned previously.

Herein, cholesterol concentrations were significantly lower in large follicles than in small ones in the pregnant group. The same observations were previously reported in camels (Albomohsen *et al.*, 2011) and dairy cattle (Leroy *et al.*, 2004). Cholesterol is considered the precursor of all steroid hormones, including estrogen and progesterone. Therefore, the low level of cholesterol in the large follicles indicates the biotransformation of cholesterol to sex steroids (Rahman *et al*., 2008).

Triglycerides are considered the storage form of lipids and their hydrolysis results in one molecule of glycerol and three molecules of fatty acids. Therefore, their lower concentrations in the large follicles as compared to the small ones in pregnant animals could be due to the continued and rapid utilization of these metabolites. Similarly, Albomohsen *et al.* (2011) noticed that the dromedary camels' follicular fluid concentrations of triglycerides were significantly higher in small follicles compared to large ones (31.3 ± 4.0 *vs.* 17.8 ± 4.2 mg/dl, respectively). On the other hand, Leroy *et al*. (2004) observed that the concentration of this metabolite in the follicular fluid increased by 43% as the follicle size increased from small (<4 mm) to large (>10 mm) in dairy cows. The discrepancy between these results may be attributed to the differences between species.

We previously showed that the levels of MDA in buffalo's FF were significantly higher in the small follicles collected during the luteal phase of the estrous cycle than those obtained from the same follicle size during the follicular phase (El-Shahat and Kandil, 2012). The results in the present study showed that the levels of MDA were significantly higher in the FF obtained from pregnant animals than those obtained from non-pregnant ones, irrespective of follicular size. This could be due to an increase in the production of reactive oxygen species (ROS). These ROS were suggested to originate mainly from steroidogenesis occurring in granulosa cells (Castillo *et al*., 2003). The higher levels of TAC obtained from large follicles of non-pregnant ovaries play an important role in the defense systems against oxidants, which implies 1) systems that prevent ROS generation, 2) antioxidant systems that inactivate oxidants and 3) systems that are able to limit the deleterious effects of oxidants by allowing the repair of oxidative damage (Cheeseman and Slater, 1993).

In conclusion, based on these data we can infer that FF compositions differ according to the reproductive status of the animal. In pregnant females, the presence of a corpus luteum on the ovary could play an important role not only in the processes of follicle growth and development but also in the concentrations of biochemical metabolites and hormonal profiles in the FF of dromedary camels. The physiological significance of this difference in the follicular fluid contents of various hormonal and biochemical metabolites in relation to reproductive status and its effect on oocyte maturation and subsequent development in dromedary camels requires further investigation.

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