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# The Involvement of Autoimmune Mechanism in Cystic and Inactive Ovaries of Dromedary Camel

Abdel-Kader A. Zaki[1,2], Abdulrhman A. Alrubayan[1,3], Abdulrahman A. Aba Alkhail [1,4] and Yousef M. Alharbi[1*]

[1]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia
[2]Department of Physiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt
[3]Ministry of Environment, Water and Agriculture, Qassim Region, Saudi Arabia
[4]National center for the Prevention & Control of Plants Pests & Animal Diseases, Qassim Region, Saudi Arabia
***Corresponding author:** yhrby@qu.edu.sa

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**ABSTRACT**

Numerous researchers have proposed work in dromedary camel immunity. It has not before been described how autoimmune etiology is involved in cystic and inactive camel ovaries. To specifically identify ELISA technique to measure anti-ovarian antibodies (Anti-OAB) in the serum, sixty female dromedary camels with various cysts and inactive ovaries during the breeding season were used. For the first time, the values of ELISA are objectively displayed in the current study. Sex evaluations were performed on each sample using the newly designed In-House indirect ELISA. Three are related to the cutoff value, two are related to the sample's initial dilution, and the last reading is related to the antibody index. The values of the Majaheem breed with follicular and luteal cyst were significantly greater than those of healthy she-camels in terms of serum OD of primary dilution; cut-off (antibody titer) value and calibrated antibody concentration against ovarian protein ($P<0.05$ and $P<0.01$, respectively). Concerning antibody index, the data demonstrated a significant difference between the healthy she- camel and Majaheem breed with follicular and luteal cyst ($P<0.05$), Majaheem breed with hemorrhagic cyst ($P<0.01$) and Majaheem and Waddah breeds with inactive ovary ($P<0.05$). Results exhibited a significant decrease in estrogen ($P<0.001$) and FSH levels ($P<0.05$) comparing to normal control group in the serum of Majaheem breed with follicular and luteal cysts and inactive ovary. It is recommended that more study be done on the blood-follicle barrier to comprehend how abnormality develops in ovarian she-dromedaries.

**Key words:** Female Dromedary Camels, Anti-Ovarian Antibodies, FSH, Estrogen, ELISA

## INTRODUCTION

Many people across the world utilize camels in their agriculture and culture. The economy of the camel is quite significant, especially in dry nations. There are specific characteristics to camelid species' reproductive physiology. In contrast to other domestic species, the primary differences include induced ovulation and the follicular dynamics in successive and superimposed waves. Reproduction issues in camels are not as thoroughly studied as they are in other mammals, including cattle. But according to the studies that are now available, camel reproductive immune infertility is scanty (Al Ramadan et al. 2021). Despite having merely, a peritoneal duplicate and without having its surface covered by peritoneum, the ovary is nevertheless regarded as an intraperitoneal organ. Although the ovary is not an immunologically favored organ, fertility suffers greatly when tolerogenic mechanisms for ovarian-specific antigens fail. The blood-follicle barrier (BFB) is one of the blood-tissue barriers in mammals, which can be discovered in the ovary's developing follicles. Along with the tight junction (TJ)-permeability barrier of the endothelial cells in the micro capillaries that surround it, the growing follicle's basement membrane, which rapidly changes in composition throughout follicle development, comprises and significantly contributes to the BFB. Intra-follicular barriers were discussed previously in detail **(**Andrade et al. 2019; Baena and Terasaki 2019).

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The participation of autoimmune mechanisms in cystic and inactive ovaries of dromedary camel was not discussed before. Various factors can cause an autoimmune attack to target the ovary and its arteries (Saeed 2017**)**. Abdominal or pelvic inflammatory process might share in the inflammation of ovaries (ovaritis) or inflammation of the substances of the ovaries, the oocytes in specific (oophoritis). Accurate assessment of the prevalence of the autoimmune form of infertility has been made impossible due to the lack of a highly sensitive and specific diagnostic tools. Mammals with polycystic ovaries and early ovarian failure are known to be affected by autoimmune disease (Shoukry et al. 2020). It has been proposed that ovarian antibodies (OAB) may aid in the identification of ovarian autoimmunity (Makled et al. 2014).

OAB is a class of autoantibodies that target various ovarian antigens. A few of the causes of OAB development include laparoscopic abdominal surgery, the removal of oocytes for use in assisted reproductive techniques, and chronic ovarian inflammation (Al-Naffakh and Risan 2020). The harvest of oocytes for use in assisted reproductive procedures, laparoscopic abdominal surgery, and chronic ovarian inflammation are only a few of the causes of OAB development (Al-Naffakh and Risan 2020). The pathological significance of OAB to various ovarian cell components can decrease pregnancy and fertilization rates, in addition to create an inadequate response to gonadotropin induction and could be responsible for implantation failure (Kirshenbaum and Orvieto 2019). OAB target the ovaries, making them to malfunction normally, stop them from generating the hormone estrogen normally, or make the release of eggs irregular. A normal organism is not intended to generate antibodies against its own ovarian tissues. The issue of OAB still exists since there is a possibility of unchecked self-destruction of its own tissues without obvious causes. As a result, it is impossible to examine the pathophysiology of antibodies against one's own antigens (Byersdorfer et al. 2005). The development of antibodies against the injured tissues may play (to some extent) a sanogenic role by facilitating their removal from the body more quickly (Rodgers et al. 2010). There have been reports of autoimmune oophoritis with lymphocytic infiltrates, cysts, and atresia of follicles (Monteleone et al. 2011). The OAB are independent predictors of the start of ovarian failure (Thabbah et al. 2015). Since OAB are known to be related with poor outcomes in subfertility, screening for their existence before the start of treatment may be advised.

Immune disruption is an important contributor to infertility, but it is not yet evident if disruption is the primary cause or just a complication of infertility problems. There is a dearth of information about OAB in infertile dromedary camels. When there is OAB, autoimmune etiology should be taken into consideration because the ovary is a common target for autoimmune attack. The current study is the initial stage in developing a new indirect solid phase ELISA technique to assess the presence of OAB in camel serum with cystic and inactive ovaries. Treatment of infertile camels can benefit from the evaluation of OAB, estradiol 17 and CamFSH as a prognostic factors.

## MATERIALS AND METHODS

### Ethical Approval

All the study's methods have been given the go-ahead by the Animal Care and Use Committee of the Experimental Animal Centre at Qassim University No. 23-2-15. All surgeries used diethyl ether as the anesthetic, and every attempt was made to decrease the pain.

### Camel Samples and Protocol for Ultra-Sonographic Procedure

Female dromedary camels (cystic ovaries: n=49 and inactive ovaries: n=11) were admitted to the veterinary educational clinic of Qassim university Kingdom of Saudi Arabia (KSA) for various reproductive complaints. Camels weighed between 425 and 575kg, with a normal body condition score (3.5-4.5) based on the scale of 1–5. Established on the phenotypic classification of Saudi Arabian camel (*Camelus dromedarius*) by Abdallah and Faye (2013), the samples used in the present study were collected from the black colored Mujahim and the white colored Wahda breeds during the breeding season (October to December, 2020-2021).

The females were restrained in sternal recumbence while the inspections were conducted on specially outfitted tractors. Each animal's reproductive system was inspected by a licensed veterinarian. Ovaries were examined utilizing real-time, B mode ultrasonography connected to a 5MHz probe and linear transducers, along with routine transrectal palpation (Aloka SSD500; Aloka Co., Ltd., Tokyo, Japan). After applying transmission gel to the transducer, the ovaries of both sides were inspected to get all the information of the existence of different types of follicles, corpora lutea, and any abnormal features (Fig.1).

### Blood Samples

Blood samples were directly drawn from the jugular vein and centrifuged at 3000rpm for 10min to separate the

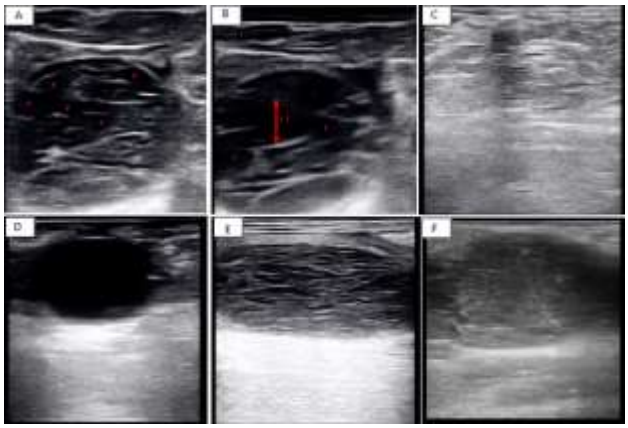

**Fig. 1:** Ultrasonography images of ovaries in different camel breeds. The ultrasonography showed cycling active ovary with normal follicles <10mm in diameter (A). The ovulating ovary with one large ovulating follicle 10mm in diameter (OF) and many medium and small follicles <10mm in diameter (B). Inactive ovary of very small diameter with no growing follicles(C). Ovary showed one large cystic follicle >25mm in diameter (D), large hemorrhagic cystic follicle(E) and luteal cyst (F).

serum. After that, samples were grouped and given numbers before being kept in a refrigerator to be tested for estrogen, CamFSH, and OAB by ELISA.

**Samples of Male-Camels**

Three male-camels were chosen in the breeding season according to the fertility case history of sexually mature for several years. Blood samples were collected and centrifuged at 3000rpm/10 minute for separation of serum and considered as negative control when measuring the existence of OAB by ELISA assay.

**Preparation of Camel Ovarian Tissue Protein and Hyperimmune Sera**

Four ovaries were taken from non-pregnant one-humped she-camels slaughtered in neighboring Qassim Abattoirs and placed in a container with ice bags (4°C). There was no recognized reproductive abnormality in the slaughtered camels. The ovaries were cleaned in phosphate buffered saline pH7.4 and macroscopically inspected, paying particular attention to the size of ovarian follicles. Ovaries showed only normal sized follicles >3mm were used. Corpora lutea, granulosa and thecal tissues and follicular fluid cells were subjected to cold homogenization (Staufen, Germany) with TRIS-HCL solution (0.05mol/LpH7.4) supplemented with sucrose (0.25mol/L) and EDTA (1mmol/L) (Rossi et al. 1987). 4mL of cold extraction buffer was added to each gram of tissue. The subsequent homogenate was centrifuged at 3,000rpm for 30min after being filtered through two plies of gauze. The supernatant was sonicated on ice for interrupted min at 10kHz (Takada et al. 1982). According to Pires et al. (2007), the sonicates were centrifuged at 10,000rpm for 30min at 4°C before the supernatant was subjected to commercial BioMerieux kits for protein analysis. The fluid from the supernatant was used immediately as the immunizing antigen. Antisera against known supernatant protein were prepared using Oguna's technique (1997). Following the steps outlined, the immunizing antigen was injected into four male rabbits (four to five months old). First, 2mL of the emulsion of equivalent volume of prepared protein and Freund's complete adjuvant (Difco inc:0639-60-6, com:0638-60-7) was administered subcutaneously. Three injections were followed, spaced by ten days, and then another final booster dosage without the adjuvant was administered after seven days. Rabbits were sacrificed, sera were gathered and kept in storage at -20°C until used.

**Checkerboard Titration**

The following materials were used in the all methods described here: ELISA microtiter plates (Nunc, Roskilde, Denmark); freshly prepared carbonate bicarbonate coating buffer (0.159% sodium carbonate and 0.293% sodium bicarbonate, pH 9.6); washing buffer (PBS pH7.2 supplemented with 0.05% Tween -20); blocking buffer (bovine serum albumin2%: pH7.2); TMB as substrate solution ([(3,3',5,5'-tetramethylbenzidine) 30 mg in 75mLDw (Sigma chemical Co.)]); stop solution (2.5M H2SO4); microplate ELISA reader (BIO-TEK, INC., ELx, 800UV Winooski, VT, USA) to measure the enzyme-mediated reaction at wavelength 492 nm; Horse Radish Peroxidase -conjugated sheep anti-rabbit IgG (product no. A-5279, Sigma co., St. Louis, U.S.A); and Horse Radish Peroxidase anti-camel-IgG (ALPHA DIAGNOSTICS INTERNATIONAL 4638 N Loop 1604 West, San Antonio, Texas 78249 USA). The best dilutions of both conjugates were obtained by the highest concentration which gave a strong positive signal. The best dilutions were 1:4000 and 1:3000 for sheep anti-rabbit IgG and anti-camel-IgG-conjugates respectively. Checkerboard titration was used to determine the ideal antigen dilution and test the potency of the prepared hyperimmune sera. Different dilutions of the tested anti sera were applied with serial dilutions of the tested antigen that was used to coat the ELISA plate and a negative control serum. Under the known dilution of conjugate and substrate, the serum dilution with the lowest concentration of antigen/well that produced a distinct signal was deemed the ideal situation and used in further testing. The antisera's ideal dilution was 1:16 when the potency of the rabbit antisera against the antigen (400 ng of protein) was tested. The volume of antigen used was determined by the potency of the prepared camel ovarian protein antisera in rabbit's curve. The equation displayed was $Y=0.01555X-0.1524$ with the $R^2$ value of 0.8643. The equation displayed from the standard log - dose response curve was $y=0.3215x-0.3285$ with $R^2= 0.923$.

**Estimation of OAB in Serum of Camel**

According to Esmailnejad et al. (2020), ovarian protein polyclonal antisera (OAB) was estimated in camel serum. In order to evenly distribute the protein in the pits, the ELISA microplate wells were coated with 100 µL/well camel ovarian proteins (400ng) diluted in carbonate bicarbonate buffer and incubated for two hours at 37°C in a shaking water bath. Adhesive tape was used to protect the plates from evaporation, and they were incubated at 4°C overnight to allow the solid phase to completely adsorb. The excess unbound ovarian proteins were eliminated by three washings with buffer. The remaining binding sites were blocked with 200µL/well of blocking buffer, and the plates were incubated for two hours at 37°C while being shaken. The plates were then washed three times and incubated for an hour at 37°C with shaking after covering with adhesive tape. For each well, 100µL of serially diluted tested serum samples were added. All the plates were filled with 100µL/well of the enzyme conjugate horseradish peroxidase labeled anti-camel IgG, which was then incubated for an hour at 37°C in a water bath that was shaking. A 100µL/well substrate buffer containing TMB was added to each well after the plates had been washed three times with washing buffer. The plates were incubated at room temperature in the dark for 7min. The reaction's distinctive coloring was found. A 50µL/well stopping solution was used to stop the reaction. At a wavelength of 492nm, the enzyme-mediated response was quantified using an ELISA reader for microplates.

**Estimated Parameters of ELISA**

ELISA is a diagnostic method frequently used in biological research to identify antibodies related to a particular antigen and distinguish between positive and negative results. Sex evaluations were performed on each sample using the newly designed In-House indirect

ELISA. Three are related to the cutoff value, two are related to the sample's initial dilution, and the last reading is related to the antibody index. Firstly, mean OD of cut off values of samples. A common formula for a cut-off value is as follows: cut-off value = a*X + f*SD (López-Ratón et al. 2014). Where a and f are two multipliers, X is the mean, SD is the standard deviation of independent negative control values. Second, mean calibrated concentration of cut off values that obtained from the standard dose response curve. Third, the mean dilution points at which the cut off values and was considered as antibody titer. A positive result is indicated if the titer of the tested samples is greater than the cut-off value, while a negative result is indicated if it is less than the cut-off value. Fourth, mean original dilution of samples that were serially diluted in an ELISA plate. Fifth, the average calibrated concentration of samples that were first diluted. Finally, according to Tabouret et al. (2001) and Bauer et al. (2002), the antibody index was determined for each sample based on comparisons with positive and negative control values using the subsequent formula:

Antibody index = (OD of sample–OD of negative control/ OD of positive control–OD of negative control) X 100.

**Estradiol 17β and CamFSH Determination:**

The evaluation of plasma Estradiol 17β and CamFSH intensities was achieved by using the purchased commercial ELISA assay Kits. The Estradiol 17β ELISA kit (Estradiol ELISA, 17β estradiol antigenic, monocent, lnc. 9025 Eton Ave, Ste C, Canoga Park, CA 91304, USA). The sensitivity of the test was stated at 8.7 pg/mL and the intra-assay CV was set as 9.2%, and the inter-assay variability CV was 8.5% with detection range of 21.23–571.44pg/mL. The CamFSH ELISA kit (Catalog No. SL 0022Cm, Sun Long Biotech, (www.sunlongbiotech.com) was used for assay FSH in the serum. There were 10% and 12% CVs for intra- and inter-assays, respectively. Assay range and sensitivity were 0.6ng/mL- 40 ng/mL and 0.1ng/mL respectively.

**Statistical Analysis**

Means and standard errors were used to represent the data values. A simple one-way analysis of variances (ANOVA) test was performed for each measured parameter. At $P<0.05$, which denoted a statistical difference, the Mann-Whitney test was utilized as post hoc analysis to compare the positive and negative control to other groups.

## RESULTS

The number of samples used in the current investigation are listed in Table 1. The results reported in Table 2 indicated the estimated ELISA assay parameters of the positive (rabbit hyperimmune sera) and negative (male camel serum) controls. Data showed that there were no significant variations between the positive and negative control in terms of the ODs of the cut-off value and calibrated concentrations at cut-off values. This similarity indicates that the ELISA method's cutoff settings were chosen correctly. A significant difference between negative and positive results was seen in the dilution at cut-off, original dilution's OD, and original dilution's concentration ($P<0.001$).

Table 3 data revealed that there were no significant variations between the examined groups in the ODs of the cut-off value and calibrated concentrations at cut-off values. This similarity indicates that the cutoff values used in each ELISA raw sample under study were chosen correctly.

The dilution at cut-off (antibody titer), ODs and concentration of antibodies related to ovarian protein antigen of original dilution of serum samples from camels with different cysts and in active ovaries were measured and presented in Table 3. Compared to a value of OD of original dilution (0.1355±0.0131) and concentration of original dilution (3.069±0.4156ng/100µL) in healthy she-camels, the values of Majaheem breed with follicular cyst were significantly higher than healthy she-camel (0.5165±0.0604 at $P<0.05$ and 82.83±5.663ng/100µL at $P<0.01$, respectively).

However, the antibody titer of Majaheem breed with luteal cyst was significantly higher than healthy control (1:384±80.95 vs. 1:11.20±1.960, $P<0.001$). Similarly, the ODs of the antibody titer in serum of Majaheem and Waddah breeds of camel with inactive ovary recorded significant difference (1:256.0±57.24, $P<0.05$ and 1:352.0±46.84, $P<0.001$, respectively) as compared to healthy she-camel respectively. In a similar manner, the value of OD of original dilution of serum samples of Waddah breed with luteal cyst and inactive ovary of both breeds were higher than healthy control with a statistically significant difference (0.5585±0.0737, $P<0.05$), (0.6457±0.1139, $P<0.05$), (0.6508±0.1025, $P<0.05$) and (0.8045±0.0694, $P<0.01$), respectively. On the other side, the concentration value of antibodies related to antigen ovarian protein of original dilution were 44.23±6.172, 36.79±7.779, 398.3±87.34 and 241.2±58.26ng/100µL ($P<0.05$), respectively than healthy control.

**Table 1:** Number of animals with follicles, luteal and hemorrhagic cysts and inactive ovary of two breeds of camels

| Ovary Breeds | Normal follicles | Follicular cysts | Luteal cysts | Hemorrhagic cysts | Inactive ovary |
|---|---|---|---|---|---|
| Mujahim | 7 | 6 | 8 | 13 | 5 |
| Waddah | 7 | 3 | 11 | 9 | 6 |
| Total | 14 | 9 | 19 | 21 | 11 |

**Table 2:** Estimated ELISA assay parameters of negative and positive controls

| Parameters | Negative/Positive control[1,2] | Mean±SE |
|---|---|---|
| Cut-off (OD) | 1 | 0.0410±0.0019 |
| | 2 | 0.0430±0.0010 |
| Concentration at | 1 | 1.427±0.1043 |
| cut-off (ng/100µL) | 2 | 1.430±0.010 |
| Dilution at cut-off | 1 | 1.667±0.3333 |
| | 2 | 341.3±85.330*** |
| OD of original | 1 | 0.0606±0.0099 |
| dilution | 2 | 1.120±0.0503*** |
| Concentration of | 1 | 1.631±0.1142 |
| original dilution | 2 | 393.50±10.500*** |
| ng/100µL) | | |

In the same parameter, values (Mean±SE) having asterisks are significantly ($P<0.001$) different from respective negative control (ANOVA-Kolmogorov-Smirnov test).

**Table 3:** Estimated ELISA assay parameters of camel serum samples

| Groups ELISA | Normal follicles | Follicular cyst | | Luteal cyst | | Hemorrhagic cyst | | Inactive ovary | | P values |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Majaheem | Waddah | Majaheem | Waddah | Majaheem | Waddah | Majaheem | Waddah | |
| Cut-off (OD) | 0.046± | 0.0982± | 0.133± | 0.1096± | 0.039± | 0.0402± | 0.041± | 0.0731± | 0.065± | 0.2543 |
| | 0.0226 | 0.028 | 0.034 | 0.0251 | 0.0011 | 0.0011 | 0.0030 | 0.0147 | 0.0141 | |
| Concentration at | 2.961± | 2.270± | 2.979± | 2.516± | 1.557± | 1.435± | 1.403± | 5.200± | 4.978± | 0.151 |
| cut-off (ng/100µL) | 0.5786 | 0.493 | 0.665 | 0.5110 | 0.0675 | 0.0094 | 0.0085 | 0.4394 | 0.3740 | |
| Dilution at cut-off | 11.20± | 341.3± | 170.7± | 384.0± | 21.33± | 105.1± | 192.0± | 256.0± | 352.0± | < 0.001 |
| | 1.960 | 85.33** | 42.67 | 80.95*** | 5.333 | 15.16 | 36.95 | 57.24* | 46.84*** | |
| OD of original | 0.1355± | 0.5165± | 0.3768± | 0.3917± | 0.5585± | 0.6457± | 0.2968± | 0.6508± | 0.8045± | < 0.001 |
| dilution | 0.0131 | 0.0604* | 0.0246 | 0.0767 | 0.0737* | 0.1139* | 0.0701 | 0.1025* | 0.0694*** | |
| Concentration of original | 3.069± | 82.83± | 19.55± | 22.82± | 44.23± | 36.79± | 14.56± | 398.3± | 241.2± | < 0.001 |
| dilution (ng/100µL) | 0.4156 | 5.663** | 1.685 | 0.4390 | 6.172* | 7.779* | 0.7100 | 87.34** | 58.26* | |
| Antibody index | 0.032± | 0.0568± | 0.010± | 0.1201± | 0.0819± | 0.2271± | 0.0750± | 0.2780± | 0.1603± | < 0.001 |
| | 0.0041 | 0.0032* | 0.0654 | 0.0064* | 0.0013* | 0.0365** | 0.0017 | 0.04321** | 0.00582* | |

Values (Mean±SE) bearing *, ** and *** are significantly different at P<0.05, P<0.01and P<0.001, respectively (ANOVA-Kolmogorov-Smirnov test).

**Table 4:** Estimated Estrogen and CamFSH assay parameters of camel serum samples

| Groups ELISA | Normal follicles | Follicular cyst | | Luteal cyst | | Hemorrhagic cyst | | Inactive ovary | | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Majaheem | Waddah | Majaheem | Waddah | Majaheem | Waddah | Majaheem | Waddah | |
| Estradiol 17β | 78.46± | 34.20± | 59.94± | 34.42± | 55.07± | 45.33± | 40.07± | 33.71± | 33.89± | <0.0001 |
| (pg/mL) | 0.847 | 0.491*** | 7.190 | 0.644*** | 8.778 | 6.346 | 4.363 | 0.3662*** | 0.413*** | |
| CamFSH (ng/mL) | 10.46± | 5.516± | 8.996± | 8.100± | 6.412± | 4.572± | 6.461± | 2.564± | 2.580± | <0.0001 |
| | 0.8758 | 1.006* | 1.197 | 1.803 | 1.228 | 0.348** | 0.944 | 0.821*** | 0.830*** | |

Mean±SE. In the same raw *, ** and *** are significantly different from apparently healthy at P<0.05, P<0.01and P<0.001, respectively (ANOVA-Kolmogorov-Smirnov test).

Concerning the breed type and ovarian lesion effect on the serum levels of antibody index, the data demonstrated a significant difference between the healthy she- camel and Majaheem breed with follicular and luteal cyst (P<0.05), Majaheem breed with hemorrhagic cyst (P<0.01) and Majaheem and Waddah breeds with inactive ovary (P<0.01 and P<0.05) respectively.

Results in Table 4 showed a significant decrease (P<0.001) in serum estradiol 17β levels comparing to normal control group of Majaheem breed with follicular and luteal cysts (34.20±0.6440pg/mL and 34.42±0.6440 pg/mL vs. 78.46±0.8474pg/mL respectively). Majaheem and Wahda breeds with inactive ovary showed a similar significant drop (P<0.001) in estradiol 17β levels compared to normal control group (33.710.3662pg/mL and 33.890.4132pg/mL vs. 10.460.8758pg/mL, respectively). However, levels of estradiol 17β in Waddah breed with follicular luteal and hemorrhagic cysts and Majaheem breed with hemorrhagic cyst were not significantly changed than that of healthy group.

Concerning the CamFSH levels, results in Table 4 showed a significant decrease in CamFSH levels comparing to normal control group in the serum of Majaheem breed with follicular and hemorrhagic cysts (5.516±1.006ng/mL, P<0.05 and 4.572±0.3481ng/mL, P<0.01vs. 10.46±0.8758ng/mL respectively). Majaheem and Waddah breeds with inactive ovary showed a similar significant drop (P<0.001) in CamFSH levels compared to the normal control group (2.5640.8216ng/mL and 2.5800.8309ng/mL, respectively). However, levels of CamFSH in Waddah breed with follicular, luteal and hemorrhagic cysts and Majaheem breed with luteal cyst were not significantly changed than that of healthy group.

## DISCUSSION

Data enumerated randomly the number of samples used with different ovarian structure in the present study without scientific solid scientific prevalence of these structures. Forty-nine she-camels with cystic ovaries and eleven with inactive ovaries in addition to fourteen showed normal follicular structure were used. Two breeds of female dromedary camels were chosen due to the majority admitted to the veterinary educational clinic of Qassim university KSA. During the mating season, proper food sources paired with ambient climate can increase ovarian activity, improve body condition scores, and maintain the ideal level of gonadotropins released from the anterior lobe of the hypophysis in she-camels (Ainani et al. 2018; Gherissi et al. 2020). Several cysts may develop in and around the ovaries, though some cysts are discovered by accident during an ultrasonographic check and others are linked to problems with fertility (Iliyasu et al. 2016). Cystic ovaries have been identified in the past with varying rates of incidence (Dawod and Hamed 2018). However, unlike in cattle or other domestic animals, dromedary cystic ovary problems are not as well reported. The most frequent ovarian abnormalities were follicular, luteal, and hemorrhagic cysts, and these abnormalities appear to contribute to ovarian dysfunction (Elshazly et al., 2018). It is generally recognized that follicular, luteal, and hemorrhagic cysts are developed normally in non-ovulatory follicles (functional cysts). These cysts suggest ovulation failure, which may be brought by insufficient LH release following copulation (Hamouda et al. 2011). A disturbance in the hypothalamo-pituitary system caused by an exogenous or endogenous component, as well as a decreased stimulatory effect of copulation, may be responsible for the absence or inadequate release of LH. Both follicular and luteal cysts can develop in the ovary of camels, however follicular cysts are more common (El-Badry et al. 2020). Ovarian follicular cysts are ovarian follicles that are excessively large on either one or both ovaries. It is a physiological variation of normal follicular dynamics that many scientists find troubling (Benaissa et al. 2015; Abd-

Elfattah et al. 2020). Follicular cysts are normally 3-5cm in diameter, but they can be larger. They usually contain a yellowish fluid, though they occasionally contain blood. A follicular cyst's growth may experience pathological changes that cause a hemorrhagic cyst, which is marked by rapid bleeding and a deposit of blood inside the cyst (Gherissi et al. 2022). On the surface of the ovary, luteal cysts are visible as a dark red mass filled with a pigmented, semi-coagulated discharge (Bekkouche et al. 2022). There have been no prior studies or findings linking immunological disruption to aberrant ovarian architecture in camels. The metabolic activity and "barrier" qualities of the follicular wall are changed dramatically during ovarian follicular growth. Infertility issues can be brought on by ovarian follicle production and persistence as well as disturbances in hormonal and metabolic balances as well as a variety of oxidative stresses (El-Badry et al. 2020).

In the current investigation, the connections between an autoimmune cause and a cyst were explored. Immunological data and levels of hormones showed that the dilution at cut-off (antibody titer), ODs and concentration of antibodies related to ovarian protein antigen of original dilution of serum samples of she-camels with different cysts were measured. The antibody titer of serum from Majaheem breed with follicular and luteal cyst were recorded significantly higher than healthy control. In a similar manner, the value of OD of original dilution of serum samples and the calibrated concentration value of antibodies of original dilution of serum from Majaheem camels with follicular cyst, Waddah breed with luteal cyst were higher than healthy control with a statistically significant difference. The data of antibody index demonstrated a significant difference between the healthy she- camel and Majaheem breed with follicular and luteal cyst, Majaheem breed with hemorrhagic cyst. Results showed a significant decrease in serum estrogen levels comparing to normal control group of Majaheem breed with follicular and luteal cysts. However, levels of estrogen in Waddah breed with follicular luteal and hemorrhagic cysts and Majaheem breed with hemorrhagic cyst were not significantly changed than that of healthy group. Regarding the FSH levels, the serum of the Majaheem breed with follicular and hemorrhagic cysts showed a significantly lower FSH level when compared to the normal control group. However, the level of FSH in the Majaheem breed with a luteal cyst and the Waddah breed with follicular, luteal, and hemorrhagic cysts did not differ substantially from that of the healthy group. We can infer that breed and lesion dependencies have a role in the emergence of OAB.

Ovarian activity in dromedary camels increases during the breeding season in the winter and spring and is influenced by plentiful food sources, favorable weather, and the physical condition of the female (Ainani et al. 2018; Gherissi et al. 2020). Paying attention is obviously necessary because ovarian inactivity may significantly affect infertility issues and interfere with reproductive effectiveness. There has not been much research done on the camel ovarian inactivity phenomena during mating season. Reduced gonadotrophin release and subsequent lack of folliculogenesis produce inactive ovaries. In this situation, neither developed follicles nor corpora lutea or its degenerative sequelae are present in the affected ovary. Lack of cyclicity may be brought on by malnutrition and prolonged, debilitating illnesses. However, some research is being done to better understand dromedary reproductive issues and increase reproductive effectiveness (Skidmore et al. 2005). The negative physical condition was given as the cause of the female camels' inactive ovaries during the breeding season (Gherissi et al. 2020). Female camels with inactive ovaries are frequently presented to the clinic with a history of multiple unsuccessful mating attempts or refusal to mate. Several camel experiments were carried out to trigger and track ovarian activity either outside or at the beginning of the mating season in dromedary camels, with different degrees of success (Quzy et al. 2013).

Serum samples from camels with inactive ovaries were obtained, and the antibody titer, ODs, and concentration of antibodies associated to ovarian protein antigen were determined immunologically. These measurements were significantly higher in Majaheem and Waddah breeds with inactive ovaries, than in healthy controls. Like this, the findings revealed a significant difference between the healthy she-camel and the Majaheem and Waddah breeds with inactive ovaries based on the value of the antibody index. Results demonstrated a significantly lower level of serum estrogen and FSH in Majaheem and Wahda breeds with inactive ovaries than in the normal control group. The findings of this study on hormone levels concur with those of most researchers, who found that pituitary gland activity was obviously lower in the case of inactive ovaries than it was in the case of active ovaries (Hegazy et al. 2004; Madhi et al. 2022). As seen in buffalo, the presence of inactive ovarian follicles, which have less LH receptors in the granule and sacral cells, may reduce the generation of follicular androgen, which then results in decreased estrogen production (AL-Jabri et al. 2019). The origin of this antibodies may be due to detected or undetected oophoritis and ovaritis that proceed the observed cyst and inactive ovary. Camel oophoritis has been documented in earlier times (Abd-El Wahab 1991). Ovaritis may result from several factors, including rough handling of the ovaries in an effort to rupture anovulatory follicles. Ovaritis, however, could have a microbiological origin as an infection rising from the uterus or develop over the course of a particular disease (Gwida et al. 2019; Belina et al. 2021).

Numerous ovarian pathologies have been connected to autoimmune pathways, including polycystic ovarian syndrome, unexplained infertility, recurrent miscarriages, endometriosis, and other conditions with poor reproductive consequences (Al-Naffakh and Risan 2020). Additional knowledge of the autoimmune effects of OAB will help to clarify the underlying processes of ovarian injury. Additionally, it will contribute to the improvement of diagnostic techniques and therapy regimens (Mahbod Ebrahimi et al. 2015). We further hypothesize that particular non-invasive diagnostics are necessary to identify concomitant diseases and to choose the camel with the best probability of regaining ovarian function and fertility with immunosuppressive medication. Finally, the values of OAB associated to ovarian lesions in dromedaries are presented in the current study objectively for the first time. To identify the true immunological

etiology of cystic and ovarian inactivity, polyclonal antibody testing alone would not be sufficient. Therefore, it is strongly advised to use complementary analyses in camels, including creating reference points and preparing monoclonal antibodies.

## Conclusion

In conclusion, camel immune system research moves slowly and only gradually acquires academic interest although the diagnosis of camel diseases frequently uses immunoassay or serological tests. The goal of recent research is to pinpoint the precise target antigens that could inspire common commercial testing. To identify antibodies, immunohistochemistry and immunoassay are used, and the test conditions and antigen preparation may have a little effect on the outcomes. Oophoritis and ovaritis are frequently referred to as inflammation of the oocytes and inflammation of the ovaries, respectively. Aside from the blood test for OAB, neither condition is clinically evident. However, the persistent oophoritis and ovaritis may be the cause of the significant results of the OAB in the current investigation of abnormal ovarian structure. The primary obstacles to the research are the lack of or poor production of the reagents necessary for a thorough examination of the immune system of the camel. The establishment of significant scientific communities and societies that support the study in this area and serve as forecasting bodies that contribute to the research viewpoint in this field are also necessary for the research in camel immune system to be significantly boosted.

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## Conflicts

There are no material financial or non-financial interests to disclose for the authors.

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