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Research Article
Dynamics of Follicular Fluid Composition in Relation to Follicular Size and Corpus Luteum in Dromedary Camels

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Abstract

Background and Objective: The concentration of biochemical metabolites in follicular fluid of the ovary fluctuates considerably with cycle stage, follicle size and follicle status and the presence of corpus luteum. Therefore, this study was designed to study dynamics of follicular fluid composition in relation to follicular size and corpus luteum in dromedary camels. **Methodology:** Two hundred ovaries were collected at slaughterhouse from hundred female dromedary camels. The ovaries were classified into ovaries with CL (CL-bearing group) and ovaries without CL (non-CL bearing ovaries). Follicular fluid of small, medium and large sized follicles were aspirated and analyzed for hormonal (estradiol-17β, progesterone, insulin and leptin) and antioxidant (nitric oxide (NO), ascorbic acid and glutathione (GSH), beside biochemical concentrations (glucose and cholesterol). **Results:** The large sized follicles contained significantly ($p<0.05$) higher leptin concentrations than medium and small ones in ovaries with or without CL. Furthermore, the concentration of Insulin was significantly higher ($p<0.05$) in FF obtained from large and small follicles in CL-bearing ovaries than those found in non-CL bearing ovaries. Furthermore, insulin concentrations were significantly higher ($p<0.05$) in the FF obtained from large and small follicles in CL-bearing ovaries than those obtained in non-CL bearing ovaries. However, GSH levels were significantly higher ($p<0.05$) in the FF obtained from large follicle compared to other follicles in CL-bearing ovaries. **Conclusion:** The FF composition was modified depending on the ovarian status. Presence or absence of the corpus luteum played a crucial role in follicular fluid composition of dromedary camels. Further studies are required to investigate the different pathways affecting camel follicular physiology.

Key words: Follicular fluid, ovary, camel, leptin, insulin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Follicular fluid was the critical environment affecting either growth or maturation of both ovarian germ and somatic cells. It contained substances involved in cell differentiation, gamete quality and rupture of the follicular wall[1]. This could reasonably expect that assaying of its biochemical composition will give better guidelines concerned with studying ovarian physiology moreover regulating of camel follicular growth and maturation. Therefore, follicular fluid might be regarded as a biological window reflecting metabolic and hormonal processes occurring in the micro-environment of the maturing oocyte before ovulation[2]. The concentrations of different metabolites the aspirated follicular fluid of collected from different sizes ovarian follicles ovarian follicles in either pregnant or non-pregnant she-camels slaughtered during breeding season were greatly varied[3]. To date, reports of comparing the effect of attendance of a corpus luteum (CL) on FF hormonal composition in camels were limited. *Corpora lutea* are considered the normal continuation of follicular maturation that formed following ovulation from the residual follicular cells. In addition, the CL was a transitory endocrine gland that secretes P4 and had a key role in establishment and conservation of pregnancy in domestic mammals[4]. The camel was known to be an induced ovulator and the corpus luteum (CL) can only be seen during pregnancy[5]. Presence of CL upon the ovaries had been reported to reduce the growth of the follicles by increasing the rate of follicular atresia[6]. As previously reported that the concentrations of different follicular composition in camel vary according to the follicular size and presence or absence[3] of CL. An inadequate knowledge about camel reproduction continues to hinder the progress towards overcoming the reproductive disorders that leads to a limited productivity. Studies on the follicular fluid microenvironment in camel might provide a valuable insight into the process of normal follicular development as well as the pathogenesis of some reproductive problems in the species[7]. Therefore, this study intended to evaluate the effects of ovarian status (presences or absence of corpus luteum (CL) on chemical compositions, hormonal profiles and antioxidant bulk of the follicular fluid follicular fluid aspirated from variable sized ovarian follicles in dromedary camels (*Camelus dromedaries*).

MATERIALS AND METHODS

The experimental design has been directed with the ethical approval of the Institutional Animal Care and Use Committee (IACUC) of Faculty of Science, Cairo University, Egypt. Approval # 2732/24/2018.

Follicular fluid collection and processing: Ovaries (N = 200) were collected from mature she-camels (5-10 years old) slaughtered during the period from January-March (2018) of unknown reproductive history at El-Basstin slaughterhouse, Cairo and transported to the laboratory within 1 h. The ovaries were gathered in pairs, placed in plastic bags normal l saline (0.9% NaCl) at 30°C. The ovaries were divided according to their status into two groups, ovaries with CL (CL-bearing group) and ovaries without CL obtained from (non-CL bearing group). Follicular diameter was measured and counted on the ovarian surface using a caliper. Follicles were classified into three main categories: small (1-3 mm), medium (4-9 mm) and large (≥10 mm). Ovaries with cystic follicles were excluded from the study. The contents of different sized ovarian follicles were aspirated using a 10 mL syringe provided with an 18-gauge blunt needle. Follicular fluids from each group from each pair of the ovaries were pooled in one sample for each individual camel. Following aspiration, the follicular fluids were subjected to centrifugation at (3000 rpm for 10 min) to separate the fluid away from the cellular portion. Supernatants were stored at -20°C until analysis. Each of the follicular fluid samples was assayed for hormonal (P4, E2, insulin and leptin) and biochemical (glucose, cholesterol, NO, ascorbic acid and GSH). Follicular fluid samples were analyzed for different hormonal and biochemical metabolites and the analysis of the concentration of each parameter in the different groups was repeated at least four times.

Assay of progesterone and estradiol 17 β: Estradiol, progesterone were assayed using DRG diagnostics (DRG, Germany, RUO in the USA) using ELISA (competitive binding ELISA). The intra and inter-assay coefficients of variation of progesterone were 6.86 and 5.59 and 11.5, 4.5, respectively[8,9].

Assay of leptin and insulin: Insulin was assayed according to Flier *et al.*[10] using EIA commercial kits ELISA (DRG, International, Inc., USA) The analytical sensitivity was calculated from the mean plus two standard deviations of 20 replicate analyses of *Zero Standard* and was found to be 1.76 μIU mL^{-1}. Intra and inter-assay coefficients of variation were 2.6 and 2.88%, respectively. Leptin was assayed using Leptin ELISA (Sandwich) GmbH Germany commercial kit, according to Considine *et al.*[11]. Intra and inter-assay coefficients of variation were 3.1 and 9.7%, respectively. Sensitivity of the assay was 1.0 ng mL^{-1}.

Assay of biochemical: Biochemical components of follicular fluid such as total cholesterol, glucose, nitric oxide, ascorbic

acid, GSH were analyzed using colorimetric commercially available ELISA kits (Biodiagnostics, Egypt). According to the methods described by Richmond[12], Caraway and Watts[13], Montgomery and Dymock[14] and Beutler *et al.*[15].

Statistical analysis: All values were expressed as Means±SEM. The data were analyzed using SPSS® Statistical Software (SPSS®11.01 for Windows, 2007)[16]. Simple one way ANOVA was done to identify the effect of ovarian status on all follicle categories and between them. The Duncan's Multiple range test was used to separate between significant means. While 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 CL on follicular levels of insulin and leptin hormonal levels: Insulin concentrations were significantly higher ($p<0.05$) in the FF obtained from large and small follicles in CL-bearing ovaries than those obtained in non-CL bearing ovaries. In the CL-bearing ovaries, the concentrations of insulin were higher ($p<0.05$) in large than medium and small follicles (3.19±0.73 vs. 1.26±0.12 and 2.98±0.98 ng mL^{-1}, respectively). On other hand, the only significant effect between the 2 groups was observed in leptin concentrations in the FF obtained from small follicles (1.67±0.01 vs. 2.49±0.06 ng mL^{-1}) in CL and non-CL bearing groups, respectively as shown in Table 1.

Effect of on follicular levels of CL on FF progesterone and estrogen hormonal levels: The concentrations of progesterone in the follicular fluid obtained from large follicles significantly ($p<0.05$) decreased in CL-bearing group as compared to those obtained from small and a medium follicles (21.62 vs. 31.32, 36.87 ng mL^{-1}, respectively). Conversely, the concentrations of estradiol-17β were significantly ($p<0.05$) increased in large and a medium follicle compared to small ones in CL-and non-CL bearing ovaries Table 2.

Effect of CL on follicular levels of glucose and cholesterol: The only significant difference ($p<0.05$) between the 2 groups was observed in glucose concentrations in the FF obtained from large follicles (97.0±10.1 vs. 80.0±7.3 mg dL^{-1} in CL and non-CL bearing groups, respectively). However, the concentrations of cholesterol was significantly ($p<0.05$) higher in small follicles of non CL-bearing ovaries than those obtained of the same follicular size of CL-bearing ovaries (135.02±15.54 vs. 18.87±0.16 mg dL^{-1}, respectively) as shown in Table 3.

Effect of CL on follicular levels of NO, ascorbic acid and GSH: NO levels were significantly higher ($p<0.05$) in the FF obtained from small follicle categories in CL-bearing ovaries (64.46±35.34 mg dL^{-1}) than those obtained in non-CL-bearing ones (46.24±20.63 mg dL^{-1}). Within the same group, the small follicles contained significantly ($p<0.05$) higher NO concentrations compared to those in medium and

Table 1: Effect of presence of CL on insulin and leptin hormones levels in the follicular fluid of dromedary camels (Mean±SEM)

		Ovaries	
Hormones	Follicular size	CL- bearing	Non CL- bearing
Insulin (ng mL^{-1})	Large	3.19 ± 0.73^{ac}	0.35 ± 0.06^{bd}
	Medium	1.26 ± 0.12^{ab}	7.04 ± 1.04^{bc}
	Small	2.98 ± 0.75^{ac}	0.98 ± 0.19^{bd}
Leptin (ng mL^{-1})	Large	2.37 ± 0.28^{bc}	2.41 ± 0.10^{bc}
	Medium	1.65 ± 0.01^{ad}	1.76 ± 0.07^{ad}
	Small	1.67 ± 0.01^{ad}	2.49 ± 0.06^{bc}

Values with different superscripts within the same row (a,b) and within the same column (c,d) are significantly different ($p<0.05$)

Table 2: Effect of presence of CL on progesterone and estrogen hormonal levels in the follicular fluid of dromedary camels (Mean±SEM)

		Ovaries	
Hormones	Follicular size	CL- bearing	Non CL- bearing
Progesterone (ng mL^{-1})	Large	21.62 ± 6.59^{ad}	31.51 ± 0.74^{bc}
	Medium	36.87 ± 0.08^{ac}	34.96 ± 0.65^{ac}
	Small	31.32 ± 1.51^{bc}	28.25 ± 3.33^{ad}
Estradiol 17-β (pg mL^{-1})	Large	1128.20 ± 55.2^{ac}	1169.80 ± 19.2^{ac}
	Medium	1213.40 ± 50.3^{ac}	1227.20 ± 33.9^{ac}
	Small	910.30 ± 98.9^{ad}	778.60 ± 209.6^{ad}

Values with different superscripts within the same row (a,b) and within the same column (c,d) are significantly different ($p<0.05$)

Table 3: Effect of presence of CL on cholesterol and glucose levels in the follicular fluid of dromedary camels (Mean±SEM)

		Ovaries	
Metabolites	Follicular size	CL- bearing	Non CL- bearing
Cholesterol (mg dL^{-1})	Large	18.88±.89^{ac}	17.78±.9.48^{ad}
	Medium	28.18±4.40^{ad}	20.34±1.82^{bd}
	Small	18.87±0.16^{ac}	135.02±15.54^{bc}
Glucose (mg dL^{-1})	Large	97.01±.10.13^{ac}	80.01±.7.35^{bc}
	Medium	63.81±3.17^{bd}	86.07±3.61^{bc}
	Small	67.91±2.06^{ad}	72.76±3.31^{ab}

Values with different superscripts within the same row (a,b) and within the same column (c,d) are significantly different ($p<0.05$)

Table 4: Effect of presence of CL on NO, ascorbic acid and GSH levels in the follicular fluid of dromedary (Mean±SEM)

		Ovaries	
Metabolites	Follicular size	CL- bearing	Non CL- bearing
NO (μmol L^{-1})	Large	19.01±1.65^{ad}	13.38±0.92^{bd}
	Medium	12.15±1.09^{ad}	13.11±0.096^{ad}
	Small	64.46±35.34^{ac}	46.24±20.63^{bc}
Ascorbic acid (μmol L^{-1})	Large	2.21±0.79^{ac}	1.80±0.46^{ac}
	Medium	6.08±4.12^{ad}	3.23±1.13^{be}
	Small	7.91±4.54^{ad}	13.53±9.60^{bd}
GSH (U mL^{-1})	Large	4.64±1.93^{ac}	3.73±1.37^{ad}
	Medium	4.13±1.22^{ac}	5.84±3.11^{ac}
	Small	2.13±1.06^{ad}	2.59±0.63^{ad}

Values with different superscripts within the same row (a,b) and within the same column (c,d,e) are significantly different ($p<0.05$)

large ones in both groups. Ascorbic acid concentrations significantly ($p<0.05$) decreased in the FF obtained from small to large follicles in both groups (Table 4). However, medium and large follicle contained high concentrations of GSH levels than those obtained in small ones in CL-bearing ovaries (4.64±1.93 and 4.13±1.22 vs. 2.13±1.06 U mL^{-1}, respectively). While the medium sized follicles contained the highest levels of GSH (5.84±3.11 U mL^{-1}) as compared to the others in non-CL bearing ovary (Table 4).

DISCUSSION

The present study evaluated the variations in the concentrations of different biochemicals (glucose and cholesterol), hormonal (estrogen, progesterone, leptin and insulin) and antioxidant (NO and GSH) in the follicular fluid obtained from different sized ovarian follicles in CL and non-CL bearing ovaries of female camels slaughtered during the breeding season. Regarding hormonal profiles, the results showed that, CL-and non-CL bearing ovaries had a positive relationship between leptin concentrations and follicle size. Leptin is classified as a "metabolism modifier" it is likely that leptin influences, through tissue-specific mechanisms, the sensitivity to insulin and hence glucose uptake by the cells, in order to direct nutrients towards organs or tissues that are metabolically[17]. Leptin levels increased as ovulation approached. Leptin also played a role during follicle growth, ovulation and corpus luteum development and modulating uterine blood flow before and following ovulation in mares[18]. Leptin and estradiol concentrations played an important role in first postpartum ovulation in Arab mares[19]. It has been proposed that leptin acts directly on GnRH neurons[20]. Follicular size has been shown to affect its insulin contents recorded herein. However, oversized follicles in dromedaries were associated with decreased concentrations of IGF-1 in follicular fluid but not in serum[21]. In cyclic cattle, insulin administration increases the diameter of large follicle by increasing follicular fluid volume[22]. It might be due to positive effect of insulin on granulosa cell proliferation[23]. The higher concentration of progesterone reported in the present study the large compared to small follicles in non CL-bearing ovaries suggested that luteinization of granulosa cells could occur in dromedary camels. Similarly, previous results concluded that progesterone level was significantly greater in camel follicular fluid aspirated from large sized follicles than that recovered[24,25]. Concerning biochemical metabolites, the results showed that the FF levels of NO were highest in small follicles in comparison to those in medium and large ones in both groups. The higher concentrations of NO obtained from small follicles suggested its active role in the follicular development during the growth phase. NO was an essential intra ovarian factor, regulates the process of follicular dynamic due to complicated role during angiogenesis, vasodilation, moreover its role in regulation of the permeability of follicular wall,

steroidogenesis and ovulation[26] the lower concentrations of NO levels of medium sized follicles recorded herein indicated that, hamper the progressive development due to decreased vascularity leading to atresia[27]. In mare it had been reported that, the higher levels of NO in estrous phase in relation to the follicular size during mutual growth phase suggested its active role in follicular growth during this phase, possibly due to its regulatory action on angiogenesis and vasodilatation[28]. Moreover, nitric oxide appears, under normal physiologic conditions, to be the most important regulator of blood flow[29]. The increased pregnancy rate as a response to the increased level nitric oxide may be due to its effects of follicular blood flow being a subsequent in improvement of the ovulated oocyte and its potential role in enhancement of luteal blood flow that help maintenance of luteal function during early pregnancy[30]. The pregnancy rates were enhanced in response to the augmented follicular blood flow in mare[31]. The presence of ascorbic acid in follicular fluid and corpus luteum had been documented previously in buffaloes[32]. However, the fluctuations in follicular fluid ascorbic acid concentration in relation to functional status of different sized follicles were described for the first time in camel. A significantly higher concentration of ascorbic acid in the small sized follicles indicated the possible prerequisite of ascorbic acid to be a specific modulator for steroidogenesis[33] especially during the periods of maximal estrogen production[34]. The lower level of ascorbic acid recorded herein in large sized follicles in the presence or absence of CL possibly leads to weakening of the follicular basement membrane leading to the rupture of follicle at ovulation[35]. In the present study, the concentration of GSH in the follicular fluid significantly increased ($p<0.05$) in CL bearing ovary and reached maximum levels in large sized follicles. In addition, medium sized follicles contain the highest levels as compared to the others in non-CL bearing ovary. In buffaloes. The GSH action was significantly augmented in the follicular fluid obtained from small, medium and large sized follicles during the luteal stage than those obtained at the follicular phase[36]. The GSH was the major non-protein sulf hydryl compound in the mammalian cells, it serves as a reservoir for cysteine and plays an vital role in the protection of the cell from oxidative damage[37]. The GSH content increased during maturation and subsequent development of the oocyte in the ovary as the oocyte approaches the time of ovulation[38]. Reduced glutathione plays an important role in oxidoreduction processes and detoxification of H_2O_2 and organic peroxides, which were ingredients produced in large quantities during inflammatory processes in living cells[39].

CONCLUSION

The results of the study had identified that concentrations of different hormonal and biochemical constituents of FF dromedary camels could be changed with the progressing of follicular growth and the presence or absence of corpus luteum. These changes might indicate the existence of a localized effect of corpus luteum. Further investigations are required to clarify the possible features and different pathways such a local effect of corpus luteum upon follicular physiology.

SIGNIFICANCE STATEMENT

This study discovers the concentration of different biochemical components of FF in dromedary camel could be changed with the progressing of follicular growth and the presence or absence of corpus luteum. It could be beneficial for studying the fluctuations in follicular fluid dynamic in CL^- compared to CL^+ ovaries. This study will help the researcher to uncover the critical areas of existence of a localized effect of corpus luteum on the follicular fluid composition that many researchers were not able to explore. Thus a new theory on possible factors and pathways such a local effect of corpus luteum upon follicular composition in camel were explored.

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