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THE INFLUENCE OF THE CORPUS LUTEUM LOCATION ON HORMONAL AND VITAMIN C COMPOSITION OF FOLLICULAR FLUID AND SERUM IN DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

The aim of this study was to compare hormonal composition of the predominant antral follicle coexisting with or without a corpus luteum (CL) in dromedary camels. Forty-seven genitalia and blood samples were collected from clinically healthy adult (7–12 years of age) non-pregnant female camels during the breeding season at a local abattoir. Follicles (0.7–1.8 cm in diameter) did not coexist with a CL were classified as follicles F1 and their blood serum labeled F1S (n=12), follicles coexist with CL on the same ovary were labeled F2 and their blood serum labeled as F2S (n=9), and follicles with contralateral CL on the other ovary labeled F3 and their blood serum named F3S (n=11). Follicular fluid (FF) and sera were subjected to biochemical and hormonal analysis. Results revealed greater ($P<0.05$) concentrations of progesterone in the FF of F1 follicles and F1S serum than that found in F3 follicles and F3S serum. The concentrations of cortisone and T3 were higher ($P<0.05$) in FF of F1 and F3, and serum of F1S and F3S in comparison to FF of follicles type F2 and serum of F2S. The thyroxine and vitamin C concentrations in FF from F1 and F2 follicles, and serum of F1S and F2S were higher ($P<0.01$) in comparison with the FF from F3 and serum of F3S. The presence of CL might exert a local effect on hormonal composition of FF and could indirectly influence follicular development and oocyte quality. It is better to use oocytes originated from ovaries without a CL in *in-vitro* fertilisation (IVF) in dromedary camels.

Key words: Antral follicles, camel, corpus luteum, hormones

The camels' oestrous cycle is described as a "follicular wave pattern" (Tibary and Anouassi, 1996; Skidmore *et al,* 2013). Camels are induced ovulators and thus ovulation and CL formation normally occur in response to mating, and the CL that develops has a lifespan of only 10-12 days (Skidmore, 2011). In camels, failure of conception and early embryonic death could occur in the presence of the CL due to low peripheral progesterone concentrations (Mostafa *et al,* 2017). In the application of assisted reproductive techniques, the salutary roles of follicular fluid (FF) on *in-vitro* maturation (IVM), IVF and subsequent embryo development have been investigated in several species (Da Silva, 2008; El-Shahat *et al,* 2019). The FF contains hormones and metabolites, which are locally synthetised and easily pass through the basal lamina to enter the antrum or escape toward circulating blood (Gérard *et al,* 2002; Blaszczyk *et al*, 2006; Fahiminiya *et al,* 2011). It has been reported that FF is rich in steroid reproductive hormones, such as oestradiol-17β (E2) and progesterone (P4) in cattle (Bearden and Fuquay, 2003), and mares (Bashir *et al,* 2016; Młodawska *et al,* 2018; Satué *et al,* 2019). There are scarce reports about comparing the effect of the absence or presence of a corpus luteum (CL) on FF hormonal composition (Kor, 2014). The composition of FF is modified according to the absence or presence of CL in the ovary in mares (Da Silva, 2008), cows (Kor *et al,* 2013), ewes (Karami *et al,* 2010) and camels (El-Shahat *et al,* 2019). These modifications could

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indicate the existence of a possible local effect of CL on the dynamic development of follicles. Corpora lutea affected ovarian follicular dynamics in both ovaries by a systemic effect with evidence for a local ipsilateral effect (Contreras-Solis *et al,* 2008). The cortisol in ovarian FF inhibits the ovarian steroidogenesis (Michael *et al,* 1993) and stimulate oocyte maturation (Fateh *et al,* 1989; Jimena *et al,* 1992). T3 concentrations were high in the FF collected from predominant follicles existing in absence of CLs (Tabatabaei *et al,* 2011). Thyroxine affects the follicular development and oocyte maturation (Fedail *et al,* 2014), and ovarian steroidogenesis (Spicer *et al,* 2001). In human, the quality of oocyte maturation and embryos is better in lower levels of vitamin C (Saffari *et al,* 2015).

As changes in the hormonal and vitamin C (Saffari *et al,* 2015) composition of FF may influence the maturation and quality of the oocyte, the aim of the present study was to compare some hormonal and vitamin C composition of the predominant antral follicle coexisting with or without a CL in dromedary camels.

Materials and Methods

Experimental materials

A total of 47 genitalia were collected from clinically healthy adult (7–12 years of age) non-pregnant female camels (*Camelus dromedarius*) during the breeding season (November–March) at a local abattoir in the eastern province of Saudi Arabia. The reproductive history of those animals was unknown. Blood sample (10 ml) was collected from each animal during exsanguinations into plane vacutainer tubes. After slaughtering, the clinically normal genitalia were transported to the laboratory in an icebox within one-hour post-slaughter. In the laboratory, ovaries were washed twice in cooled 0.9% NaCl and blotted dry. Paired ovaries bearing oversized follicle (>20 mm in diameter; Tibary and Anouassi, 1997; Ghoneim *et al,* 2013) or ovarian cyst were excluded from the investigation. Predominant antral follicles without a corpus luteum (CL; Fig 1; F1), follicles coexist with CL on the same ovary (F2), and follicles with contralateral CL on the other ovary (Fig 2a and 2b; F3) were considered for measuring using a Vernier caliper. The follicular diameter ranged from 0.7–1.2 cm (Skidmore, 2011). Follicular fluid (FF) of the predominant follicles was aspirated using sterilised 22-gauge hypodermic needles and syringes. The FF and blood samples were centrifuged at 1500 g at 4°C for 10 minutes. The supernatant fluid and serum were harvested and stored at -80°C until analysis. Serum samples were labeled as F1S (Serum of camels bearing follicles without CL; n= 20), F2S (Serum of camels bearing follicles coexist with CL on the same ovary; n= 12), and F3S (Serum of camels bearing follicles with contralateral CL on the other ovary; n= 15).

Hormonal and Vitamin C analyses

The follicular fluid and serum concentrations of camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ mL; Catalog No. MBS9381137), camel progesterone (P4; ng/ mL; Catalog No. MBS7606970), camel cortisone (ng/ mL; Catalog No. MBS062065), camel insulin-like growth factor-II (IGF-II; ng/mL; Catalog No. MBS058122), camel ultra-sensitivity triiodothyronine (T3; nmol/L; Catalog No. MBS056436), general thyroxine (T4; ng/mL; Catalog No. MBS289276) and vitamin C (µg/ml; Catalog No. MBS2700398) were estimated using Enzyme immune assay kits My BioSource® (USA).

The laboratory reported intra- and inter-assay coefficient of variances of the studied hormonal concentrations were 4.4% and 5.1% for E2, 2.3% and 4.8% for P4, 4.4% and 5.1% for cortisone, 3.1% and 3.9% for IGF-II, 2.3% and 4.1% for T3, 3.2% and 5.3% for T4, 3.9% and 7.4% for vitamin C, respectively. The sensitivity of assays for E2, P4, cortisone, IGF-II, T3, T4 and vitamin C were 0.1 ng/ml, 0.188 ng/ml, 1.0 ng/ml, 1.0 ng/ml, 0.1 nmol/L, 0.0975 ng/ml and 173.5 µg/ml, respectively. All measurements were carried out according to the manufacturers' guidelines. The optical densities were measured using an ELISA reader (Absorbance Microplate Reader ELx800TM, Bio Tek®, Highland Park, VT, USA and Microplate Strip Washer (ELx800 TM, Bio Tek®, Highland Park, VT, USA).

Statistical analysis

The data of follicular fluid hormonal and vitamin C compositions are presented as means ± SEM. Analyses were conducted by ANOVA using SPSS statistical software programme (2013), version 22.0.

Results and Discussion

The mean concentrations of E2 in FF of F3 follicles were higher ($P<0.01$) compared with that of F2 follicles (Tables 1 and 2). The concentrations of P4 were higher ($P<0.05$) in the FF harvested from F1 follicles than that collected from F3 follicles (Tables 1 and 2). The concentrations of cortisone and T3 were higher ($P<0.05$) in FF harvested from F1 and F3, in comparison to FF of follicles type F2 (Tables 1 and

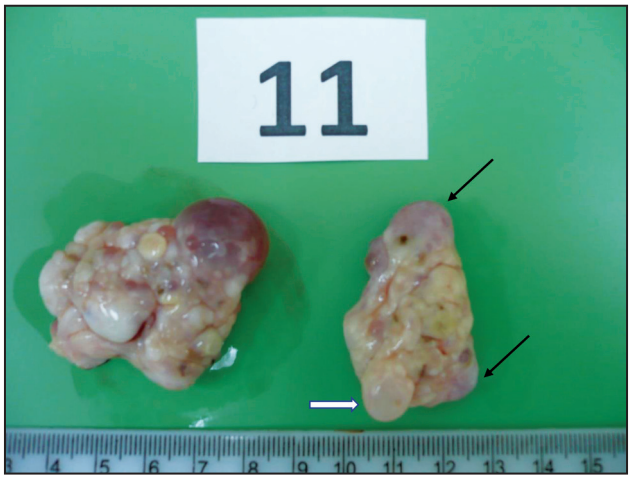

Fig 1. Congested follicle (1.8 cm in diameter) showed on the left ovary and regressed CLs (Black arrows) and predominant antral follicle (0.8 cm in diameter; White arrow) present on the right ovary.

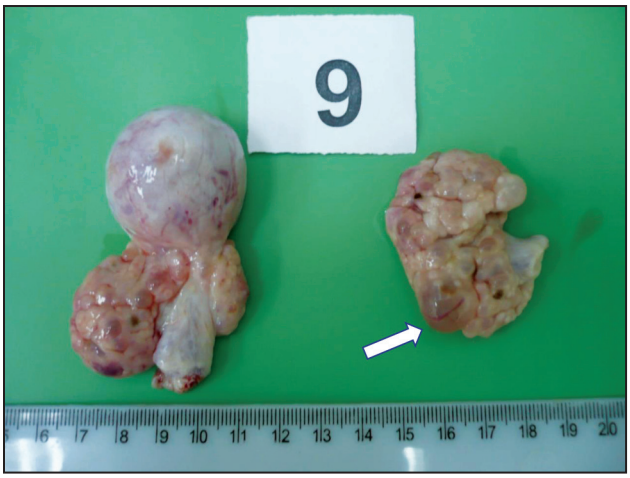

Fig 2. A. Mature CL showed on the left ovary and predominant antral follicle (0.8 cm in diameter) present on the right ovary.

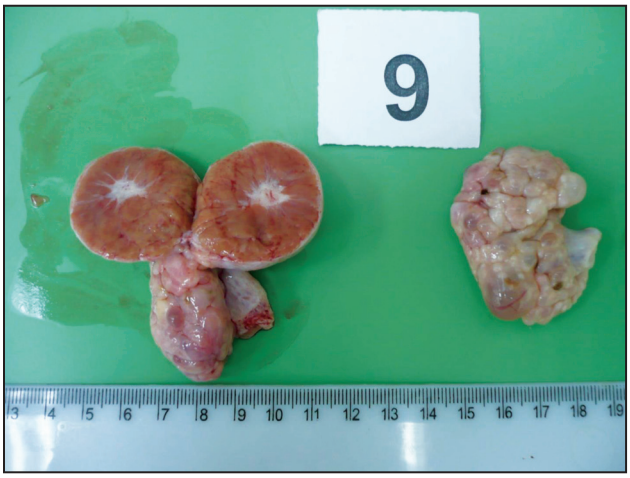

Fig 2. B. The mature CL present on the left ovary after dissection.

2). The IGF-II concentrations in the FF from F1 and F3 were lower (P<0.001) compared with that of FF from F2 (Tables 1 and 2). As shown in tables 1 and 2, the T4 concentrations in FF from F1 and F2 follicles were higher (P<0.01) compared with the FF from F3. Vitamin C concentrations in the FF harvested from F1 follicles were higher (P<0.001) than the concentrations present in F2 and F3 follicles (Tables 1 and 2).

The mean concentrations of E2 in serum of F3S were higher (P<0.01) compared with that of serum of F2S camels (Tables 1 and 2). The concentrations of P4 were higher (P<0.05) in serum of F1S camels than that present in F3S serum (Tables 1 and 2). The concentrations of cortisone and T3 were higher (P<0.05) in serum of F1S and F3S in comparison to serum of F2S (Tables 1 and 2). The IGF-II concentrations in serum collected from F1S and F3S were lower (P<0.001) compared with that serum from F2S (Tables 1 and 2). As shown in Tables 1 and 2, the T4 concentrations in serum of F1S and F2S were higher (P<0.01) compared with serum of F3S. Vitamin C concentrations in serum of F1S camels were higher (P<0.001) than the concentrations present in serum from F2S and F3S (Tables 1 and 2). The hormonal and vitamin C concentrations in FF were many folds higher than those of serum, and there was no correlation in these concentrations between the FF and peripheral circulation.

The assessment of some hormonal and vitamin C composition of the predominant antral follicles coexisting with or without CL were the goals of this study. FF holds different biochemical metabolites that are derived from serum or synthesised locally in the follicles and shared in the metabolic activities of follicular cells (Edwards, 1974; Gérard *et al,* 2002). The constituents of the FF change through the growth and development of each follicle (Wise, 1987). This study reported a significant increase in the concentrations of E2 of FF from F3 and serum of F3S compared with FF from follicles F2 and serum F2S. The ability of antral follicles to produce large amounts of E2 is a distinctive sign of health status of the follicle (Beck *et al,* 2003; Kobayashi *et al,* 2006). Granulosa and theca cells secret large amounts of progesterone which act as a precursor for androgen and subsequently oestrogen production (McNatty *et al,* 1984). It has also been suggested that E2 is important in the oocyte acquisition of fertilisation competence (Yoshimura *et al,* 1987; Dode and Graves, 2003). In mares, intrafollicular E2 concentrations were significantly higher in non-CL-bearing ovaries than in CL-bearing

ones (Satué *et al,* 2019). However, in bovine, location of follicles relative to the CL had no influence on oestradiol-17 beta concentrations (Brantmeier *et al,* 1987).

In the prsent study, P4 concentrations in both FF of follicles F1 and serum F1S were higher than these concentrations in FF of F3 and serum of F3S. The source of the significant high peripheral P4 concentrations reported in camels bearing predominant follicles without CL may be the adrenal cortex (Asher *et al,* 1989). Moreover, in mares, FF P4 concentrations of ovaries not bearing CL were higher than that present in FF of the CL bearing ones. Thus, CL modifies the intrafollicular P4 concentrations

Table 1. Hormonal and vitamin C compositions of follicular fluid in presence or absence of the corpus luteum in camels (Mean ± SEM).

Hormones & vitamin C	Follicles without CL [F1] (n= 20)	Follicles coexist with CL on the same ovary [F2] (n= 12)	Follicles with contralateral CL on the other ovary [F3] (n= 15)	P-value
Camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ml)	0.290^{ab} ± 0.038 C. I.* 0.205 – 0.375	0.131^{a} ± 0.028 C. I. 0.069 – 0.193	0.347^{b} ± 0.063 C. I. 0.203 – 0.490	P<0.01
Camel progesterone (P4; ng/ml)	0.280^{a} ± 0.006 C. I. 0.267 – 0.295	0.253^{ab} ± 0.015 0.220 – 0.286	0.224^{b} ± 0.019 0.182 – 0.267	P<0.05
Camel cortisone (ng/ml)	0.236^{a} ± 0.033 C. I. 0.161 – 0.311	0.080^{b} ± 0.019 C. I. 0.036 – 0.124	0.245^{a} ± 0.055 C. I. 0.122 – 0.368	P<0.05
Camel IGF-II (ng/ml)	41.140^{a} ± 1.288 C. I. 38.227 – 44.053	57.271^{b} ± 1.041 54.915 – 59.627	45.810^{a} ± 2.331 40.537 – 51.083	P<0.001
Camel Ultra sensitivity triiodothyronine (T3; nmol/L)	3.849^{a} ± 0.023 C. I. 3.797 – 3.901	3.591^{b} ± 0.055 3.466 – 3.716	3.872^{a} ± 0.111 3.620 – 4.124	P<0.05
General Thyroxine (T4; ng/ml)	9.409^{a} ± 0.010 C. I. 9.387 – 9.431	9.491^{a} ± 0.406 8.572 – 10.410	8.349^{b} ± 0.187 7.927 – 8.771	P<0.01
Vitamin C (µg/ml)	26.920^{a} ± 0.081 C. I. 26.738 – 27.102	19.921^{b} ± 0.519 18.748 – 21.094	19.511^{b} ± 1.020 17.203 – 21.819	P<0.001

Means with different superscripts are significantly different.

*Confidence Interval.

Table 2. Serum hormonal and vitamin C concentrations in presence or absence of the corpus luteum in camels (Mean ± SEM).

Hormones & vitamin C	Serum of camels bearing follicles without CL [F1S] (n= 20)	Serum of camels bearing follicles coexist with CL on the same ovary [F2S] (n= 12)	Serum of camels bearing follicles with contralateral CL on the other ovary [F3S] (n= 15)	P-value
Camel oestradiol 17-Beta-Dehydrogenase (E2; pg/ml)	0.053^{ab} ± 0.019 C. I.* 0.011 – 0.095	$0.0.024^{a}$ ± 0.014 C. I. 0.004 – 0.049	0.063^{b} ± 0.032 C. I. 0.011 – 0.124	P<0.01
Camel progesterone (P4; ng/ml)	0.139^{a} ± 0.012 C. I. 0.111 – 0.167	0.126^{ab} ± 0.030 0.091 – 0.162	0.111^{b} ± 0.038 0.076 – 0.151	P<0.05
Camel cortisone (ng/ml)	0.018^{a} ± 0.008 C. I. 0.001 – 0.037	0.006^{b} ± 0.005 C. I. 0.0002 – 0.015	0.019^{a} ± 0.013 C. I. 0.001 – 0.044	P<0.05
Camel IGF-II (ng/ml)	61.014^{a} ± 1.713 C. I. 57.138 – 64.890	84.938^{b} ± 1.384 82.082 – 87.830	67.940^{a} ± 3.100 60.591 – 75.245	P<0.001
Camel Ultra sensitivity triiodothyronine (T3; nmol/L)	4.118^{a} ± 0.098 C. I. 3.895 – 4.341	3.842^{b} ± 0.234 3.555 – 4.135	4.143^{a} ± 0.473 3.713 – 4.589	P<0.05
General Thyroxine (T4; ng/ml)	7.832^{a} ± 0.160 C. I. 7.470 – 8.194	7.900^{a} ± 0.406 6.821 – 9.045	6.950^{b} ± 0.292 6.308 – 7.621	P<0.01
Vitamin C (µg/ml)	24.712^{a} ± 0.976 C. I. 22.504 – 26.920	18.287^{b} ± 0.254 15.779 – 20.952	17.911^{b} ± 2.290 14.479 – 21.672	P<0.001

Means with different superscripts are significantly different.

*Confidence Interval.

(Satué *et al,* 2019). On the contrary, there were significant higher P4 concentrations in the FF collected from pregnant camels (presence of a CL) than those obtained from non-pregnant ones (El-Shahat *et al,* 2019; Fawzy *et al,* 2021). The presence of the CL on the ovaries could play an important role in follicle growth, development, and control the concentrations of biochemical metabolites and hormonal profiles in the FF of dromedary camels (El-Shahat *et al,* 2019). Moreover, in sheep (Dufour *et al,* 1972; Rexroad and Casida, 1977) and cattle (Kor, 2014), the higher P4 concentration in FF of ovaries bearing CL than the FF of ovaries have no CL may relate to the presence of the CL. There is a relationship between the development of the CL and the development of follicles (Dufour *et al,* 1972; Rexroad and Casida, 1977). Corpora lutea affect the dynamics of follicles in both ovaries by a systemic effect with evidence for a local ipsilateral effect (Contreras-Solis *et al,* 2008).

Similar to the data reported in this study, the serum concentrations of E2, P4 and cortisol are many folds lower than those of follicular fluid in dromedary camels (Rahman *et al,* 2008) and mares (Satué *et al,* 2019). There are no correlations between the serum and FF of P4 in the preovulatory period or transitional mares (Bøgh *et al,* 2000; Satué *et al,* 2020).

In the present study, the cortisone concentrations in the FF of F1 and F3, and serum of F1S and F3S camels were higher than those present in FF of F2 and serum of F2S. Glucocorticoids in follicular fluid are derived from the general circulation. The glucocorticoid status of ovarian FF is prognostic to oocyte quality (Lewicka *et al,* 2003). The 11 beta-hydroxysteroid dehydrogenases convert cortisol to its inactive metabolite cortisone and *vice versa* (Quinkler *et al,* 2003). Cortisol inhibits the ovarian steroidogenesis (Michael *et al,* 1993) and stimulate oocyte maturation (Fateh *et al,* 1989; Jimena *et al,* 1992). During the LH surge, an increase in total and free cortisol occurs in the ovarian follicle (Harlow *et al,* 1997; Yong *et al,* 2000). Formation and function of the CL benefits from a high local concentration of free cortisol, whereas the surrounding developing follicles may experience negative effects (Andersen, 2002).

In the present study, camel IGF-II concentrations in the FF of F2 and serum of F2S were higher than those concentrations in the FF of F1 and F3 and serum of F1S and F3S. The IGFs play a significant role in follicular and luteal development in the bovine ovary, and locally produced IGF-II is probably an important regulator of follicular growth in cattle (Perks *et al,* 1999). In primate species, IGF-II is the predominant circulating and intraovarian form of IGFs (Giudice, 2001; Tkachenko *et al,* 2021). Besides, IGF-II stimulates granulosa steroidogenesis (Giudice, 2001).

The T3 concentrations in the FF of F1 and F3, and serum of F1S and F3S camels were higher than those found in FF of F2 and serum of F2S. Parallel with these results, T3 concentrations were significantly high in the FF collected from predominant follicles existing in absence of CLs (Tabatabaei *et al,* 2011). T4 concentrations were high in the FF of F1 and F2 and serum harvested from F1S and F3S. However, the majority of thyroxine present in follicular fluid appear to be derived from peripheral blood and enter follicles through theca interna cells (Cai *et al,* 2019). Thyroid hormones control the growth, differentiation, and metabolism in almost all somatic tissues (Ingbar and Wieber, 1981). Moreover, thyroxine affects the follicular development and oocyte maturation (Accardo *et al,* 2004; Ashkar *et al,* 2010; Verga Falzacappa *et al,* 2012; Zhang *et al,* 2013; Fedail *et al,* 2014), and ovarian steroidogenesis (Cecconi *et al,* 1999; Spicer *et al,* 2001). T3 appears to synergise with follicle-stimulating hormone (FSH) to induce development of granulosa cells (Shabankareh *et al,* 2013). The concentrations of T3 and T4 in the FF were higher in absence of CL when compared with those in follicles coexist with CL on ovaries (Kor, 2014). In this study, Vitamin C concentrations were high in the FF of F1 and serum of camels' type F1S when compared with those concentrations in the FF of F2 and F3, and serum of dromedaries' type F2S and F3S. The ovaries store high amounts of ascorbic acid within the granulosa, thecal, and luteal cells (Deane, 1952). Ascorbic acid endorses steroidogenesis (Sanyal and Datta, 1979), acts as an antioxidant (Goralczyk *et al,* 1992; Luck *et al,* 1995) and assets in remodeling the basement membrane during follicular growth (Murray *et al,* 2001). However, in human, the quality of oocyte maturation and embryos is better in lower levels of vitamin C compared to higher levels (Saffari *et al,* 2015).

The presence of CL might indicate the existence of a local drastic effect on hormonal and vitamin C composition of FF and could indirectly influence follicular development and oocyte quality.

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