

UTERINE HISTOPATHOLOGICAL FINDINGS OF INFERTILE FEMALE CAMELS (*Camelus dromedarius*)

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ABSTRACT

The current study was carried out on 100 infertile female dromedaries (5 - 18 years old). A complete gynaecological examination was performed. Four categories of infertility problems were recorded: pyometra (34%), repeat breeder (28%), endometritis (24%) and mucometra (14%). The effects of season and age of females on these infertility problems were declared. Uterine biopsies from 24 dromedaries, clinically diagnosed as endometritis were examined and graded microscopically. The results revealed a significant ($p < 0.05$) effect of season on infertility as high percentages of endometritis during autumn, repeat breeder during winter and pyometra during spring. Besides, infertility was significantly ($p < 0.05$) influenced by the age of females. High rates of repeat breeders and endometritis were noticed in the young age. While, middle-aged females had high percentages of pyometra. Results of uterine biopsy were variable. One female (4.17%) had a normal uterus, which was graded 1A. Four dromedaries (16.67%) had minor uterine abnormalities that were graded 1B. Five dromedaries (20.83%) had appreciable endometritis that was graded 2A. Ten dromedaries (41.67%) had endometritis with gland fibrosis that was graded 2B. Four dromedaries (16.67%) had notable uterine gland fibrosis that was graded 3A. Uterine biopsy could be considered as a good diagnostic tool of endometritis in female camels.

Key words: Dromedaries, endometritis, infertility, reproduction, uterine biopsy

Camelids are important animal production resource in many areas of the world (Tibary *et al*, 2006). The reproductive efficiency of dromedary camels is generally considered low (Tibary and Anouassi, 1997c; Kaufmann, 2005; Tibary *et al*, 2005). The objective of the breeders is to produce the maximum number of live, healthy calves from the female camels bred during the previous season. Perhaps, the biggest obstacle to achieve this aim is the female camel infertility. Establishing the cause of infertility in any species relies on knowing the full breeding history of the patient and performing an extensive examination of the reproductive tract (Tibary and Anouassi, 2001). The latter should include a general physical examination, palpation and ultrasonography of the reproductive tract (Tibary and Anouassi, 2001). Endometrial biopsy samples should also be collected to evaluate pathological changes in the endometrium (Powers *et al*, 1990; Tibary and Anouassi, 1997a; Tibary, 2004).

There is high incidence of reproductive failure among female camels admitted to the Veterinary Teaching Hospital. Therefore, the main objective of the present paper is to study the causes of

reproductive problems with great emphasis on the uterine histopathological changes accompanying infertility in the female dromedaries.

Materials and Methods

Animals

One hundred infertile female dromedaries (5 - 18 years old) admitted to the Veterinary Teaching Hospital during the period from November to April in 2 successive years were used in this study.

History and clinical evaluation

Previous breeding history of the female camels was obtained. In addition, physical condition and general health were assessed. A complete gynaecological examination of female dromedaries was performed by palpation and transrectal ultrasonographical examination (Tibary *et al*, 2006).

Uterine biopsy

Uterine biopsy was considered in females failing to conceive despite being bred to fertile males and after the occurrence of an apparently normal ovulation, and in females experiencing early embryonic loss or abortion (Tibary and Anouassi, 2001). The technique

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used in camels was similar to that used in the mare (Powers *et al*, 1990).

Histopathology

Endometrial biopsies (24 cases of endometritis) fixed in 10% formalin and processed to paraffin wax, and 4 µm sections were cut and stained with haematoxylin and eosin (HE).

A classification system of endometrial biopsy was similar to that used in llamas (Powers *et al*, 1990). The classification included 3 grades:

- Grade 1A: endometria showing no histopathological abnormalities.
- Grade 1B: endometria showing minor histopathological abnormalities.
- Grade 2A: endometria showing acute endometritis.
- Grade 2B: endometria showing chronic endometritis characterised by periglandular fibrosis.
- Grade 3A: endometria showing chronic endometritis characterised by massive periglandular fibrosis.
- Grade 3B: endometria showing neoplasia.

Statistical analysis

The data was divided according to season and age of the female dromedaries. Seasons were autumn (November - December), winter (January - February) and spring (March - April). The age groups were A (4 - 6 years), B (7 - 9 years), C (10 - 12 years) and D (14 - 15 years). Chi square, t-test and analysis of variance (ANOVA) were calculated using commercial software (Statistics for windows, 1993).

Results

Clinical examination of 100 female camels was evaluated and classified into 4 infertility problems: pyometra (34%), repeat breeder (28%), endometritis (24%) and mucometra (14%). The effect of season and age on these infertility problems was declared in the following tables.

As shown in Table 1, the frequency of repeat breeders was significantly higher ($P<0.05$) during winter than the other 2 seasons. However, the percentage of endometritis was significantly higher ($P<0.05$) during autumn than during winter and spring.

There was significant ($P<0.05$) high percentages of endometritis (41.94%) during autumn, repeat breeder (44.74%) during winter and pyometra (41.94%) during spring (Table 2).

A significant ($P<0.05$) high percentages of pyometra were found in the age groups B (29.41%) and C (41.18%). Moreover, repeat breeders were significantly higher ($P<0.05$) in the age groups A (35.71%) and B (39.29%), respectively. Simultaneously, endometritis and mucometra were significantly higher ($P<0.05$) in the age groups B (37.50%) and C (42.86%), respectively (Table 3).

There was a significant ($P<0.05$) difference between the percentage of repeat breeders (43.48%) and the other cases of infertility in the age group A. Similarly, in the age group C there was a significant ($P<0.05$) difference between the percentage of pyometra (51.85%) and the other cases of infertility (Table 4).

Microscopic examination of endometrial biopsies from cases of endometritis (24 cases, 24%) was assigned to one of the following grades:

Table 1. Effect of season on causes of female camel infertility.

Season	Pyometra (%)	Repeat Breeder (%)	Endometritis (%)	Mucometra (%)
Autumn	26.47(n=9)	17.86 ^b (n=5)	54.17 ^a (n=13)	28.57(n=4)
Winter	35.29(n=12)	60.71 ^a (n=17)	16.67 ^b (n=4)	35.71(n=5)
Spring	38.24(n=13)	21.43 ^b (n=6)	29.17 ^b (n=7)	35.71(n=5)
All groups	34	28	24	14

Percentages in the same column not sharing common superscript letters differ significantly $P<0.05$

Table 2. Effect of season on frequencies of female camel infertility.

Season	Pyometra (%)	Repeat Breeder (%)	Endometritis (%)	Mucometra (%)	Total
Autumn	29.03 ^a (n=9)	16.13 ^a (n=5)	41.94 ^b (n=13)	12.90 ^a (n=4)	31
Winter	31.58 ^a (n=12)	44.74 ^a (n=17)	10.53 ^b (n=4)	13.16 ^b (n=5)	38
Spring	41.94 ^a (n=13)	19.35 ^b (n=6)	22.58 ^b (n=7)	16.13 ^b (n=5)	31

Percentages in the same row not sharing common superscript letters differ significantly $P<0.05$

Grade 1A was observed in one case (4.17%). There was no obvious histopathological alterations, and the endometrium seemed to be normal and healthy (Fig 1). The normal surface of endometrium consisted of simple cuboidal or columnar. Beneath the epithelium was a loose or dense area of connective tissue. The upper lamina propria contained a few uterine glands and the deeper lamina propria had less dense connective tissue and contained numerous glands.

Grade 1B was observed in 4 cases (16.67%). There was few polymorphonuclear cells (PMNs) and sub-epithelial haemorrhage associated with siderophages. The endometrial glands showed often secretory activity (Fig 2). Mild to moderate degrees of congestion and oedema were also observed.

Grade 2A was observed in 5 cases (20.83%). The lining epithelium of endometrium was invaded by

numerous polymorphonuclear cells (PMNs) mostly seen in the upper lamina propria (Fig 3). It also showed vacuolation with necrotic changes and focal desquamation. The blood vessels were severely congested associated with focal haemorrhages and varying degrees of oedema. The endometrial stroma and glands were also heavily infiltrated by polymorphonuclear cells. Some glands were degenerated and replaced with the aggregations of polymorphonuclear cells (PMNs).

Grade 2B was observed in 10 cases (41.67%). The endometrium showed slight to moderate mononuclear cells infiltration, mostly lymphocytes and macrophages. The inflammatory cells were either distributed throughout the endometrium, being most common in the deeper lamina propria around the endometrial glands and the blood vessels. Sometimes, few cells were seen in the lumen of the glands.

Table 3. Effect of age on causes of female camel infertility.

Age (years)	Pyometra (%)	Repeat Breeder (%)	Endometritis (%)	Mucometra (%)
A (4 - 6)	11.76 ^a (n=4)	35.71 ^a (n=10)	25.00 ^b (n=6)	21.43 ^b (n=3)
B (7 - 9)	29.41 ^b (n=10)	39.29 ^a (n=11)	37.50 ^a (n=9)	28.57 ^{ab} (n=4)
C (10 - 12)	41.18 ^b (n=14)	7.14 ^b (n=2)	20.83 ^b (n=5)	42.86 ^a (n=6)
D (12 <)	17.65 ^a (n=6)	17.86 ^b (n=5)	16.67 ^b (n=4)	7.14 ^b (n=1)
All groups	34	28	24	14

Percentages in the same column not sharing common superscript letters differ significantly P<0.05

Table 4. Effect of age on frequencies of female camel infertility.

Age (years)	Pyometra (%)	Repeat Breeder (%)	Endometritis (%)	Mucometra (%)	Total
A (4 - 6)	17.39 ^b (n=4)	43.48 ^a (n=10)	26.09 ^b (n=6)	13.04 ^b (n=3)	23
B (7 - 9)	29.41 ^a (n=10)	32.35 ^a (n=11)	26.47 ^a (n=9)	11.76 ^b (n=4)	34
C (10 - 12)	51.85 ^a (n=14)	7.41 ^b (n=2)	18.52 ^b (n=5)	22.22 ^b (n=6)	27
D (12 <)	37.50 ^a (n=6)	31.25 ^a (n=5)	25.00 ^a (n=4)	6.25 ^b (n=1)	16

Percentages in the same row not sharing common superscript letters differ significantly P<0.05

Table 5. Classification of endometrial biopsy and potential effect on fertility (adapted from Powers *et al*, 1990).

Categories	Characteristics	Effect on fertility	Prognosis
Grade 1A	Normal endometrium	Normal	Very good
Grade 1B	Few polymorphonuclear cells and sub-epithelial haemorrhage associated with siderophages	Slightly decreased	Good if treated promptly
Grade 2A	The lining epithelium of endometrium is invaded by numerous polymorphonuclear cells mostly seen in the upper lamina propria	Reduced conception rate, Increased early embryo death	Good if recent, poor if the female has been barren for > 1 year
Grade 2B	Mild to moderate degrees of fibrosis of the endometrial stroma and fibrous tissue is arranged in whorls of <10 layers around the endometrial glands	Reduced conception rate, Increased early embryo death	Good if recent, poor if the female has been barren for > 1 year
Grade 3A	Endometrial stroma is severely fibrosed and fibrous tissue is arranged in whorls of >10 layers around the endometrial glands	Increased early embryonic loss or abortion	Poor

The large and medium sized blood vessels showed hyalinisation of the tunica media and narrowing of the lumina. Mild to moderate degrees of fibrosis of the endometrial stroma occur in most cases. The fibrous tissue was arranged in whorls of <10 layers around the endometrial glands (Fig 4). In some cases, the endometrial glands were dilated by accumulations of secretions and desquamated cells (Fig 5).

Grade 3A was observed in 4 cases (16.67%). The endometrium showed marked mononuclear infiltration, with increase in number of lymphocytes and macrophages (Fig 6). Mast cells were occasionally seen in some cases. In most cases, the endometrial stroma was heavily infiltrated with lymphocytes and macrophages. In few cases, granulomatous foci, consisting of macrophages, epithelioids and small lymphocytes, situated through the endometrium were

seen (Fig 7). The endometrial stroma was severely fibrosed in most cases, the fibrous tissue was arranged in whorls of >10 layers around the endometrial glands (Fig 8). Most of the endometrial glands appeared cystic with accumulations of secretions and others were fibrosed and completely disappeared. The large and medium sized blood vessels also showed hyalinisation of the tunica media and narrowing of the lumina.

Such classification system of endometrial biopsies according to pathological findings has been proposed for fertility prognosis (Table 5).

Discussion

The uterus of female dromedary, as in other female animal species, could be a site of acquired abnormalities that might seriously affect the fertility

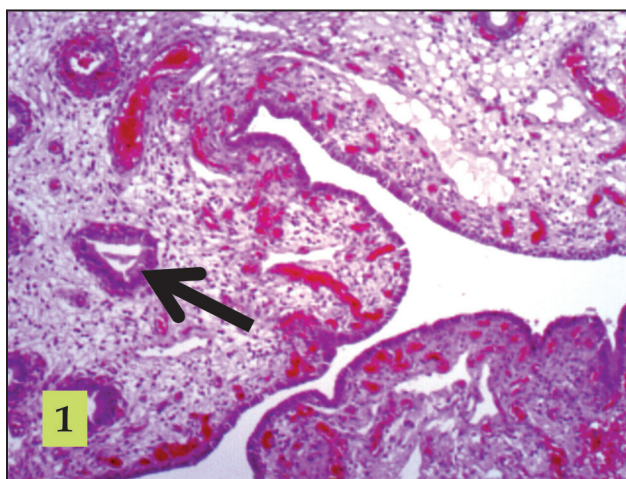


Fig 1. Grade 1A: uterus showing normal endometrium with normal uterine glands (arrow). HE X100.

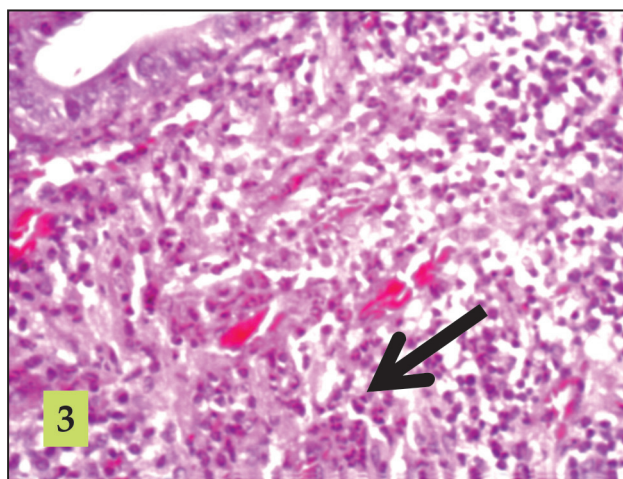


Fig 3. Grade 2A: uterus showing extensive numbers of polymorphonuclear cells (PMNs) in lamina propria of stratum compactum (arrow). HE X 400.

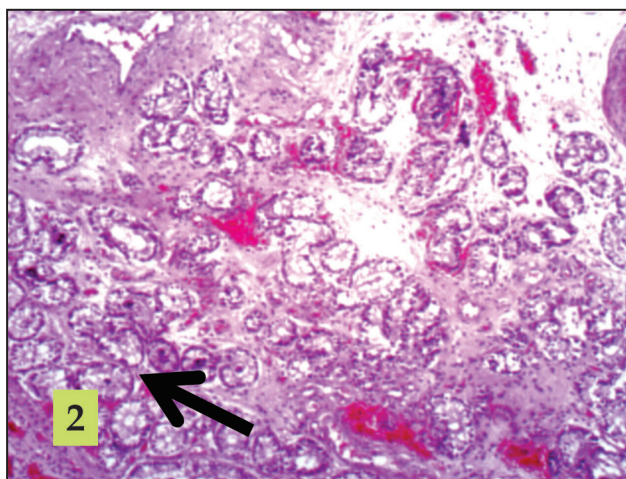


Fig 2. Grade 1B: uterus showing few polymorphonuclear cells with increased secretory activity of uterine glands (arrow). HE X 100.

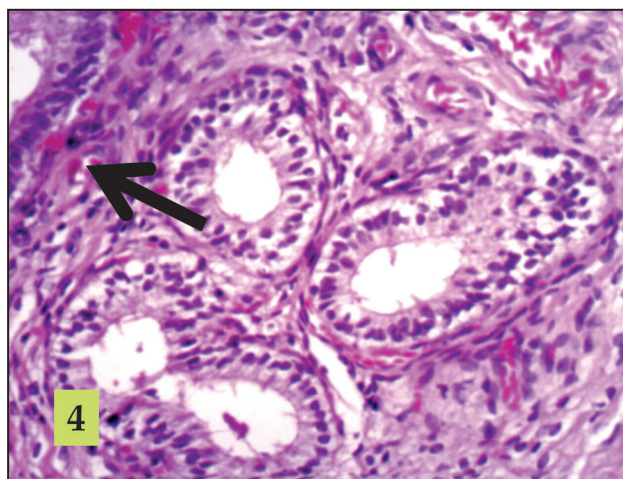


Fig 4. Grade 2B: uterus showing periglandular fibrosis (less than 10 fibrocytic layers, arrow). HE X 400.

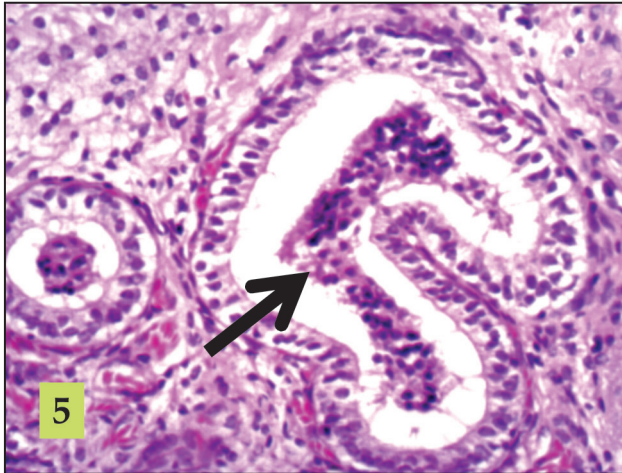


Fig 5. Grade 2B: uterus showing cystic dilatation of uterine glands with secretions and desquamated cells (arrow). HE X 400.

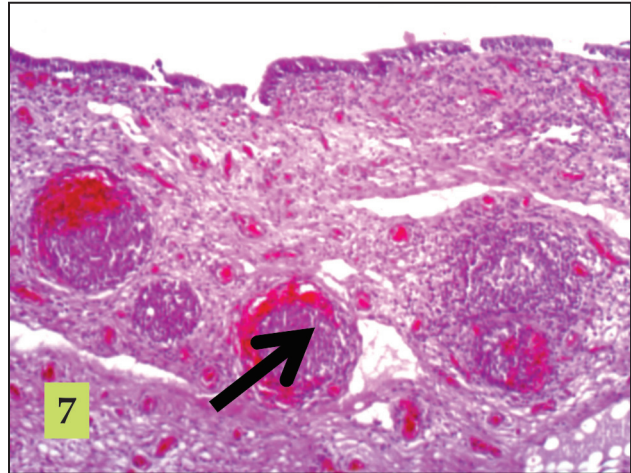


Fig 7. Grade 3A: uterus showing granulomatous foci consisting of macrophages, epithelioids and lymphocytes throughout the lamina propria (arrow). HE X 100.

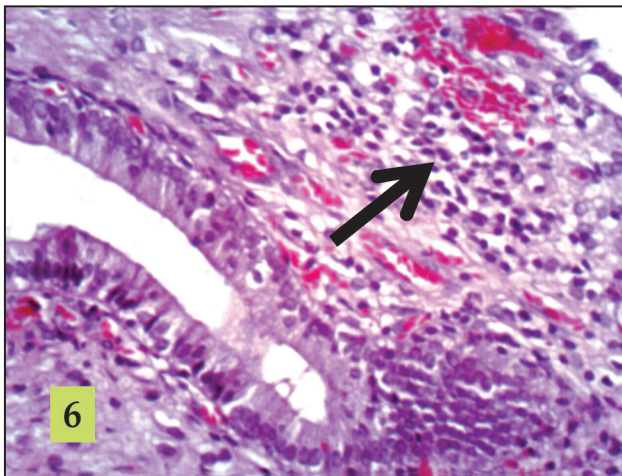


Fig 6. Grade 2B: uterus showing mononuclear cells infiltration mostly lymphocytes and macrophages (arrow). HE X 400.

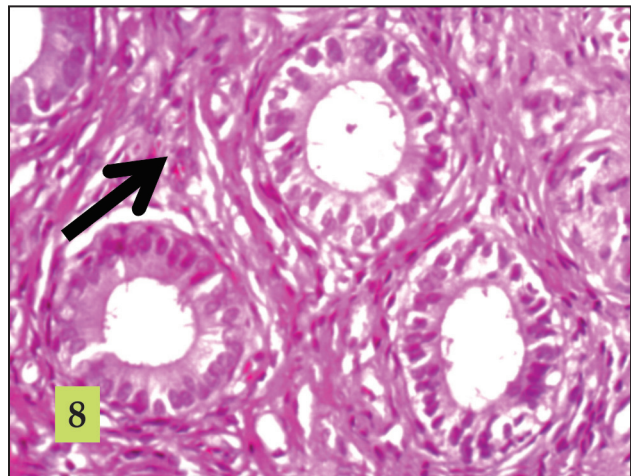


Fig 8. Grade 3A: uterus showing periglandular fibrosis (more than 10 fibrocytic layers, arrow). HE X 400.

of the female (Tibary and Anouassi, 1997b). Amongst the most common acquired uterine abnormalities reported in dromedaries were repeat breeder, endometritis, pyometra and mucometra (Tibary and Anouassi, 1997b). In the present study, clinical diagnosis of infertile female dromedaries revealed similar abnormalities.

In dromedary camels of Arabia, mating begins in mid-November and continues until mid-April (Abdel-Rahim and El-Nazier, 1990; Abdel-Rahim *et al*, 1994; Sghiri and Driancourt, 1999). Seasons of this period are autumn, winter and spring. The data reported in the current study were found during these 3 seasons.

The incidence of repeat breeders was 28% and mostly recorded in Winter and in the young aged females. Nevertheless, higher percentages (76% &

40%) were reported earlier (Al-Ani *et al*, 1992; Tibary and Anouassi, 1997b) and mainly found between October and December (Autumn) due to the reduced follicular maturation that was measured by reduced oestradiol output observed at the beginning of the breeding season (Sghiri and Driancourt, 1999). In the young aged females, the high incidence of repeat breeding was attributed to the proportion of active ovaries presenting active corpora lutea significantly depressed in young age (≤ 5 years old, 21.4%) as opposed to 52.9% of the older (≥ 16 years old) female dromedaries (Sghiri and Driancourt, 1999).

The incidence of pyometra was 34% of the total female dromedaries examined which was much higher than ranges (0.4 – 12.1%) reported in an earlier studies (Shalash, 1965; Nur, 1984; El-Wishy, 1990; Ribadu *et al*, 1991). The majority of pyometra cases

(41.94%) were found during spring. Approximately, all parturitions of female dromedaries occur during March (spring) due to the influence of season on gestation length (Elias *et al*, 1991; Musa *et al*, 1993). Pyometra with an open cervix and vaginal discharge is usually seen in the immediate postpartum period and is due to postpartum complications (El-Wishy, 1990; Chauhan and Kaushik, 1992; Tibary *et al*, 2006). In the non-parturient female, pyometra is generally associated with vaginal or cervical adhesions (the most prevalent in dromedaries) following dystocia or obstetrical manipulation trauma (Tibary and Anouassi, 1997b; Tibary *et al*, 2006).

Pyometra has been reported in all types of camelidae including dromedaries (Shalash and Nawito, 1963; Nawito, 1973; Ali *et al*, 1987; Chauhan and Kaushik, 1992; Tibary *et al*, 2006).

In view of current findings, pyometra was much evident in the age groups B (7 – 9 years old) and C (10 – 12 years old). The most plausible explanation for these findings was that female dromedaries were at the peak of their reproductive performance during these periods of age with frequent mating, parturitions, postpartum complications and cervical adhesions. Furthermore, in most management systems, dromedaries are not bred until 4 years, resulting in an age at first calving of 5 years or more (Beniwal and Chaudhry, 1984; Wilson, 1986; Sghiri, 1988).

Mucometra was found in 14% of the total cases and it was prevalent (42.86%) in the age group C (10 – 12 years old). All cases of mucometra were associated with vaginal adhesions. Similar results were reported previously (Tibary and Anouassi, 1997b).

The incidence of endometritis in female dromedaries was 24%. Meanwhile, prior studies recorded that uterine infections in camelidae were the most common acquired reproductive problems resulting in infertility (Nur, 1984; Wernery and Ali, 1989; Wernery, 1991; Wernery and Wernery, 1992).

There was significantly ($P < 0.05$) high percentage of endometritis found during autumn. In this respect, autumn is the beginning of the breeding season in camelidae where uterus is exposed to the risks of infection with various microorganisms that carried from the posterior part of the genitalia or from the environment into the uterine cavity because the cervical barrier is wide open (Tibary and Anouassi, 1997b).

The percentage (37.50%) of endometritis was the highest significantly ($P < 0.05$) in the age group B (7 – 9

years old). During this age period, either the majority of female dromedaries are at the puerperium period of first parturition or at breeding period after the first parturition and those are times of high risk of uterine infection (Tibary and Anouassi, 1997b).

Endometrial tissue specimens from 24 dromedaries with clinical diagnosis of endometritis had abnormal histological findings in all but one (4.17%). A high ratio (16.70%) of normal histological specimens was reported in llamas (Powers *et al*, 1990).

In female dromedaries, percentages of endometritis grades 1B, 2A, 2B and 3A were 16.67%, 20.83%, 41.67% and 16.67%, respectively. These grades of endometritis were 25.60%, 50.00%, 3.30% and 2.20%, respectively in llamas (Powers *et al*, 1990). Female dromedaries had either grade 2A or 2B endometrium, indicative to endometritis that was of sufficient degree to interfere with conception or cause early embryonic death unless it is treated in the early stages of pathogenesis. Four dromedaries had uterine gland fibrosis severe enough to be classified as grade 3A. In mares with grade-3 endometrium, pregnancy rate was 70.3%, but foal production rate was only 35.1%, indicating appreciable resorption or abortion rate related to gland fibrosis (Shideler *et al*, 1978; Shideler *et al*, 1982).

As from the present results, it could be speculated that the effect of endometritis on future fertility of dromedaries is good in grades 1B, 2A and 2B with a total percentage of 79.17%. On the same direction, Powers *et al* (1990) recorded similar overall percentage (78.90%) in llamas.

In conclusion, infertility in female dromedaries due to uterine infection was a common acquired reproductive problem; therefore, uterine biopsy was considered as a good diagnostic tool in cases of uterine infection especially endometritis.

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