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## Research Article

# Prevalence of Non-Neoplastic Ovarian Disorders in Non-Pregnant She-Camels (*Camelus dromedaries*) and their Correlation to Bacteriological Isolation

MO Elshazly<sup>1</sup>, Sahar S Abd El-Rahman<sup>1\*</sup>, Dalia A Hamza<sup>2</sup> and Merhan E Ali<sup>1</sup>

<sup>1</sup>Department of Pathology; <sup>2</sup>Department of Zoonosis, Faculty of Veterinary Medicine, Cairo University, Egypt

\*Corresponding author: saaharsamirmah@cu.edu.eg; saaharsamirmah@hotmail.com

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### ABSTRACT

Reproduction is a key role of effective production of camel, thus healthy sex organs play important roles in successful reproductive functioning. Hence, the current study was undertaken to verify various pathological abnormalities in she-camel ovaries that may interfere with the reproductive efficacy. The ovaries of 500 she-camels were collected from three abattoirs in Giza governorate, Egypt during the period of January 2016 to January 2018. Both bacteriological and histopathological examinations were carried out for each abnormality. A total of 113 (22.6%) camels were found with a variety of ovarian pathological abnormalities included; cystic ovary (8.2%), paraovarian cyst (4%), ovario-bursal adhesion (1.2%), oophoritis (4%), brown pigmentation of interstitial cells (4.4%) and inactive ovary (1.2%).

**Key words:** Dromedary camel, Ovary, Histopathology, Bacteriology, Egypt

### INTRODUCTION

Camelids are considered as important animal's production resource in many areas of the world. The reproductive potency of Camelidae, notably dromedary camels, is usually thought-about low (Kaufmann, 2005). Decreased herd productivity could be primarily a result of genital tract diseases and consequently render rearing these animals uneconomical (Mutebi, 2006). The forms and incidence of reproductive disorders in Camelidae livestock have been mentioned by limited studies. Hence, scarcity of scientific publications regarding that issue in camels has pushed scientists to give more attention on research and broaden the knowledge about this species.

Depending on the nature and extent of the abnormality, ovarian lesions could be either unilateral or bilateral and may be associated with varying degrees of infertility. It was reported that ovarian malformations are rare among small ruminants but usually occur in camels but in association with diseases of other organs of the reproductive system (Jones *et al.*, 1997).

They are usually described among freemartins and other forms of intersexes in large ruminants (Buergelt, 2000). Ovario-bursal adhesions and cystic degeneration in ovaries are among the ovarian abnormalities that are responsible for considerable number of long-standing infertility problems in farm animals including she-camels

(Musa, 1983; Tibary and Anouassi, 2001; Kubar and Jalakas, 2002).

In non-pregnant dromedary camels, the ovaries are oval, flattened and lobulated organs. The ovarian content of follicles and corpora lutea determines the size and shape of the ovaries (Yagil and Etzion, 1980). The irregularity of the ovarian surface is fundamentally due to the presence of numerous small follicles or the presence of corpora lutea. However, the ovarian color ranges from light red to pink of variable intensities. The age and size of the animal as well as the stage of the reproductive cycle determine the size and weight of the ovaries (Yagil and Etzion, 1980). Generally, camel's ovary measures about 3 cm in length, 2 cm in width and less than 1 cm in thickness (Abdalla, 1965), however, slight variations from these measurements were reported (Arthur *et al.*, 1985). Regarding the weight of the ovary, it depends mainly on its contents; the non-functioning ovary weighs about 4 to 5 g (Iliasu *et al.*, 2016). Ovarian inactivity occurs as a result of decreased release of gonadotrophins and subsequently failure of folliculogenesis (Arthur *et al.*, 1983). In such case, the affected ovary lacks presence of mature follicles and corpora lutea or its degenerative derivatives. Malnutrition and chronic debilitating diseases could be a cause of lack of cyclicity (Morrow, 1986). In addition, ovarian quietness is associated with weight loss which influences the secretion of ovarian hormones (Tanaka *et al.*, 2003).

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Pathological examination of the ovaries provides definite information which is more accurate than that obtained by clinical or other para-clinical examinations. Hence, this research was designed to detect the probable ovarian abnormalities in she-camels (*Camelus dromedaries*) breed in Giza, Egypt with the purpose to identify the common ovarian abnormalities that could interfere with the reproductive performance or directly responsible for infertility among camels. In addition to a trial to identify the probable causative agent that may be incorporated with such pathological abnormalities.

## MATERIALS AND METHODS

### Animals

Surveyed camels were slaughtered for human consumption at El-basateen, Elmonib and Kerdasa abattoirs, Giza, Egypt, during the period from January 2016 to January 2018. The ovaries of 500 non-pregnant one-humped she-camels, aged between 6-15 years old were collected and examined. The breeding history of those animals was unknown. This protocol was approved by the Institutional Animal Care and Use Committee at Cairo University (IACUC), CUIIFC3118.

### Examination procedures and sampling

The collected ovaries were thoroughly examined visually and by palpation for any abnormalities. Specimens from all of the collected ovaries were collected and fixed in 10% buffered neutral formalin, another specimen from each ovary was kept frozen at -20°C in sterile package under aseptic condition for bacteriological examination. Aspiration of fluid from cases of cystic ovary and paraovarian cysts was carried out using 5ml sterile syringe for bacterial isolation.

### Histopathological examination

Formalin fixed tissue specimens were routinely processed, dehydrated in series of alcohol, cleared in xylene, embedded in paraffin and then sectioned at 4-6 µm thickness. Sections were routinely stained with hematoxylin and eosin (H&E) according to Bancroft and Gamble (2008). The collected ovaries were sorted into normal and abnormal according to the gross and histopathological evaluations, and the observed lesions were arranged on the basis of gross and microscopic examinations.

### Bacteriological examination

#### Isolation and identification of Enterobacteriaceae

Specimens collected for bacteriological examination were inoculated directly into Trypticase soya broth and incubated at 37°C for 24 hrs. A loop-full of the previously inoculated broth was cultured onto MacConkey agar (Oxoid) and Eosin methylene blue agar (EMB) (Oxoid). Morphological, cultural and biochemical characters were examined according to Brenner and Farmer (2005). RapID™ONE (Remel), Oxoid, test was used for identification of various members of the family Enterobacteriaceae according to manufacturer's instructions.

#### Isolation and identification of *Pseudomonas aeruginosa*

The collected specimens were inoculated directly into Trypticase soya broth and incubated at 37°C for 24 hrs. A

loopful from the previously incubated broth was inoculated on *Pseudomonas* agar base containing (C-N) supplement (oxoid) and incubated at 37°C for 24-48hrs. Further microscopic and biochemical identification were done according to Brenner and Farmer (2005). Biochemical identification using API 20NE, (Analytical profile index) (bioMérieux) was carried out according to manufacture pamphlet.

#### Isolation and identification of *Salmonella*

As a pre-enrichment step; buffered peptone water 225 ml was inoculated with the test portion 25 g sample, and then incubated at 37°C for 18 hrs. followed by enrichment step on Rappaport-Vassiliadis medium with soya (RVS broth) that inoculated by 0.1 ml of the pre-enrichment broth and incubated at 41.5°C for 24 hrs. A loopful from the Rappaport broth was plated on the Xylose lysine deoxycholate agar (XLD agar) (Brenner and Farmer, 2005).

#### Isolation and identification of *Staphylococcus aureus*

All samples were pre-enriched in brain heart infusion broth and were incubated for 24 hrs. at 37°C, and then each broth was spread onto a dry surface of Baird parker agar plate and incubated at 37°C for 48 hrs. The purified *S. aureus* isolates were identified using different biochemical tests; catalase test and coagulase test (tube test) (Quinn *et al.*, 2002) as well as using Staphylect Plus kits.

#### Isolation of *Bacillus* species

Ten grams of sample were aseptically transferred into a sterile blender flask containing 90ml of sterile peptone water followed by homogenization for 2 minutes, 0.1 ml was evenly spread over the dry surface of duplicate plates of dextrose tryptone agar medium. The inoculated and control plates were incubated at 55°C for thermophilic bacteria and at 32°C for mesophilic bacteria for 48 hours. (Varnam and Evans, 1991).

#### Isolation of *Candida albicans*

One gram from each sample was suspended in a sterilized glass bottle containing 99.0 ml of sterile physiological saline (0.85% NaCl) supplemented with chloramphenicol (10.0 mg/ml). The mixture was left at room temperature for about 10-15 min to complete dissolving of dropping, and then shaken vigorously for 4-5 min. The bottle was left for complete precipitation. From the supernatant fluid of each prepared sample, a loopful was streaked onto plates of Sabouraud dextrose agar with chloramphenicol and incubated at 30°C for 48 hrs. *Candida albicans* species were phenotypically identified by their color and microscopic morphology on Sabouraud dextrose agar (Elias *et al.*, 2009).

#### Statistical Analysis

Statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA) to determine the seasonal relationship with the incidence of ovarian abnormalities. Different values were compared using one-way ANOVA test followed by Fisher's least significant difference (LSD) test ( $P > 0.05$ ). A p value of less than 0.05 was considered statistically significant.

**RESULTS**

The overall incidence of pathological lesions observed in she-camels' ovaries were (22.6%) as presented in (Table 1 and Fig. 1). The ovarian abnormalities were more pronounced in summer than other seasons of the year as demonstrated in Fig. 2.

The non-neoplastic lesions included; cystic ovaries (polycystic ovary, follicular cyst, follicular cyst with partial luteinization, luteal cysts and hemorrhagic cyst), paraovarian cysts, oophoritis, brown pigmentation of interstitial cells and inactive ovary. Most of these abnormalities were diagnosed on microscopic examination except for cystic ovary, paraovarian cyst, inactive ovary which could be diagnosed on PM examination and microscopic examination as well.

**Gross and histopathological findings**

**Polycystic ovary**

Polycystic ovaries were detected in two cases (0.4%). They were unilateral and characterized by presence of multiple cysts on the ovarian surface with a grape like appearance (Fig. 3A). Histologically, they were multiple large dilated and distorted follicular cysts, mostly without ova, distributed throughout the ovarian cortex (Fig. 3B). Those dilated cysts exerted pressure that compressed the surrounding cells and tissues of the ovarian parenchyma. Sometimes, the lining granulosa cells appeared detached and scattered throughout the eosinophilic fluid within the follicles.

**Follicular cysts**

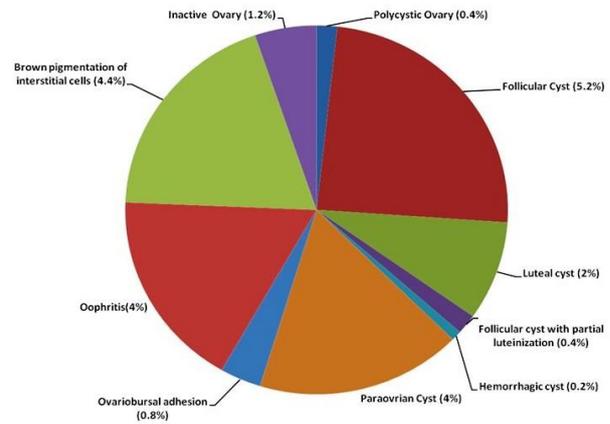
Follicular cysts were seen in 26 cases (5.2%). Grossly, all cysts were thin walled. The wall was semitransparent, well vascularized in most cases and slightly opaque with little vascularization in few ones. Cysts were unilateral in eighteen cases and bilateral in eight cases. They appeared either solitary or multiple cysts on the ovarian surface (Fig. 3C), They were filled with a straw yellow colored fluid. Microscopically, the ovum and the surrounding cells were completely degenerated or mostly absent with presence of faint eosinophilic fluid in the center of the cyst. The wall of the cyst was lined by 1-3 layers of compressed or degenerated granulosa cells (Fig. 3D).

**Follicular cyst with partial luteinization**

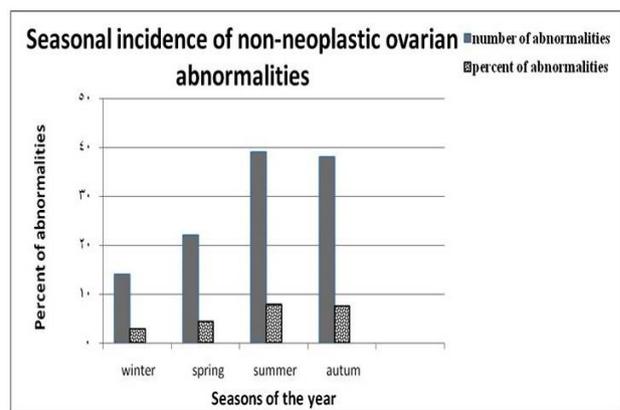
It was observed in only two cases. Microscopically, the cyst had pale eosinophilic fluid in the center with absence of ovum, the wall consisted of three to four layers of degenerated granulosa cells admixed with some clear vacuolated polygonal lutein cell.

**Luteal cyst**

It was observed in 10 cases (2%). Grossly, these cysts were thick walled, opaque and grayish yellow in color. They were unilateral in all cases. Histologically, the cyst wall consisted of multiple layers of lutein granulosa cells, at which the granulosa cells showed vacuolization and luteinization forming granulosa lutein cells which appeared polyhedral with large vesicular nuclei and vacuolar cytoplasm (Fig. 3E). The cysts were surrounded by thick fibrous capsule.



**Fig. 1:** Prevalence of various ovarian pathological lesions detected among camels.



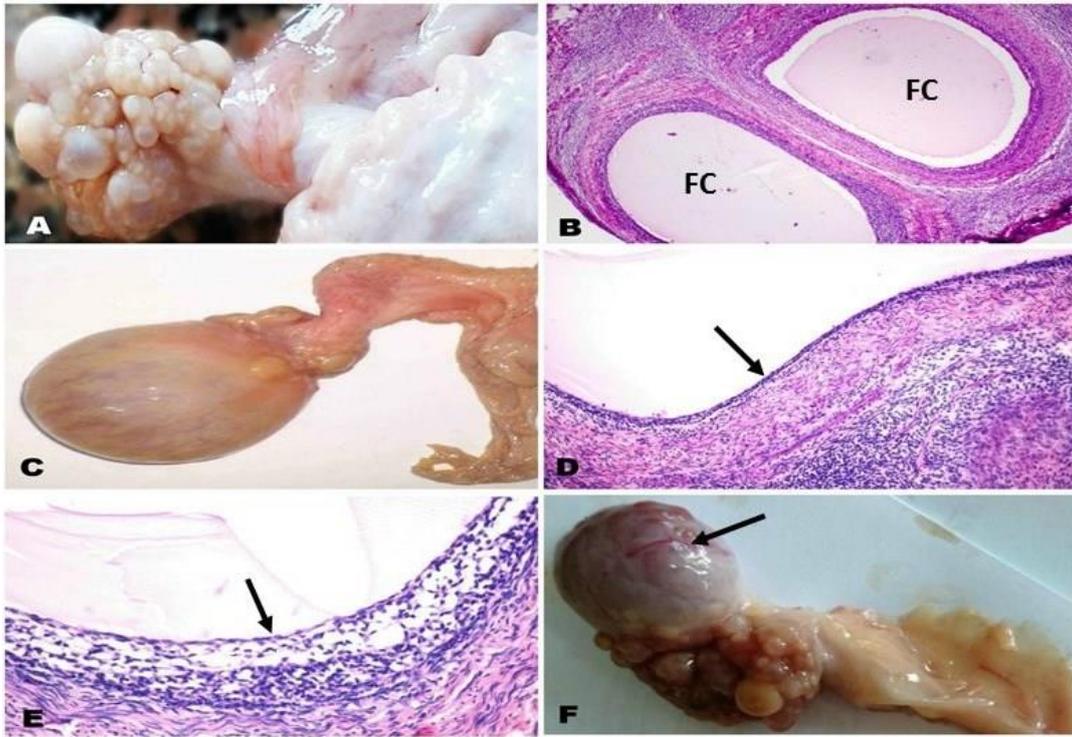
**Fig. 2:** Seasonal incidence of various ovarian abnormalities detected among camels.

**Table 1:** Incidence of various pathological lesions observed in the ovaries of she-camels.

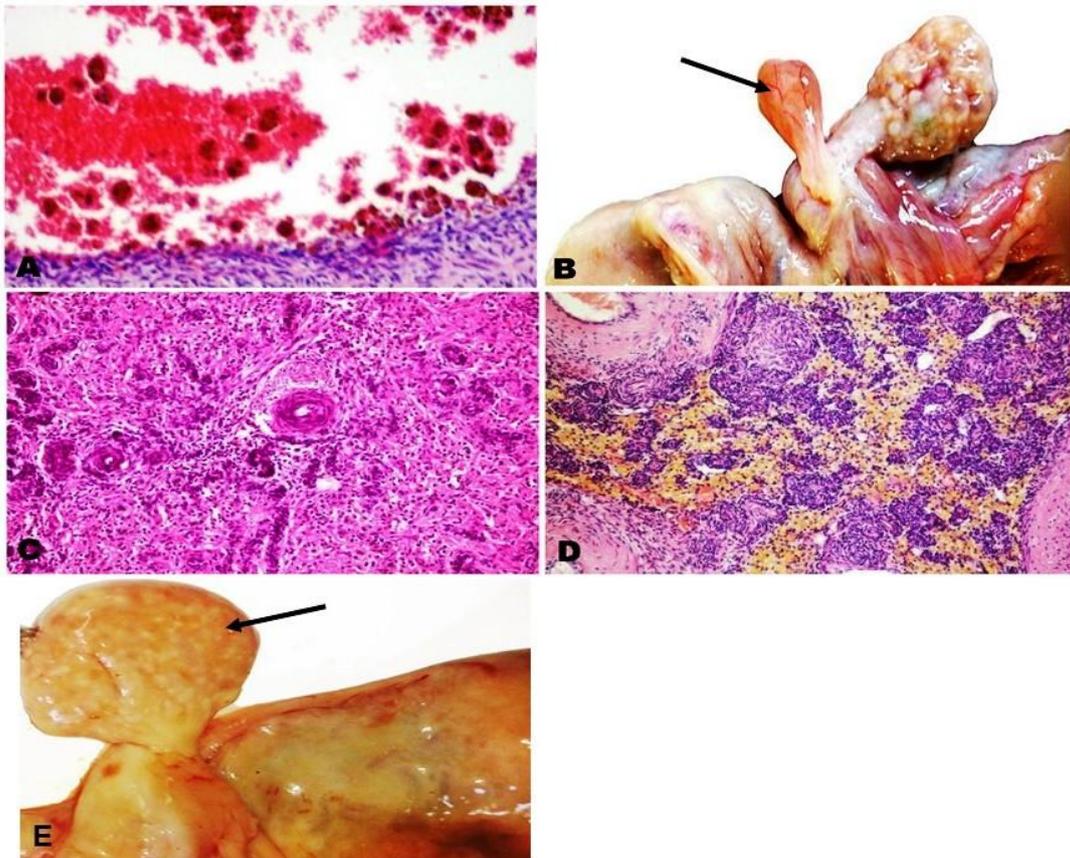
The observed lesions	Number of affected animals	Percentage from examined cases (500) (%)
Cystic ovary (total)	41	8.2
Polycystic ovary	2	0.4
Follicular cyst	26	5.2
Follicular cyst with partial luteinization	2	0.4
Luteal cyst	10	2
Hemorrhagic cyst	1	0.2
Paraovarian cyst	20	4
Ovario-bursal adhesions	4	0.8
Oophoritis	20	4
Brown pigmentation of interstitial cells	22	4.4
Inactive ovary	6	1.
Total	113	22.6

**Table 2:** The prevalence of the isolated microorganisms.

Isolated microorganism	Number of isolates	Incidence of each isolate (%)
<i>S. aureus</i>	23	34.8
<i>E.coli</i>	20	30.3
<i>Klebsiella pneumoniae</i>	6	9.09
<i>Enterobacter</i>	6	9.09
<i>Candida albicans</i>	6	9.09
<i>Pseudomonas aeruginosa</i>	5	7.57
Total	66	58.4



**Fig. 3:** Ovaries of She-camels showing; Polycystic ovary; composed of grape-like multiple cysts on the ovarian surface (A) and multiple large dilated follicular cysts (FC) without ova distributed throughout the ovarian cortex (B). (C) Follicular cyst with semitransparent well vascularized wall that lined by compressed and degenerated granulosa cells (arrow). (E) Luteal Cyst lined by granulosa lutein cells (arrow)with large vesicular nuclei and vacuolar cytoplasm. (F) Hemorrhagic cyst (arrow) showing thick highly vascular wall resembling hematoma (H&E, X200).



**Fig. 4:** Ovaries of She-camels showing; (A) The antral fluid of hemorrhagic cyst is replaced by a large amount of hemorrhage. (B) Paraovarian cysts, thin transparent wall and contained a clear yellowish serous. (C) Oophoritis; the ovarian medulla is highly infiltrated by inflammatory cells. (D) Brown pigmentation of interstitial cells in ovary with normal histological structure. (E) Inactive Ovary showing no follicular structure on the ovarian surface. (H&E, X200).

### Hemorrhagic cyst

It was observed in one case (0.2%). The cysts had thick highly vascular wall resembling hematoma (Fig. 3F). In cut section, brownish material occupied the antral cavity. Histologically, the antral fluid was replaced by a large amount of hemorrhage (Fig. 4A). The cyst wall was composed of a thick connective tissue capsule.

### Paraovarian cysts

Twenty cases (4%) with paraovarian cysts were noticed. By naked eye, the cysts were fluctuating, spherical, oval or irregular, freely mobile and contained a clear yellowish serous fluid with thin transparent wall (Fig. 4B). They were unilateral in ten cases and bilateral in the other ten. The cysts were either attached to the mesovarium or the mesosalpinx. Histologically, the cysts were lined with one layer of cuboidal or flattened epithelium and surrounded by a thin layer of connective tissue.

### Ovario-bursal adhesions

It was observed in 4 cases (0.8%). Such ovaries were firmly adhered to the bursa and involved the corresponding fallopian tube as well. The site of adhesions consisted of fibrous bands. Sometimes, fibrous threads were found between the outer surface of the uterus, broad ligaments and the surrounding structures.

### Oophoritis

Oophoritis was diagnosed in twenty cases (4%), only during microscopic examination. The ovarian medulla was congested and infiltrated by inflammatory cells including macrophages and lymphocytes, sometimes in a focal manner and of variable degrees of severity (Fig. 4C). The blood vessels in the vicinity showed edema and hyalinization of their walls. The ovarian cortex revealed presence of antral and atretic follicles. A yellowish to yellowish brown pigment comparable with lipochrome pigment was observed in ten cases. It was distributed mostly in the ovarian medulla (inflamed area), either free or engulfed in the cytoplasm of macrophages.

### Brown pigmentation of interstitial cells

It was observed in 22 cases (4.4%). As previously mentioned, it accompanied oophoritis in ten cases and was observed in ovaries of twelve cases with normal histological architecture (Fig. 4D).

### Inactive Ovary

It was diagnosed in six she-camels' ovaries (1.2%). It was bilateral, the affected ovaries were very small, oval shaped, with almost smooth surface. No follicles were seen neither by naked eye nor palpated externally (Fig. 4E). Microscopically, scarce or complete absence of the follicles was a remarkable finding accompanied by excessive proliferation of stromal cells and fibroplasia.

### Microbiological examination

The samples which were sent for microbiological examination were only for those ovaries which showed pathological abnormalities. The ovarian samples of 113 cases yielded 66 isolates with a percent of 58.4% (Table 1). The most prevalent isolated organisms were; *S. aureus*

(34.8%) and *E.coli* (30.3%), followed by *Klebsiella pneumoniae* (9.09%), *Enterobacter* (9.09%), and *Candida albicans* (9.09%), while the least prevalence was for *Pseudomonas aeruginosa* (7.57%). Cases of luteal cyst, oophoritis, inactive ovary and the aspirated fluid of follicular and paraovarian cysts were positive for the bacterial isolation.

## DISCUSSION

The present study revealed that the total incidence of non-neoplastic ovarian affections was 22.6% of all of the examined ovaries, that incidence is considered high compared with previous results obtained in Egypt by Shawky *et al.* (2004). That difference could be attributed to nutritional, seasonal and genetics causes (Al-Afaleq *et al.*, 2012).

The current study revealed that the highest prevalence of ovarian abnormalities was in summer. This is fairly consistent with Hussein *et al.* (2008); Iliasu *et al.* (2016), who mentioned that cystic ovarian degeneration and inactive ovary reached their peak in hot season that could be attributed to the higher temperature associated with adverse nutritional status of animal during the summer season. But in contrary with Hamouda *et al.* (2011) who mentioned that there was no effect of season on ovarian pathological affection in Saudi Arabia. This could be imputed to the fact that she-camels are seasonal breeder. Seasonal variation of reproductive disorders in ruminants could be related to seasonal or non-seasonal reproduction pattern (Benaissa *et al.*, 2015).

The present work disclosed that the non-neoplastic abnormalities constituted 22.6%. Cystic ovarian diseases encountered the highest non-neoplastic ovarian abnormality (8.2%) followed by brown pigmentation of interstitial cells (4.4%), paraovarian cyst (4%), oophoritis (4%), inactive ovary (1.2%), and finally ovario-bursal adhesion (0.8%). These lesions were more or less in agreement with Ali *et al.* (2010); Hamouda *et al.* (2011); Al-Afaleq *et al.* (2012); Wajid (2015).

Cystic ovarian degenerations in the present work were of several types included; polycystic ovary (0.4%), follicular cyst (5.2%), follicular cyst with partial luteinization (0.4%), luteal cyst (2%) and hemorrhagic cyst (0.2%), those types were similar to those reported by Chen *et al.* (1983) and Elwishy (1990). Polycystic ovary has been reported in goat (Pawaiya *et al.*, 2004), animals with polycystic ovaries usually tend to be infertile or sterile, this is because polycystic ovary could induce disturbances in the hypothalamo-hypophysio-gonadal system that consequently leads in irregular estrous cycle as a result of high level of estrogen and finally ends in reproductive failure (Salveti *et al.*, 2004).

In regard to follicular and luteal cysts, their incidences were higher than that reported previously in Egypt by Elwishy (1990) but were nearly similar to that reported by Shawky *et al.* (2004) who recorded 5.2% and 1% for follicular and luteal cysts respectively. Both follicular and luteinized cysts were of major clinical significance and constituted the lump of cystic ovarian diseases (Morrow, 1986; Maclachlan and Kennedy, 2002). Generally, in she-camels' various cysts may develop in and around the ovaries, although some cysts are incidental

findings at postmortem examination; others are associated with fertility disturbances (Iliasu *et al.*, 2016). Follicular, luteal and hemorrhagic cysts are well-known as normal evolution of non-ovulatory follicles (functional cysts). The presence of these cysts indicates ovulation failure which may be caused by inadequate LH release in response to copulation (Skidmore *et al.*, 1995). That lack or insufficient LH release could be due to a disturbance of hypothalamo-pituitary function by exogenous and/or endogenous factor or as an after reduced stimulatory effect of copulation. The incidence of hemorrhagic cyst in the present work was lower than that reported by Shawky *et al.* (2004) (1.4%) in Egypt and Hamouda *et al.* (2011) (1.33%) in Saudi Arabia. Hemorrhagic cyst could be a consequence of some pathological changes during growth of a follicular cyst resulting in quick bleeding with accumulation of blood within the cyst forming a hemorrhagic cyst (Shawky *et al.*, 2004). *Staph aureus* was isolated from the aspirated fluid of the follicular cyst of thirteen cases while *Candida albicans* was isolated from ovaries with luteal cyst in six cases.

Twenty cases of paraovarian cysts were detected with an incidence of 4%, which is nearly like those mentioned by Fetaih (1991) and Shawky *et al.* (2004) who reported that, the incidence of paraovarian cysts in she-camels was 2.10% and 1.8% respectively. Meanwhile higher incidence (8.02%) was recorded by Shalaby (1986) and lower incidence (0.22%) was reported by Omar *et al.* (1984). Paraovarian cysts are suspected to arise from persistent embryonal structures which are vestiges of Wolffian ducts. Additionally, paraovarian cysts are known to be derived from mesonephric tubules, paramesonephric ducts, uterus and the mesosalpinx (Maclachlan and Kennedy, 2002). Alam (1984) has clarified that the presence of paraovarian cysts in cows does not interfere with reproductive performance until compression of the lumen of the oviduct occurs. Bacterial isolation of the aspirated fluid of ten cases revealed isolation of *Staph aureus*.

The prevalence of ovario-bursal adhesion in our study constituted (0.8%) which was nearly similar to that (0.5%) reported by Hamouda *et al.* (2011). Ovario-bursal adhesions could be a result of hemorrhage produced by harsh manipulation during examination of the ovaries or during attempts to rupture anovulatory follicles, or may be a consequence of oophoritis, and leading to ovarian hydrobursitis and peritonitis (Tibary and Anouassi, 2001).

Oophoritis was previously recorded in camels (Abd-El Wahab, 1991), in the current work, it was observed in twenty cases. Several causes could be incorporated in the occurrence of ovarian inflammation such as harsh manipulation of the ovaries in attempts to rupture anovulatory follicles. However, ovarian inflammation could be of microbial cause, as an ascending infection from uterus or arise during the course of specific disease such as tuberculosis, brucellosis and campylobacteriosis (Tibary and Anouassi, 1997). In our work, bacteriological investigation in cases of oophoritis revealed isolation of *Pseudomonas aeruginosa* from five cases, *E.coli* from ten cases and mixed *Klebsiella* and *E.coli* from five cases. This result is more or less agreed with Arthur *et al.* (1985) who found that the main reproductive disease and abortion in Saudi camels were due to *Salmonella* species

(spp), *Proteus* spp, *Escherichia* spp, *Serratia* spp, *Klebsiella* spp, *Pseudomonas* spp., *Streptococcus* spp., *Staphylococcus* spp. and *Corynebacterium* spp.

Regarding the observed brown pigmentation of interstitial cells that was either accompanying other lesions or not; yellow to brown pigment could be aging change. Generally, this brown pigmentation may be due to accumulation of lipofuchsin, hemosiderin or ceroid. Administration of compounds that interfere with steroid synthesis may result in accumulation of lipofuchsin, while, accumulation of hemosiderin and hematoidin may be associated with vascular lesions resulting in hemorrhage into follicles or corpora lutea. On the other hand, ceroid is the pigment that the most frequently seen, mainly accumulates in the cytoplasm of interstitial cells (Montgomery and Alison, 1987). Inactive ovary was observed in six cases with a percent of (1.2%) which is lower than that reported by Ali *et al.* (2010) (3.6%) in Saudi Arabia. This problem is not fully investigated in Camelidae as in the other domestic animals (Wajid, 2015). Bacterial examination of these cases revealed isolation of *Enterobacter* and *Klebsiella pneumoniae* from one case and *Enterobacter* and *E. coli* from the remaining five cases. Bacterial infection to the genital tract or even bacterial products, both have adverse effect on the reproductive function of farm animals by disturbing the ovarian follicular growth function and ovulation via suppressing pituitary LH secretions (Monghaddam and Mamoei, 2004).

## Conclusions

Ovarian abnormalities could interfere with reproductive efficacy and may constitute important infertility problems, and of course paying attention is a must. In addition, ovarian cysts were the most common observed non-neoplastic ovarian disorders in she-camel' ovary in Egypt, followed by paraovarian cysts, oophoritis, inactive ovary and finally ovario-bursal adhesion. Most of the isolated pathogens could be of zoonotic importance during ovarian examination.

## Author contributions

All authors contributed to the reagents/ materials/ analysis tools, collected the material, analyzed the data and wrote and revised the manuscript.

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