Pathological Findings on Genital Abnormalities in Female Camel in the El Oued Region, Algeria

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Abstract

The present study was performed to give a detailed histopathological description of genital abnormalities found in normal, clinically healthy female camels. A total of 165 apparently healthy female camels were randomly selected at the El Oued slaughterhouse at the south east of Algeria. Histopathological analysis was carried out on 25 pathological formations of ovaries (follicular, haemorrhagic and luteinized cysts and teratoma), uterine infections (clinical, chronic and pyometra), uterine serosal inclusion cyst, uterine agenesis and ovarian infundibular cyst, by establishing elementary lesions related to each macroscopic condition. A significant impairment of normal structure of each studied reproductive organ was recorded. The prevalence of animals showing genital abnormalities was 15%. The frequency incidence of ovarian lesions was ranked first (56%) followed by uterine affections (28%) and finally ovarian bursa abnormalities with four cases (16%). The incidence of genital conditions was as follows: follicular cysts (28%), haemorrhagic cysts (16%), hydrobursitis (16%), chronic endometritis (12%), luteinized follicular cysts (8%), clinical metritis (4%), pyometra (4%), dermoid cysts (4%), uterine agenesis (4%) and uterine serosal inclusion cysts (4%). The main lesions of the uterine infection were congestions, oedema, endometrial epithelium and glandular degeneration and infiltration by inflammatory cells. Uterine agenesis was characterised by a lack of endometrial glands and hyalinization of the myometrium. The mean lesions for the infundibular cyst were congestion, haemorrhage and hemosiderophages, infiltration by inflammatory cells, endometrial degeneration and vacuolation and pseudo-glandular dilations. The ovarian dermoid cyst showed a keratinized and scaly epithelium housed in fibrous connective tissue containing hair follicles, and sebaceous and sweat glands. The ovarian cysts showed thin or enlarged granulosa and internal theca with luteinization or vacuolation of the antral cavity. Finally, serosal inclusion cyst of the

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uterus appeared as dilation between the myometrium and the perimetrium with homogenous content. Concerning the above, the reported elementary genital abnormalities are indicated as responsible for the delayed reproduction, infertility and unsatisfactory camel livestock outcomes.

Key words: *Dromedary camel; Follicular cyst; Uterine Infection: Hydrobursitis; Uterine agenesis*

Introduction

The incidence and prevalence of the genital pathologies in Algerian camel herds (Benaissa et al., 2015; Gherissi et al., 2018a) and worldwide (Ali et al., 2017) reflects their prejudices on the reproductive performance and productivity of camel herds. Most of these disorders are usually associated with infertility due to repeat breeding syndrome, early embryonic deaths, foetal deaths and abortions (Gherissi et al., 2020b; Ali et al., 2021). Data on the pathogenesis and treatment of reproductive disorders of camels are very limited and diagnosis in this species is usually approached in the same way described for cows and mares.

The standard genital tract examination through history, loco-regional inspection, physical examination, palpation and/or ultrasonography and vaginoscopy provides useful information (Barlund et al., 2008; Hanzen, 2015; Ali et al., 2017). However, this routine clinical examination is often insensitive, non-specific and does not provide accurate information on fertility defects (Hanzen, 2015). Nerveless, genital biopsy constitutes an important confirmatory diagnostic method after the standard approach (Barlund et al., 2008). In cattle, histopathological examination is considered the gold standard to directly visualize both acute and chronic alterations (Garoussi et al., 2010). It provides information in different levels of epithelial disruption associated with reduced reproductive performance and has the best correlation with other complementary diagnosis methods (Pascottini et al., 2016; Casarin et al., 2018). This complementary examination finds its main application in the equine species (McCue 2014; Rua et al., 2018). In camelids, Tibary et al. (2001) reported the particular interest of this diagnosis method for infertile females. The objective of this study was to identify the elementary lesion modifications induced by the various pathologies of the genital tract of female camels (*Camelus dromedaries*).

Materials and methods

Study region

The survey was conducted in the El Oued region situated in the extreme arid region of the southeastern of Algeria (N33°5′ E6°11′, average altitude 80 m, average annual temperature 25°C, and mean annual precipitation 80 mm).

Sampling

The study was conducted between February 2013 and August 2014 on 165 randomly selected female dromedary camels with normal clinical condition. Among them, 25 females showed underlying macroscopic anomalies of their genital tracts which were sampled and analysed by microscopy. They studied animals were pubertal and non-pregnant with body condition score ranging from 2 to 4 on a scale of 5 (Faye et al., 2001), and the reproductive history was unknown. They were raised through a pastoral system and subjected to environmental local conditions. Female camels undergo an

ante-mortem inspection and external examination with great attention to search any loco-regional signs (Hanzen, 2015).

Incidence of genital abnormalities

For each genital pathological condition, the incidence was calculated as the percentage of affected cases (Fig 1).

Macroscopic study

After slaughter, the genital tract was separated carefully from the pelvis and surrounding structures. A standard genital examination was adapted for an *in vitro* diagnosis (Hanzen, 2015). Macroscopic examination was performed thorough inspection and palpation in progressive ascending sequence (vulva, vagina, cervix, uterus, oviducts and ovaries). Genital tracts with macroscopic abnormalities were sampled according the method described by Slaoui and Fiette (2011). Specimens of 2–3 cm^3 were taken from different genital parts, and kept in 10% neutral buffered formalin until histopathological examination.

Histopathological evaluation

The preparation of histological samples was performed in the Veterinary Anatomy and Pathological Cytology Laboratory of the Pasteur Institute of Algiers according to the procedures described by Gherissi et al. (2018b). All specimens fixed in formalin were dehydrated, embedded in paraffin wax and sectioned on microtome at thickness 5 μm then stained by haematoxylin and eosin (H&E) stain. Microscopic slides were examined under light microscope at magnification of 100x, 400x and 1000x oil lens for different lesions to recognize the main varieties of pathological processes: cellular, vascular, immune, chronic or acute inflammation and neoplastic. Different histopathological descriptions were verified by a board-certified pathologist.

Ethical statement

The camels used in this study had their origin on pastoral farms with a traditional management system. They were intended for the production of meat for human consumption. Slaughter conditions correspond to recommendations issued by two orders (August 1, 1984 and July 15, 1996). With the purpose of obtaining effective data for the trial, animals were submitted to ante-mortem, slaughter and post-mortem practices standardized and regulated by international guidelines for animal welfare (Terrestrial Animal Health Code 2018, section 7. Art 7.5.1) and national executive decree No. 95-363 of November 11, 1995 (Algeria).

Results

Incidence of genital abnormalities

Figure 1 shows the incidence of genital tract abnormalities sampled for histopathological investigation. Macroscopic examinations found 25 (15%) affected genital tracts. Ovarian lesions were most common (56%) followed by uterine conditions (28%) and finally ovarian bursa anomalies with four cases (16%). The incidence of genital conditions was (Fig 1): follicular cysts (28%), haemorrhagic cysts (16%), hydrobursitis (16%), chronic endometritis (12%), luteinized follicular cyst (8%), clinical metritis (4%), pyometra (4%), dermoid cyst (4%), uterine agenesis (4%) and uterine serosal inclusion cyst (4%).

Follicular cysts

Histological section on follicular cysts showed a total absence by degeneration of the cumulo- oocyte complex. The granulosa was formed of 1 to 3 cell layers. The Slavjanski membrane was preserved and the layers of the inner and outer theca pronounced (Fig 2a, 3a).

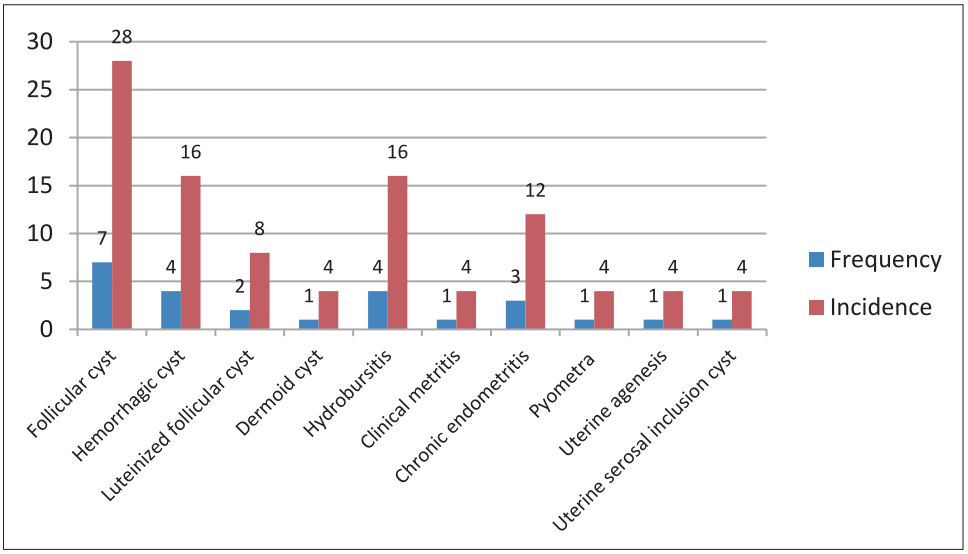

Figure 1. Incidence (%) of female camel genital tract abnormalities sampled for histopathological examination (total animals *n*=165, affected animals *n*=25)

Haemorrhagic cysts

Follicular haemorrhagic cysts were largely occupied of the antral cavity, which contained a large quantity of haemorrhagic blood. The granulosa cells and internal theca appeared hypertrophied with multiple vacuolisations in the antral cavity. Follicular cysts were encapsulated by thick connective tissue (Fig 2b, 3b).

Luteinized follicular cysts

The luteinized follicular cysts showed general hyperplasia of the internal and external theca. Granulosa cells were luteinized with a polyhedral form and large vesicular nuclei and vacuolar cytoplasm. Cysts were surrounded by a thick fibrous conjunctive capsule (Fig 2c, 3c).

Ovarian teratoma- dermoid cyst

The ovarian teratoma (*dermoid cyst*) appeared as a mature and benign cystic structure. It was composed of cystic structures surrounded by a keratinized and squamous epithelium lodged in fibrous connective tissue containing hair follicles, and sebaceous and sweat glands (Fig 2d, 3d).

Hydrobursitis - infundibular cysts

The ovarian bursa revealed multiple extensive foci of congestion and high frequencies of focal inflammation of mixed histiocytic and lymphocytic cells. The stroma was oedematous with degenerative process and vacuolation of the cell layers of the inner border and the outer mesothelium. The tissue disorganization which leads to pseudo-glandular dilations appearance had a conspicuous histological feature. Haemorrhagic foci and macrophages loaded with hemosiderin (hemosiderin laden macrophages) were also observed (Fig 2e, 4) in one of four sampled animals.

Clinical metritis

The uterine lumen contained a serous exudate tinged with blood and the uterine wall was hyperaemic and oedematous. Microscopically, the myometrium showed irregular

infiltration by inflammatory cells. In contrast, endometrium presented several neutrophilic infiltrations throughout the stromal compartment. The endometrium showed highly proliferated and activated stromal cells and focal vascular congestion and haemorrhage (Fig 2f, 5).

Pyometra - cystic hyperplasia of the endometrium

Endometrium lesions included multifocal oedema and congestions with multiple interglandular foci of infiltrations by polymorphonuclear inflammatory cells (histiocytes and lymphocytes). The endometrial epithelium and the endometrial glands showed advanced tissue degenerative features (atrophy and necrosis). Clusters of inflammatory cells were infrequently scattered between myometrium muscle fibres (Fig 2h, 6).

Chronic endometritis

Animals with chronic endometritis showed conspicuous endometrial

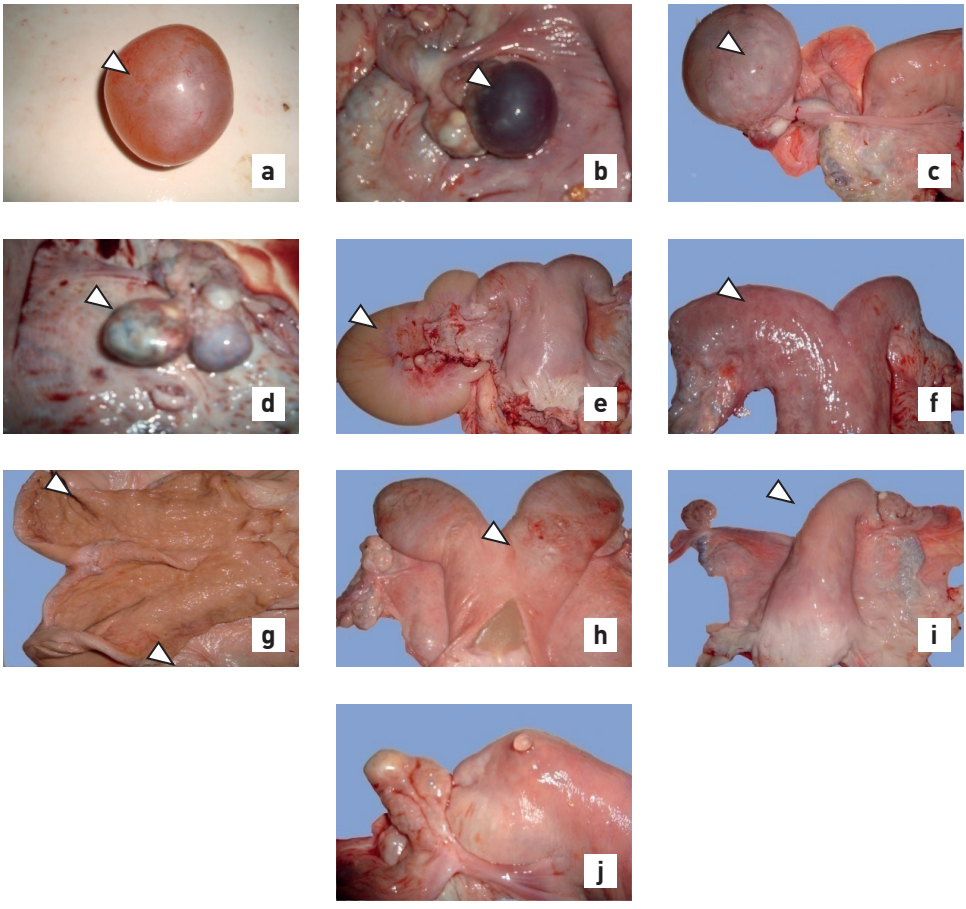

Figure 2. Macroscopic aspect of the genital affections encountered in female camels in southeast Algeria. a. follicular cyst, b. haemorrhagic cyst, c. luteinized follicular cyst, d. ovarian teratoma (dermoid cyst), e. hydrobursite (infundibular cyst), f. clinical metritis, g. chronic endometritis, h. pyrometra, i. uterine agenis, j. uterine serosal inclusion cyst

features as stromal myxematous changes, hyperplasia of the uterine glands, vascular dilatation, extravascular erythrocyte and peri-glandular and peri-vascular fibrosis which sometimes extended into the blood vessels. The endometrium and the myometrium were moderately to severely infiltrated by monocytes and polynuclear cells (Fig 2g, 7).

Uterine agenesis

The remained uterine horn showed normal stratification layers. The endometrium was lined by cuboidal epithelium and contained many endometrial glands and proliferated stroma. The same endometrial areas contained perivascular oedema and blood vessels with thickened endothelium. The myometrium muscle fibres showed mainly focal degeneration and hyalinization (Fig 2i, 8).

Uterine serosal inclusion cyst

The serosal cyst of the left uterine horn measures about 10 mm and contained a clear fluid. Microscopic examination revealed cystic dilation between the myometrium and the perimetrium. The contents of the cyst and its inner wall showed a homogeneous, amorphous and eosinophilic content (Fig 2j, 9)

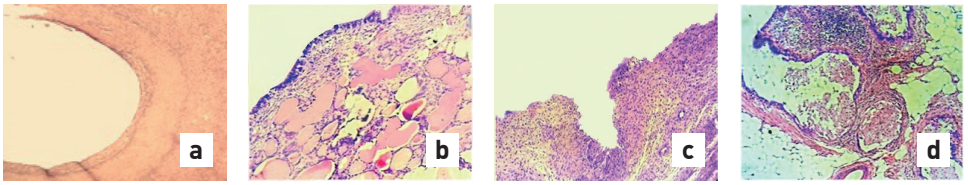

Figure 3. Histopathological aspect of the ovarian structures in female dromedary camels from southeast Algeria: a. follicular cyst (H&E, 100x); b. haemorrhagic cyst (H&E, 40x); c. luteinized follicular cyst (H&E, 100x); d. ovarian teratoma (H&E, 100x)

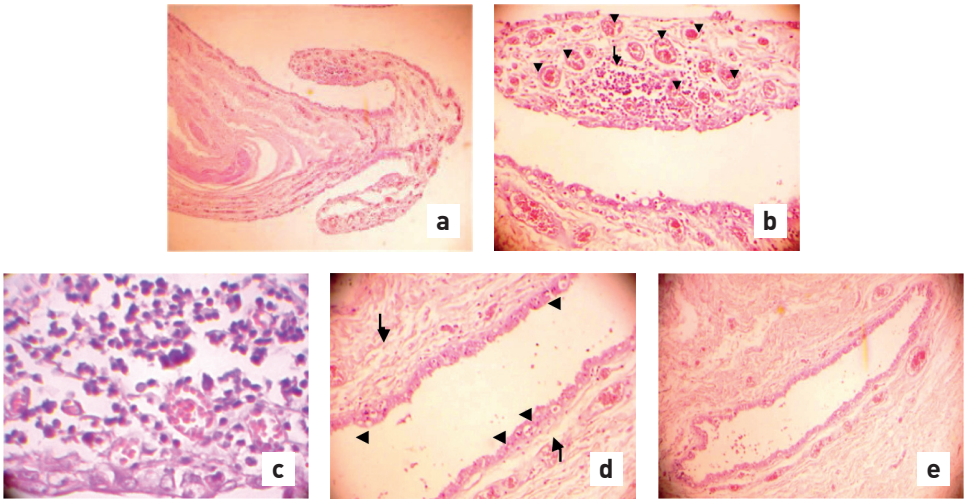

Figure. 4. Histopathological aspect of the ovarian bursa in female dromedary camels from southeast Algeria: a. General appearance of histopathological lesions of the ovarian bursa (H&E, 40x); b. foci of congestion (arrowheads) and infiltration by inflammatory cells (arrow) (H&E, 100x); c. infiltration by inflammatory cells (H&E, 400x); d. stroma oedema (arrows) with degeneration and vacuolation (arrowheads) (H&E, 100x); e. pseudo-glandular dilations (H&E, 100x)

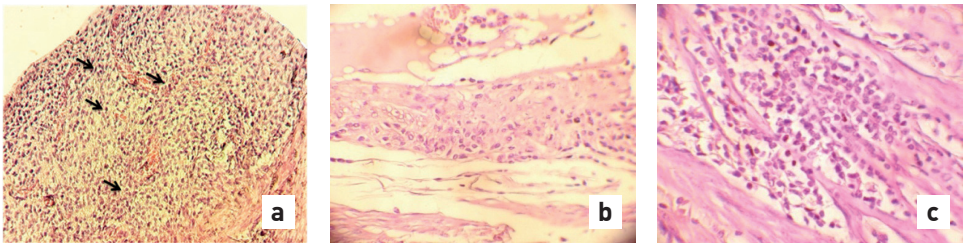

Figure 5. Histopathological aspect of the clinical metritis in female dromedary camels from southeast Algeria: a. endometrial infiltration by inflammatory cells, proliferation and activation of the endometrial stroma, vascular congestion and haemorrhagic foci (H&E, 100x); b. stromal oedema and endometrial infiltration by inflammatory cells (H&E, 400x); c. inflammatory cells within myometrium fibres (H&E, 400x)

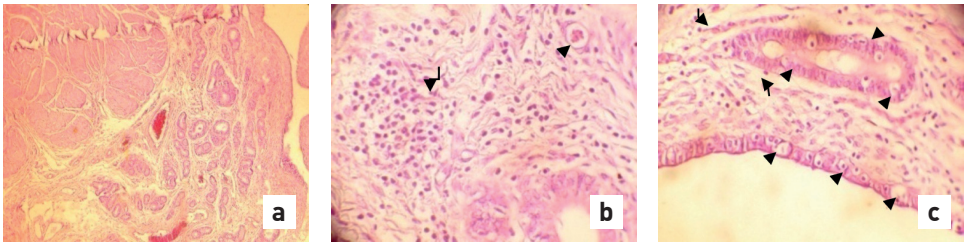

Figure 6. Histopathological aspect of pyometra in female dromedary camels from southeast Algeria: a. general aspect of the endometrium and myometrium (H&E, 100x); b. foci of inter-glandular infiltration by inflammatory cells (arrow) and vascular congestion (arrowhead) (H&E, 400x); c. multifocal peri-glandular oedemas (arrows), degenerations of the endometrial epithelium and the endometrial glands (arrowheads) (H & E, 400x)

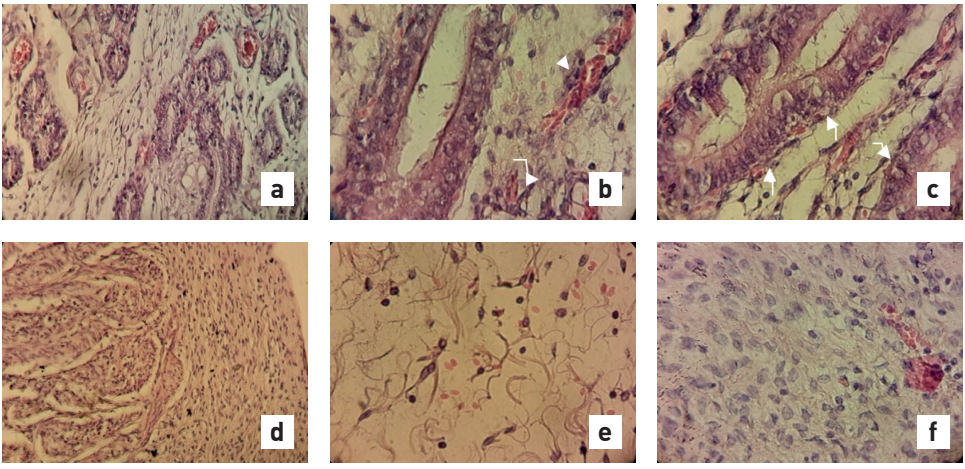

Figure 7. Histopathological aspect of chronic endometritis in female dromedary camels from southeast Algeria: (H&E; a. 100x, b. 400x, c. 400x). a, b, c. myxomatosis and hyperplasia of the uterine glands; b, c. vascular dilation (arrowhead) and peri-glandular fibrosis (arrow) (400x); d. endometrial infiltration by inflammatory cells (400x); e, f. extra-vascular erythrocytes (1000x); b, c, e. peri-glandular and perivascular fibrosis

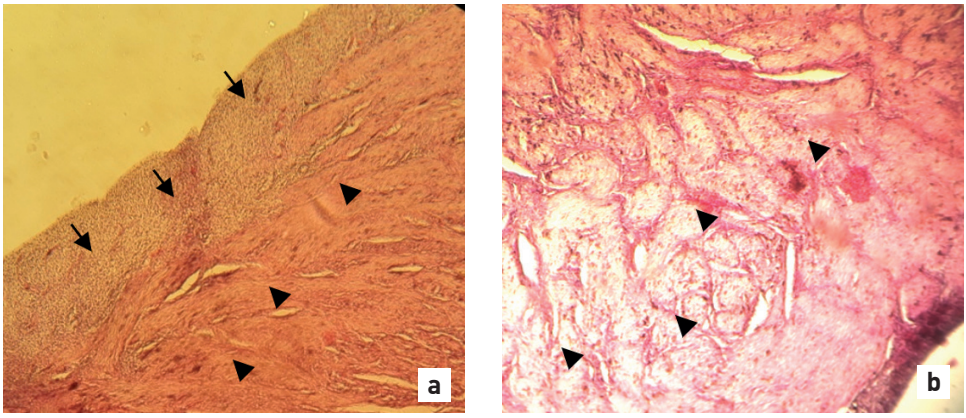

Figure 8. Histopathological aspect the remained uterine horn in female dromedary camel with uterine agenesis: a. endometrium with endometrial glands agenesis (arrows) and myometrium with hyalinization lesions (arrowheads) (H&E, 100x); b. hyalinization of the myometrial layer (arrowheads) (H&E, 400x)

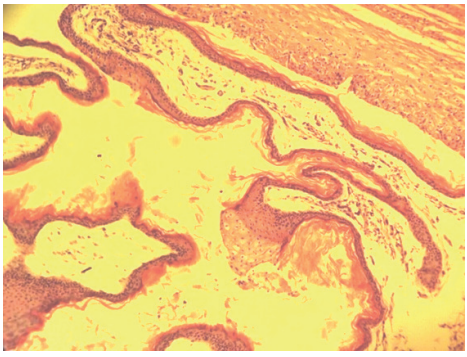

Figure 9. Histopathological aspect of the uterine serosal inclusion cyst in female dromedary camels from southeast Algeria: (H&E, 100x). Cystic dilation between the myometrium and the perimetrium with homogeneous, amorphous and eosinophilic content of the cyst and its inner wall

Discussion

The genital tract defects in female camel may have congenital, functional or infectious origin and they manifest in nulliparous or primiparous females (Gherissi et al., 2019). This has led to declines in herd reproduction and production performance and increased the early reform rate secondary to longer waiting period, "repeat breeding", mating refusal and early embryonic mortality (Ali et al., 2010, Gherissi et al., 2020b).

The prevalence of genital disorders observed in the studied female camels from the extreme arid condition of southeast Algeria was lower than that recorded by Belina et al. (2021) and Getahun et al. (2021) in Ethiopia; Gherissi et al. (2019) and Benaissa et al. (2015) in Algeria; and Wajid (2015) in Iraq and Ali et al. (2010) in Saudi Arabia. However, the obtained prevalence was higher than that reported by Hussein and Saad (2017) in Sudan.

The reported histopathological changes accompanying the oversized follicles are similar to those registered by Waheed et al. (2017) and Hussein and Saad (2017). Ovarian follicular cysts may occur as result of non-ovulation (functional cyst) of mature follicle that continues to grow due to failure of luteinizing hormone (LH) release in response to mating (Skidmore et al., 1995; Hamouda et al., 2011). They would be luteinized and then produce progesterone for several weeks (Tibary and Anouassi, 1997; Nourani and Khodakaram-Tafti, 2004). For luteal cyst, the levels of LH, progesterone and oestrogen significantly

increase in parallel with a concomitant decrease in FSH compared to females with active ovaries (Hegazy et al., 2004).

The available data indicate that teratoma shows irregular arrangement of different tissues, such as cartilage, pulmonary, bronchial and gland-like tissue, epidermis, and hair. These aspects have been reported sporadically in farm animals (Catoneet al., 2004; Srivastava and Pande, 2016; Singh et al., 2019). The set of histopathological and pathogenic findings agree for the hypothesis that it is not a neoplastic lesion (Pires et al., 2019). The pre-meiotic or somatic origin of teratomas has been proposed (Oliveira et al., 2004).

In mammals, the definitions of uterine infection often lack precision and vary among research groups, as it is the case of the term endometritis that is incorrectly includes metritis, endometritis and pyometra (Sheldon et al., 2006). It is important to underline that the histological descriptions of uterine infections reported in this study correspond to the cases obtained and classified clinically according to the definitions recommended by the 15th International Congress on Animal Reproduction (Gilbert, 2004), which can be readily adopted by researchers and veterinarians. The main elements of the histopathological diagnosis of clinical metritis were: endometrial and periglandular infiltration, endometrial stroma proliferation and activation, vascular congestion and hemorrhagic foci on the endometrium.

Some additional features of acute catarrhal endometritis were reported by Elshazly et al. (2020), such as focal epithelial desquamation, pyknosis of the glandular nuclei, vasculitis and focal hyalinization. Chronic endometritis is mainly characterized by uterine glands hyperplasia, vascular dilation, extravascular erythrocytes, periglandular and perivascular fibrosis and endometrial infiltration by monocytes and polymorphonuclear cells. Several histopathological appearances reported in clinical and chronic endometritis are consistent with those reported by Waheed et al. (2009) and Benaissa et al. (2017) in camel species. The same authors indicated presence of oedemas and haemosiderin in some cases. Recently, Elshazly et al. (2020) reported three varieties of chronic endometritis observed in Egyptian camels, namely chronic granulomatous endometritis, chronic fibrosing endometritis, and chronic cystic endometritis. In pyometra, histological diagnosis reported multifocal oedema and congestions with foci infiltration by inflammatory cells. The endometrium is degenerated and the myometrium shows cluster areas of inflammatory cells. Several authors have pointed out the same elementary lesions on uterine tissue in dromedary camel and domestic animals (Carreira and Pires, 2016; Elshazly et al., 2020).

The literature lacks information on the incidence of congenital anomalies on camel reproduction. Previously, we reported one case of this congenital anomaly associated to total agenesis of endometrial glands in female camel in the same study area (Gherissi et al., 2020a). Similar conditions have been communicated in ewes, bitches and mares (Gray et al., 2002; Dockweiler et al., 2017; Witkowski et al., 2017). It has been suggested that this genital defect has genetic and/or hormonal backgrounds. Chromosomal aberrations, gene mutation or epigenetic strategies, both hormonal disturbances and improper distribution of hormone receptors, are responsible for incomplete adenogenesis or total UGKO (Uterine Gland Knockout) (Witkowski et al., 2017). A basic lesion associated with uterine agenesis is the hyalinization of uterine tissue, which is comparable to focal or segmental hyalinization lesions reported in some congenital disorders of

sex development (Arboleda and Vilain, 2009), glomerulopathy and congenital heart diseases (Seely et al., 2018).

The serosal cyst of the uterus is characterized by an amorphous encystment of homogeneous eosinophilic content. It develops during the postpartum period over the involution process following rapid contractions of the myometrium (Cortés-Beltrán et al., 2020). The clinical impacts are unknown. Similar histological features have been described in domestic animals like bitch, queen, cow, buffalo, ewe and camel (Tafti and Darahshiri, 2000; Aguirra et al., 2015; Wajid, 2015; Raval et al., 2016).

Elementary lesions found in the hydrobursitis were vascular congestions, infiltration by immune cells, interstitial oedema, glandular vacuolation and the presence of macrophages with hemosiderin. Additionally, Ali et al. (2011) and Benaissa et al. (2015) reported the degeneration and hyperplasia of the lining epithelium and tiny haemorrhages. The pathogenesis of the infundibular cyst disease is not yet clear. Infectious, parasitic or functional factors remain to be studied (Al-Eknah and Ali, 2001; Ali et al., 2010, 2012; Zaher et al., 2017; Khalifa et al., 2018).

This study provides an assessment of the histopathological disorders in the genital tract of female camels. We highlight some elementary epithelial lesions which cannot be identified during the basic breeding examination. In our opinion, this complementary diagnosis could be a useful tool for appropriate diagnosis, prognosis and treatment of female camel infertility related to genital tract defects.

Conclusion

We evaluated the pathological aspects of internal reproductive organs in the female camels from southeast Algeria. The incidence and pathological features of genital organs differ according to histo-anatomical localisation, elementary histopathological abnormalities, type and severity of engendered lesions, and the nature of the congenital or acquired anomalies. The most frequent anomalies are ovarian cysts, uterine infection and hydrobursitis. The most common lesions were functional changes in the ovarian cysts and inflammation in the uterus and ovarian bursa.

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Patološki nalazi na genitalnim abnormalnostima u ženki deva u regiji El Oued, Alžir

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Studija patološki nalazi na genitalnim abnormalnostima u ženki deva u regiji El Oued u Alžiru provedena je u svrhu dobivanja detaljnog patohistološkog opisa nekih genitalnih abnormalnosti prikupljenih od klinički zdravih ženki deva. Ukupno 165, naizgled zdravih ženki deva, nasumice je izabrano u klaonici El Oued na jugoistoku Alžira. Patohistološka analiza je provedena na 25 patoloških tvorbi na jajnicima (folikularne, hemoragične i luteinske ciste i teratomi), infekcijama maternice (kliničke, kronične i piometra), seroznoj inkluzijskoj cisti maternice, agenezi maternice i infundibularnoj cisti jajnika utvrđivanjem elementarnih lezija povezanih sa svakim makroskopskim stanjem. Zabilježeno je značajno oštećenje normalne strukture svakog ispitanog reproduktivnog organa. Prevalencija životinja koje su pokazale genitalne abnormalnosti bila je 15 %; incidencija učestalosti lezija jajnika bila je na prvom mjestu (56 %), nakon čega slijede bolesti maternice (28 %) te na kraju abnormalnosti burze jajnika sa 4 slučaja (16 %). Padajući niz prema incidenciji genitalnih stanja jest sljedeći: folikularne ciste (28 %), hemoragične ciste (16 %), hidroburzitis (16 %), kronični endometritis (12 %), luteinska folikularna cista (8 %), klinički metritis (4 %), piometra (4 %), dermoidna cista (4 %), ageneza maternice (4 %) te serozna inkluzijska cista maternice (4 %). Glavne lezije infekcije maternice bile su kongestija, edem, endometralni epitel i žljezdana degeneracija i infiltracija upalnih stanica. Ageneza maternice bila je okarakterizirana nedostatkom endometralnih žlijezda i hijalinizacijom miometrija. Glavne lezije za infundibularnu cistu bile su: kongestija, krvarenje i hemosiderofagi, infiltracija upalnih stanica, degeneracija endometrija i vakuolizacija i pseudoglandularne dilatacije. Dermoidna cista jajnika pokazala je keratinizirani i ljuskavi epitel smješten u fibroznom veznom tkivu koje sadrži folikule dlake, žlijezde lojnice i žlijezde znojnice. Ciste maternice pokazale su tanke ili uvećane granuloze i interne kapsule s luteinizacijom ili vakuolizacijom antralne šupljine. Zaključno, serozna inkluzijska cista maternice javila se kao dilatacija između miometrija i perimetrija s homogenim sadržajem. Obzirom na navedeno, zabilježene osnovne genitalne abnormalnosti su, čini se, odgovorne za reproduktivne poremećaje, neplodnost i nezadovoljavajuće rezultate uzgoja deva.

Ključne riječi: *jednogrba deva, folikularna cista, infekcija maternice, hidroburzitis, ageneza maternice*