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ENDOMETRIAL BIOPSY TECHNIQUE AND HISTOPATHOLOGICAL FINDINGS OF ENDOMETRITIS IN CAMELS (*Camelus dromedarius*)

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ABSTRACT

This study described the technique of uterine biopsy and its application in diagnosis of endometritis in dromedary camels. Twenty two endometrial biopsies were collected from female dromedaries clinically diagnosed as suffering from endometritis. Uterine tissue specimens were microscopically examined and graded. These grades were 9.1%, 54.5% and 36.4% for normal endometrium, minor histopathological changes and chronic endometritis, respectively. In conclusion, dromedary endometrial biopsy is a safe and reliable technique for providing detail on endometrial inflammation.

Key words: Biopsy, camel, dromedary, endometritis, histopathology

Endometrial biopsies were initially performed in mares in the 1960s as a tool for investigating infertility (Brandt and Manning, 1969). Endometrial biopsy has been revealed to have both diagnostic and prognostic value in evaluating fertility in cattle (Bonnett *et al,* 1991; Chapwanya *et al,* 2010), equine (Van Camp, 1988; Snider *et al,* 2011) and IIama (Powers *et al,* 1990). When carried out appropriately endometrial biopsy is a safe and reliable technique for assessing uterine health (Bauersachs *et al,* 2008; Herath *et al*, 2009; Chapwanya *et al,* 2010). The endometrial biopsy technique can enhance research by obtaining high quality and meaningful samples to detect physiologic, immunologic or pathologic changes in the uterus (Chapwanya *et al,* 2010). It is now possible to perform quality histologic, cellular and molecular assessment on tissue obtained using biopsy (Bonnett *et al,* 1991). Various uterine disorders have been described in camelids and may play an important role in reduced fertility in these species (Tibary and Anouassi, 1997c). Similar to other domestic animal species, uterine infections are the most common in camelids (Nur, 1984; Johnson, 1989; Wernery and Wernery, 1992; Wernery and Kumar, 1994; Fowler, 1998; Tibary and Anouassi, 2000), but unlike other species, little is known about their pathogenesis and evolution in camelids. Consequently, many practitioners diagnose the endometritis in female camelids similar to described for cows and mares (Johnson, 1989). The current study aims to describe the technique for endometrial biopsy in dromedaries and subsequent effects on health as well as assessment of the histopathological findings associated with endometritis in dromedary camels.

Materials and Methods

During breeding seasons (from November to April), a total of 88 female camels (*Camelus dromedarius*) aged between 5 to 22 years were examined at the Teaching Veterinary Hospital of King Faisal University, Saudi Arabia. Camels had a history of failing to conceive after more than two services. The investigation involved the breeding history (interval since last calving, number of times bred, milking status, male fertility, and herd fertility). All camels were clinically examined by visual appraisal for any signs of abnormal vulval discharge, rectal palpation of the reproductive tract and ovaries (Tibary and Anouassi, 1997), vaginal examination (Tibary and Anouassi, 1997b; Tibary and Anouassi, 2000; Ali *et al,* 2010) as well as transrectal ultrasound (Tibary and Anouassi, 1997a; Tibary and Anouassi, 2000; Tibary and Anouassi, 2001; Ali *et al,* 2010) using linear-array 5 MHz transducer (UST-588U-5, SSD-500V, ALOKA, Co., Japan). Based on history, rectal, vaginal examination and ultrasound examination, 22 animals were diagnosed as suffering

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from endometritis. These animals were candidates for endometrial biopsy examination.

Endometrial biopsy technique

Endometrail biopsies were obtained using sterilised biopsy punch instruments (Equi-Vet® Kruse). Animals were restrained in sitting position (Arthur *et al,* 1985) on operating table. The tail of the animal was wrapped with gauze and diverted away from perineal region. The rectum was cleared from the faecal matter by back racking. Camels were sedated with mixture of xylazine (0.15mg/kg) and ketamine (2.5 mg/kg) administered intravenously (White *et al,* 1987). The perineal region was then scrubbed with povidone-iodine and dried with clean towels. After cleaning the perineum and external genitalia, the lips of the vulva were parted and the biopsy instrument in a protective metallic sheath was introduced into the vagina. The instrument was guided into cervix with the aid of a finger and the examiner's gloved arm was inserted into the rectum.Following per rectum manipulation the instrument alone (without the metallic protective sheath) was guided into the left horn. Afterward, the biopsy jaws were opened and by the aid of the hand in the rectum, the medial uterine wall was gently pressed into the instrument jaws. Endometrial tissue was clipped off by closing jaws and withdrawing the instrument. Biopsies were gently removed from the biopsy punch with fine tip forceps and transferred to a vial of 10% buffered formalin (Brandt and Manning, 1969) and processed to paraffin wax. Sections (4 µm) were cut and stained with Haematoxylin and Eosin (Schlafer, 2007).

Clinical findings

Postbiopsy, animals were exposed to twice daily clinical examination with particular regard to signs of abnormal vulval discharge or discomfort for 48 h after endometrial biopsy. Vital parameters of temperature, pulse rate and respiration rate were also recorded for each camel at each time point.

Histological assessment

A classification system of endometrial biopsy was similar to that used for mare endometrial biopsy specimens (Shideler *et al,* 1978; Shideler *et al,* 1982). The classification includes three grades viz., Grade 1A (endometria had no histopathological abnormalities), Grade 1B (endometria had minor histopathological abnormalities), Grade 2A (endometria had acute endometritis), Grade 2B (endometria had chronic endometritis characterised by periglandular fibrosis). Grade 3A (endometria had chronic endometritis characterised by massive periglandular fibrosis) and Grade 3B (endometria had neoplasia).

Results

Rectal temperature (36.3 to 36.8 C), pulse rate (49 to 52 pulse per minute), respiration rate (12 to 14 breaths per minute), and ocular mucosal colour (pink, moist) were all normal. No camel required antimicrobial treatment during the study.

The microscopic examination of endometrial biopsies revealed that two cases (9.1%) were allocated in Grade 1A.The endometrium appeared normal and 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 (Fig 1a). Twelve cases (54.5%) were assigned in Grade 2B. There was a necrobiotic change in the endometrial epithelium. The lumens of most uteri occupied with desquamated cells and few numbers of inflammatory cells. The endometrial stroma 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 (Fig 1b). Sometimes, few cells were seen in the lumen of the glands. The large and medium sized blood vessels showed hyalinisation of the tunica media. Mild to moderate degrees of fibrosis of the endometrial stroma occur in most cases. The fibrous tissue was arranged in whorls of 1-10 layers around the endometrial glands (Fig 1c). In some cases, the endometrial glands were dilated by accumulations of secretions and desquamated cells (Fig 1d).

Grade 3A was observed in eight cases (36.4%). The endometrial surfaces of most uteri were completely necrosed. The endometrium stroma showed marked mononuclear infiltration, with increase in number of lymphocytes and macrophages (Fig 2a). 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, epitheloids and small lymphocytes, situated through the endometrium were seen (Fig 2b). The endometrial stroma was severely fibrosed in most cases. The fibrous tissue was arranged in whorls of more than 10 layers around the endometrial glands (Fig 2c). Most of the endometrial

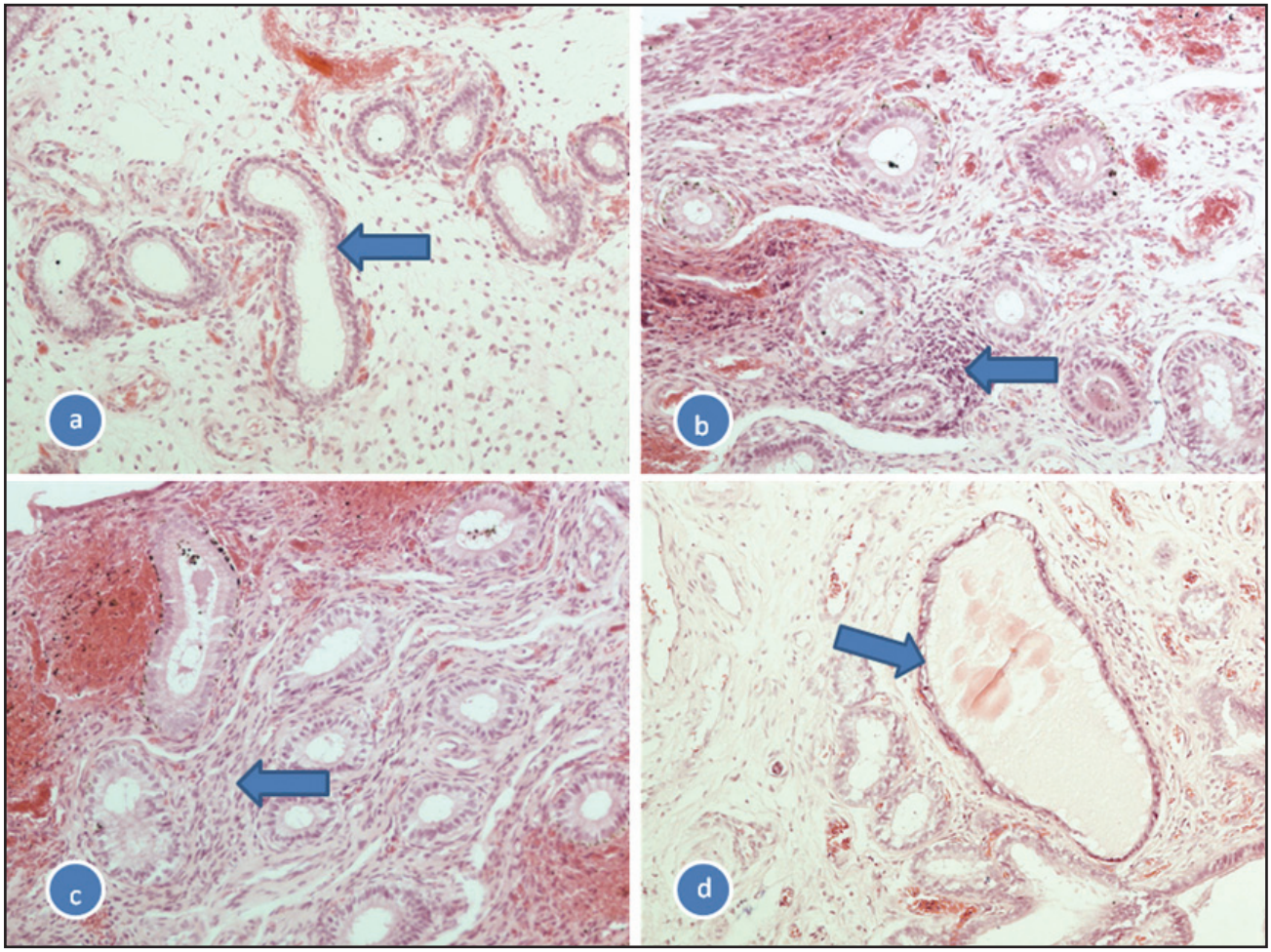

Fig 1. **a:** Normal endometrium, notice normal uterine glands (arrow). H&E. X200. **b:** Grade 2B, notice mononuclear cells around uterine glands (arrow). H&E. X200. **c:** Grade 2B, notice fibrous tissue arranging in whorls of 1-10 layers around the glands (arrow). H&E. X200. **d:** Grade 2B, notice accumulations of secretions inside the dilated endometrial glands (arrow). H&E. X200.

glands appeared cystic with accumulations of secretions and others were fibrosed and completely disappeared (Fig 2d). The large and medium sized blood vessels showed also hyalinisation of the tunica media.

Discussion

Various uterine disorders have been described in camelids and may play an important role in reduced fertility in these species (Tibary and Anouaassi, 1997a). This study revealed that 25% (22 out of 88) animals suffered from infertility were clinically diagnosed as suffering from endometritis. Uterine infections are considered to be the most common cause of reproductive failure in camelidae (Tibary and Anouassi, 2001; Tibary, 2004; Wernery and Kumar, 1994; Ali *et al,* 2010). There is a lack of standardised method for diagnosis (Tibary, 2008). Endometrial biopsy is still a relatively poorly studied technique in this species (Tibary, 2008). In the current study, neither disturbance in the general health condition nor bleeding was recorded in biopsied animals. These findings are in agreement with other studies in llamas (Powers *et al,* 1990), cows (Chapwanya *et al,* 2010) and mares (Van Camp, 1988) that concluded that endometrial biopsy is safe and reliable if it is performed with due care. On other hand Betsch (2000) reported a slight bleeding from the uterine biopsy in mares. Gross and LeBlanc (1984) found seasonal changes of the endometrium affected quantitative assessment of fibrosis in mares. Two cases out of 22 (9.1%) were diagnosed by histopathological examination as noninflammed. This means overestimation of the endometritis diagnosed with rectal examination combined with sonography when compared with histopathological examination. Traditional assessment of the dromedary uterus involves palpation per

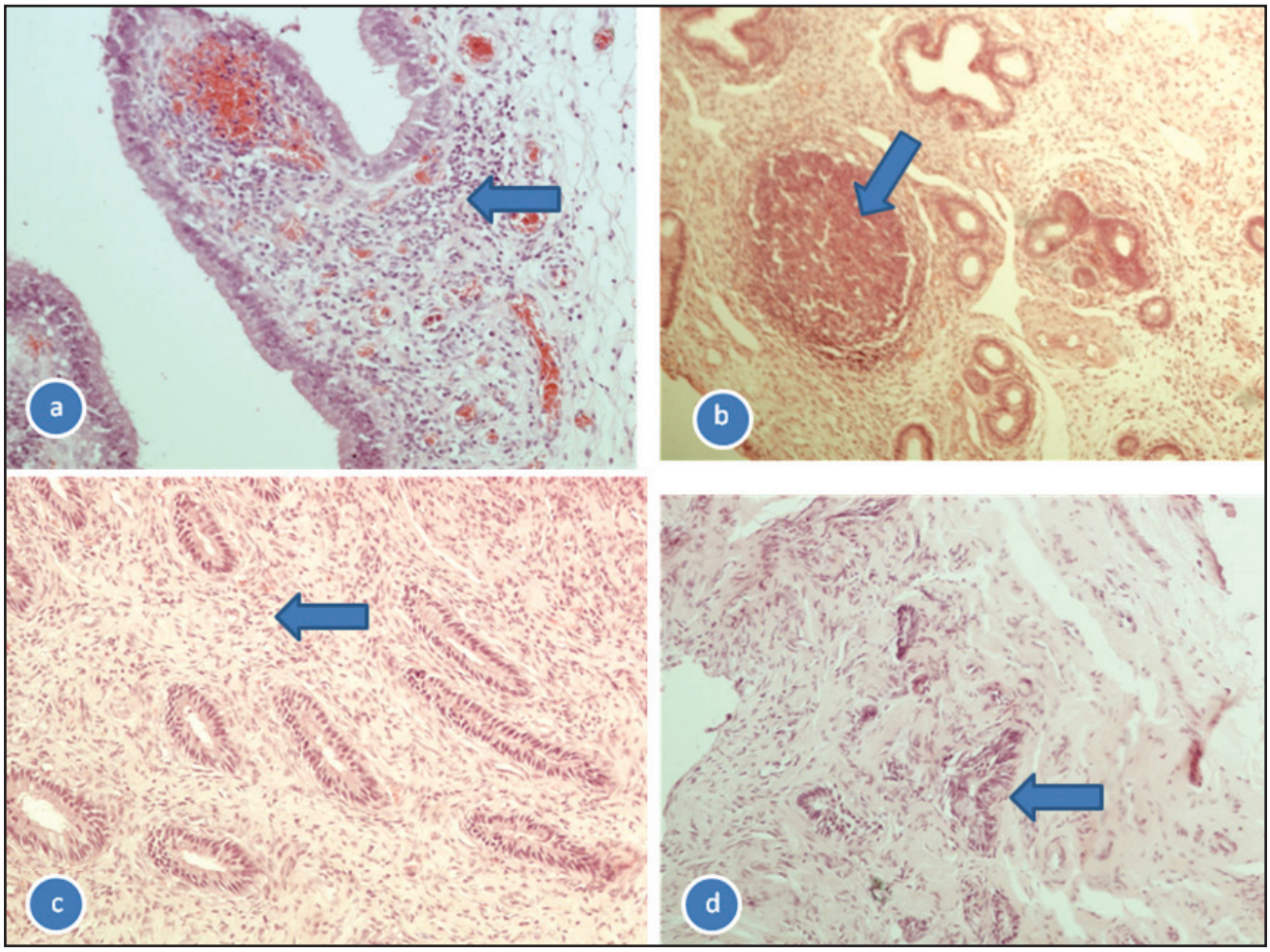

Fig 2. **a:** Grade 3A, notice mononuclear cells infiltration underneath the endometrium epithelium (arrow). H&E. X200. **b:** Grade 3A, notice granulomatous foci situated through the endometrium (arrow). H&E. X200. **c:** Grade 3A, notice fibrous tissue arranging in whorls of more than 10 layers around the endometrial glands (arrow). H&E. X200. **d:** Grade 3A, notice atrophy and complete fibroses of the endometrial glands (arrow). H&E. X200.

rectum and/or transrectal ultrasonographic imaging (Anouassi, 2001). These techniques are limited as they do not obtain tissue for a detailed and objective analysis of the pathologic processes occurring. In dromedaries, Waheed *et al* (2009) pointed to 4.17% normal histological specimens. In llamas, Powers *et al* (1990) reported 16.70% of normal histologic specimens. In present study, single biopsy sample was collected for diagnosis of endometritis. Waelchli *et al* (1989) concluded that in most cases a single biopsy examination, in combination with a thorough clinical examination, should be adequate to assess the mare's endometrium. Both Ricketts (1979) and Bergman and Kenney (1975) suggested that in absence of any palpable abnormality of the uterus, a single mid-horn biopsy sample would be representative of the histopathology of the entire endometrium. Blanchard *et al* (1987) reported that inter-sample variability could occur in only 1 of 60 samples. The current study used 10% buffered neutral formalin as a fixative for the biopsied tissues. 10% buffered neutral formalin is reported as a satisfactory fixative for the uterine tissues (Van Camp, 1988). Other author (Sertich, 1996) prefers using Bouin's fixative because it produces a firmer specimen than formalin, with less tissue distortion and artifact on sectioning. In dromedaries, histological changes of the uterus in connection with an endometritis have been reported by various scientists (Hegazy *et al,* 1979; Laila *et al,* 1987 and Fetaih, 1991). However, the results of these investigations exclusively stem from slaughtered camels with no reproductive history. So far dearth reports are available of uterine biopsies taken from living dromedary camel. In agreement with persent results of the microscopic examination of uterine biopsies in grade 1A (normal uterus), Strikankumar *et al* (2001) and Porjoosh *et al* (2010) reported that the endometrial lining consists of a single layer of

columnar epithelium supported by a broad highly cellular connective tissue stroma with simple tubular glands. Parallel with other studies (Waelchli *et al,* 1988; Strikankumar *et al,* 2001), the polymorphonuclear cells were detected in the endometrial epithelium and stratum compactum in the histological sections in different categories of endometritis.

Conclusion

Dromedary endometrial biopsy is a safe and reliable technique for providing detail on endometrial inflammation.

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