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Comparison of some biochemical and hormonal constituents of oversized follicles and preovulatory follicles in camels (*Camelus dromedarius*)

I.M. Ghoneim [a], M.M. Waheed [a,*], S.M. El-Bahr [b], A.K. Alhaider [a], M.M. Al-Eknah [a]

[a] Department of Clinical Studies, College of Veterinary Medicine and Animal Resources, King Faisal University, Kingdom of Saudi Arabia
[b] Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Animal Resources, King Faisal University, Kingdom of Saudi Arabia

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ABSTRACT

The current study was carried out to compare some biochemical and hormonal constituents in follicular fluids from oversized follicles, preovulatory follicles, and serum in camels (*Camelus dromedarius*). Follicular fluids from oversized follicles (N = 10), preovulatory follicles (N = 10), and sera were harvested from 20 dromedaries. The follicular fluids and sera were subjected to biochemical and hormonal analysis. The results indicated no significant differences in the concentrations of ascorbic acid, glucose, cholesterol, acid phosphatase, and alkaline phosphatase between follicular fluid from oversized follicles and preovulatory follicles. In addition, there were no significant variations in the level of ascorbic acid, glucose, cholesterol, and acid phosphatase in the serum of animals with oversized follicles and those with preovulatory follicles. Serum alkaline phosphatase was significantly greater ($P < 0.05$) in camels with oversized follicles. The concentrations of estradiol-17β (E2) and insulin-like growth factor-1 (IGF-1) in the follicular fluid of oversized follicles were significantly lower ($P < 0.01$) than that from preovulatory follicles. There were no differences in the concentrations of progesterone, tri-iodothyronine, and thyroxin between follicular fluid from oversized follicles and that of preovulatory follicles. The concentrations of E2, progesterone, tri-iodothyronine, thyroxin, cortisol, and IGF-1 were not different in the serum of camels with oversized follicles and camels with preovulatory follicles. The current study revealed that the significant differences of biochemical and hormonal constituents between follicular fluids from oversized follicles and preovulatory follicles were restricted on E2 and IGF-1. Relaying on the aforementioned outcome we can suggest that oversized follicle phenomenon is a form of follicular atresia of anovulatory follicles.

1. Introduction

The one humped she camel is considered an induced ovulator that ovulates only in response to mating stimuli [1]. In absence of mating or ovulation-inducing treatment, the fate of the mature follicle follows one of two possible courses: atresia and disappearance in the ovarian stroma or cystic degeneration [2]. The ovarian cysts have been described in the dromedary [1], bacterian camel [3], llama [4] and alpaca [4]. In dromedary camels, there are discrepancies about the incidence of ovarian cysts. If they are not bred, 30% to 40% of female camels tend to develop some forms of cystic ovaries [2]. Relaying on postmortem examination of the female genitalia, the incidence of ovarian cyst was estimated at 6.9% [5]. Rectal examination revealed that the frequency of ovarian cyst in the camel is 4.7% [6]. The effect of the ovarian cysts on camel fertility is doubtful. Previous investigations suggested that ovarian cysts are physiologic variation of follicular dynamics [7], and others regarded camel ovarian cysts to be pathologic on the basis of

* Corresponding author. Tel.: +966561009906; fax: +96635816635.
E-mail address: mmwaheed@kfu.edu.sa (M.M. Waheed).

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size alone [8]. However, it has been reported that non-ovulatory cysts do not appear to affect the fertility and other smaller follicles might continue to grow normally [9]. The endocrinology of cystic ovarian condition is not well documented in camels [3] when compared with cattle [10]. The follicular fluid is closely correlated with the oocyte and granulosa cell; therefore, it can serve as a reliable indicator for the functional status of the ovarian follicle [11]. The objective of the current study was to compare some biochemical and hormonal constituents of follicular fluids from oversized follicles and preovulatory follicles in the dromedary camel which might disclose the nature of oversized follicles.

2. Materials and methods

2.1. Experimental materials

During the breeding season (October–April), 112 ovarian pairs were recovered from clinically healthy adult (6–15 years of age) nonpregnant female camels (*Camelus dromedarius*) at local abattoir in the Kingdom of Saudi Arabia. A 10 mL blood sample was collected from each animal during exsanguinations into nonheparinized tubes. Preslaughter information about the reproductive status of these animals was not available. After slaughtering, macroscopic examination showed clinically normal reproductive organs. Immediately after collection, ovaries and blood samples were kept in an ice box and transported to the laboratory within 1 hour postslaughter. Upon arrival to the laboratory, ovaries were washed twice in cooled 0.9% NaCl and blotted dry. Paired ovaries bearing corpus luteum were excluded from the investigation. Two different follicle classes, based on follicle diameter (measured by Vernier caliper) were considered for puncture: thin wall oversized follicles of >20 mm in diameter, [7] and preovulatory follicles of 15 to 17 mm in diameter [9]. Oversized follicles filled with sanguineous fluid were excluded. Follicular fluids were aspirated from 10 preovulatory follicles and 10 oversized follicles by means of sterilized 22-ga hypodermic needles and syringes. The follicular fluid was centrifuged at 1250 × *g* at 4 °C for 10 minutes. The supernatant was harvested and stored at −20 °C pending analysis. Blood sera from selected animals were separated and stored at −20 °C until analysis.

2.2. Estimation of biochemical constituents in serum and follicular fluid

Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of glucose (mg/dL; Catalog No. EP37L-660), cholesterol (mg/dL; Catalog No. EP24-660), acid phosphatase (IU/L; Catalog No. EP02-295), and alkaline phosphatase (IU/L; Catalog No. EP04L-660). The analyses were conducted using a full automated chemistry analyzer (Elipse, Rome, Italy). Ascorbic acid (mM/mL) was determined using Enzyme Immuno-assay (EIA) kits (Catalog No. K661-100) produced by Biovision Research Products (Mountain View, CA, USA). The procedures for analysis and calculation were adopted as recommended by the manufacturer.

2.3. Estimation of hormonal constituents of serum and follicular fluid

Estradiol-17β (E2; pg/mL) and progesterone (P_4; ng/mL) in blood serum and follicular fluids were analyzed using EIA kits (Cayman Chemical Company, Ann Arbor, MI, USA). The intra- and interassay CVs were 5.3% and 4.7%, and 4.9% and 2.5%, for E2 and P_4, respectively. Tri-iodothyronin (T_3) and total thyroxin (T_4) concentrations (μg/mL) were analyzed using EIA kits obtained from BioCheck (Foster city, CA, USA). The intra- and interassay CVs were 4.1% and 2.3%, and 5.1% and 3.1%, for T_3 and T_4, respectively. Cortisol analysis (ng/mL) was determined by EIA kits (Oxford Biomedical Research Inc., Oxford, MI, USA). The intra- and interassay CVs were 7.2% and 5.1%, respectively. Bovine insulin-like growth factor-1 (IGF-1) (ng/mL) was assayed using ELISA kits (Catalog No. MBS701164; Biosource Inc., San Diego, CA, USA). The intra- and interassay CVs were 3.2% and 4%, respectively. The assay kit recognized the bovine IGF-1 was of no significant cross-reactivity or interference with other species like monkey, chicken, human, mouse, rat, pig, rabbit, dog, and guinea pig. All assays were performed according to the manufacturer's directions, and the optical densities were measured using an ELISA reader (Absorbance Microplate Reader ELx 800 and Microplate Strip Washer ELx800; BioTek; Highland Park, VT, USA).

2.4. Statistical analysis

The data analysis of biochemical constituents and hormones in oversized follicles, preovulatory follicular fluid, and blood serum was carried out using a general linear model procedure and means were compared by least significant difference using SPSS 16.0 statistical software (2007) [12].

3. Results

Data presented in Table 1 revealed no significant differences between the values of ascorbic acid, glucose, cholesterol, acid phosphatase, and alkaline phosphatase between follicular fluid of oversized follicles and preovulatory follicles. No significant differences were recorded in the concentrations of ascorbic acid, glucose, cholesterol, and acid phosphatase between serum of camels with oversized follicles and preovulatory follicles (Table 2). However, the concentration of alkaline phosphatase was higher ($P < 0.05$) in the serum of camels bearing oversized follicles than

Table 1
Concentrations (mean ± SEM) of different biochemical constituents in follicular fluids from oversized follicles and preovulatory follicles.

Constituents	Fluid from oversized follicles	Fluid from preovulatory follicles
Ascorbic acid (mM)	1.60 ± 0.07	1.54 ± 0.06
Glucose (mg/dL)	195.88 ± 36.75	153.66 ± 19.45
Cholesterol (mg/dL)	15.40 ± 3.24	8.29 ± 2.90
Acid phosphatase (IU/L)	2.34 ± 0.59	4.57 ± 0.24
Alkaline phosphatase (IU/L)	28.57 ± 4.65	22.49 ± 2.28