Original Research

Endometrial Cytology in the Diagnosis of Uterine Pathology in Canines

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Abstract

Endometrial cytology reflects the structural and physiological status of the endometrium in bitches. Forty bitches brought with the history for breeding management, uterine pathology and infertility were taken for the study and divided into four groups (n=10 each) as Group I,II,III and IV which included animals with normal reproductive capacity at any stage of estrus cycle (proestrus, estrus, diestrus and anestrus), cystic endometrial hyperplasia (CEH), pyometra and animals with reproductive failure (failed to conceive even after 3 consecutive cycles of breeding with proven male dogs) respectively. The study proved that the cellular pattern and morphological features in endometrial cytology changed throughout the reproductive cycle and in pathological conditions of pyometra and Cystic Endometrial Hyperplasia (CEH) with strong statistical variation. Endometrial cytology can be employed successfully as a diagnostic aid to detect infertility in bitches due to uterine pathology before the onset of clinical signs.

Key words: Bitches, Endometrial Cytology, Infertility, Uterine Pathology

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Introduction

Subfertile or infertile conditions in canines are mainly due to improper breeding management but many other cryptic factors also play a role. The cervical cytological examination in women (Buccoliero *et al.*, 2007) and endometrial examination in cows (Kasimanickam *et al.*, 2004) were studied. Endometrial cytology is considered as a major diagnostic procedure used to evaluate possible reproductive lesions in humans and domestic animals (Dubuc *et al.*, 2010), but same is not true with canines due to manipulative difficulties of canine reproductive tract, collection of endometrial samples from animals *in-vivo* poses a problem. After the innovations of vaginoscopic instruments coupled with video aide helps in manipulations through trans-cervical cannulations and ease in endometrial sample collections. The primitive cytological

population in canines are normally represented by endometrial epithelial cells, erythrocytes, neutrophils, lymphocytes, eosinophils, macrophages, plasma cells and cervical or incidental vaginal cells of varied proportions and morphology are considered as differentiate indicators of normal reproductive status to diseased ones (Groppetti *et al.*, 2010). The present study was conducted to evaluate the utilization of the endometrial cytology in detection of various uterine pathology in canines.

Materials and Methods

Forty bitches aged 2-8 years of different breeds brought to Madras Veterinary College Teaching Hospital for breeding advice and also with the history of uterine pathology and infertility were selected based on reproductive history, clinical signs, vaginal exfoliative cytology, radiography and ultrasonography of uterus and divided into four groups of 10 each as Group I bitches with normal reproductive capacity at any stage of estrous cycle (proestrus, estrus, diestrus and anestrus); Group II bitches with cystic endometrial hyperplasia (CEH); Group III bitches with pyometra and Group IV bitches with reproductive failure and that failed to conceive even after 3 consecutive cycles of breeding with proven male. Endometrial samples were collected from the bitches using transcervical cannulation technique in standing posture as per Watts *et al.* (1998) using a rigid 3 mm diameter cytoscope (Storz, Karl Storz – Endoskope, Germany) fitted with channel operating sheath of 5 mm diameter and a canine insemination catheter of 5 French diameter with a length of 69 cm. The endoscope was lubricated and inserted into the vagina and cervical os was identified at the end of vaginal median fold behind the cervical tubercle. The catheter was introduced through the operating sheath and advanced through the cervical canal into the uterine body. One to 3 ml of sterile warm saline solution was introduced into the uterus for lavage and aspirated back gently. The fluid thus obtained was centrifuged at 4500 rpm for 15 minutes and 10µL sediment was spread on glass slide and stained with Haematoxylin and Eosin. The stained smears were examined under microscope and each slide was analyzed for the presence of single endometrial epithelial cells, groups of normal and degenerated endometrial epithelial cells, naked nuclei, erythrocytes, neutrophils, lymphocytes, eosinophils, macrophages, plasma cells and amorphous material. The assessment of the number of different cellular types was carried out by counting the cells present in 20 fields per slide at 100 X and calculating the mean number of cells per field. Statistical analysis of data was performed by using computer software SPSS Version 20 (International Business Machine (IBM) corp., Chicago, USA).The percentage of cellular distribution (mean ± SE) of different types of cell/field were performed by Independent Student's 't'test.

Result and Discussion

Presence of single endometrial epithelial cells, groups of normal and degenerated endometrial epithelial cells, naked nuclei, erythrocytes, neutrophils, lymphocytes, eosinophil's, macrophages and plasma cells in each field were evaluated and statistical differences were tabulated as mean ± SE in Table 1 and 2.

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Table 1: Mean ± SE Cellular distribution of uterine smears in Group I, II, III and IV bitches

Group	Stage	Normal group of cells	Deg. group of cells	Naked nuclei	Single cells	Erytho	Neutro	Lympho	Eosino	Plasma cells	Macro	Cervical cells
I	Proestrus	3.5±0.2	0.10±0.2	4.9±0.3	2.0±0.4	1.3±0.1	4.4±0.7	0.1±0.09	0.02±0.1	0	0	0
	Estrus	2.1±0.3	0	2.1±0.2	1.0±0.3	1.1±0.1	1.5±0.6	0.02±0.1	0.01±0.02	0	0	0.04±0.02
	Diestrus	3.1±0.3	2.1±0.2	1.8±0.6	2.8±0.4	1.4±0.1	1.0±0.7	0	0	0	0	0.04±0.01
	Anestrus	0.1±0.2	2.0±0.2	1.4±0.6	1.1±0.2	1.2±0.1	0.3±0.9	0.1±0.1	0	0	0.8±0.2	0
II	CEH	0.2±0.3	1.6±0.2	0.2±0.6	2.0±0.4	1.0±0.1	0.5±0.3	0	0	0	0	0
III	Pyometra	0.05±0.3	0.9±0.2	0.3±0.7	0.8±0.4	1.2±0.1	60.2±4.9	2.1±0.1	0	2.2±0.2	1.9±0.3	0
IV	Case 1	0.2±0.3	1.5±0.2	0.3±0.6	2.0±0.3	1.0±0.1	0.5±0.4	0	0	0	0	0
	Case 2	0.3±0.2	1.6±0.2	0.2±0.6	2.1±0.4	1.0±0.1	0.4±0.3	0	0	0	0	0
	Case 3	0.3±0.1	1.4±0.2	0.2±0.5	2.0±0.3	1.0±0.1	0.4±0.3	0	0	0	0	0
	Case 4	0.04±0.2	0.7±0.2	0.3±0.6	0.8±0.4	1.2±0.1	60.4±4.7	2.1±0.1	0	2.2±0.2	1.9±0.3	0
	Case 5	0.05±0.3	0.8±0.2	0.3±0.7	0.7±0.4	1.2±0.1	60.2±4.8	2.1±0.1	0	2.2±0.2	1.9±0.3	0
	Case 6	3.0±0.3	2.1±0.3	1.7±0.5	2.8±0.4	1.4±0.1	1.0±0.7	0	0	0	0	0.04±0.01
	Case 7	0.1±0.2	2.0±0.2	1.4±0.6	1.1±0.2	1.2±0.1	0.3±0.9	0.1±0.1	0	0	0.8±0.2	0
	Case 8	0.1±0.2	2.0±0.2	1.4±0.6	1.1±0.2	1.2±0.1	0.3±0.9	0.1±0.1	0	0	0.8±0.2	0
	Case 9	3.1±0.3	2.1±0.2	1.8±0.6	2.8±0.4	1.4±0.1	1.0±0.7	0	0	0	0	0.04±0.01
	Case10	0.1±0.2	2.0±0.2	1.4±0.6	1.1±0.2	1.2±0.1	0.3±0.9	0.1±0.1	0	0	0.8±0.2	0

CEH-Cystic Endometrial Hyperplasia; PYO-Pyometra; Deg.group-Degenerated group of cells; Erythro-Erythrocytes; Neutro-Neutrophils; Lympho- Lymphocytes; Eosino-Eosinophils; Macro-Macrophages; Cer.Cells-Cervical cells.

Table 2: Statistical analysis of cellular distribution of uterine smears in Group I, II, III and IV bitches

Stage	Normal group of cells	Deg. Group of cells	Naked nuclei	Single cells	Erytho	Neutro	Lympho	Eosino	Plasma cells	Macro	Cer. Cells
PE vs ES	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.000	-	0.000
ES vs DE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.000
DE vs E	0.000	0.134 NS	0.000	0.000	0.002	0.000	0.000	-	-		-
PE vs AE	0.000	0.000	0.000	0.000	0.024	0.000	0.356 NS	0.000	-	0.000	0.000
DE vs CEH	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
DE vs PYO	0.000	0.000	0.000	0.000	0.008	0.000	0.000	-	-	0.000	-
CEH vs PYO	0.000	0.000	0.134 NS	0.000	0.134 NS	0.000	0.000	-	-	0.000	-
CASE 1 vs DE	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-	-
CASE 1 vs CEH	1.000 NS	0.024	0.000	0.000	-	0.134 NS	-	-	-	-	-
CASE 1 vs PYO	0.000	0.000	0.234 NS	0.000	0.134 NS	0.000	0.000	-	-	0.000	-
CASE 2 vs DE	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
CASE 2 vs CEH	0.000	0.356 NS	1.000 NS	0.000	-	0.134 NS	-	-	-	-	-
CASE 2 vs PYO	0.000	0.000	0.134 NS	0.000	0.134 NS	0.000	0.000	-	-	0.000	-
CASE 3 vs DE	0.000	0.000	0.000	0.000	0.000	0.000	-	-	-	-	-
CASE 3 vs CEH	0.000	0.000	1.000 NS	-	0.000	0.134 NS	-	-	-	-	-

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CASE 3 vs PYO	0.000	0.000	0.134 NS	0.000	0.134 NS	0.000	0.000	-	-	0.000	-
CASE 4 vs DE	0.000	0.000	0.000	0.000	0.002	0.000	0.000	-	-	0.000	-
CASE 4 vs CEH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-	0.000	-
CASE 4 vs PYO	0.000	0.000	0.134 NS	0.356 NS	0.134 NS	0.854 NS	0.134 NS	-	-	0.000	-
CASE 5 vs DE	0.000	0.000	0.000	0.000	0.002	0.000	0.000	-	-	0.000	-
CASE 5 vs CEH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-	0.000	-
CASE 5 vs PYO	1.000 NS	0.000	0.134 NS	0.024	0.134 NS	0.430 NS	0.000	-	-	0.000	-
CASE 6 vs DE	0.134 NS	0.134 NS	0.134 NS	0.356 NS	1.000 NS	-	-	-	0.000	-	-
CASE 6 vs CEH	0.000	0.000	0.000	0.000	0.000	0.000	-	-	0.000	-	0.000
CASE 6 vs PYO	0.000	0.000	0.000	0.000	0.008	0.000	0.000	-	0.000	0.000	0.000
CASE 7 vs AE	1.000 NS	-	1.000 NS	0.356 NS	1.000 NS	1.000 NS	1.000 NS	-	-	0.000	-
CASE 8 vs AE	1.000 NS	-	1	-	1.000 NS	1.000 NS	1.000 NS	-	-	0.000	-
CASE 9 vs DE	0.134 NS	0.134 NS	0.134 NS	0.390 NS	0.134 NS	-	-	-	0.000	-	0.210 NS
CASE 9 vs CEH	0.000	0.000	0.000	0.000	0.000	0.000	-	-	0.000	-	0.210 NS
CASE 9 vs PYO	0.000	0.000	0.000	0.000	0.002	0.000	0.000	-	0.000	0.000	0.210 NS
CASE 10 vs AE	1.000 NS	-	1.000 NS	-	1.000 NS	1.000 NS	1.000 NS	-	-	0.000	-

During proestrus and estrus, the endometrial epithelial cells were seen as single or as cellular nests with uniformly stained cytoplasm and regularly shaped nucleus. Neutrophils and lymphocytes were higher during proestrus than estrus. Some cervical cells were evident during estrus period than that of proestrus stage (Plate 1). Diestrus was characterized by the presence of both normal and degenerated endometrial epithelial cells with cytoplasm filled with large lipid droplets and pyknotic nucleus.

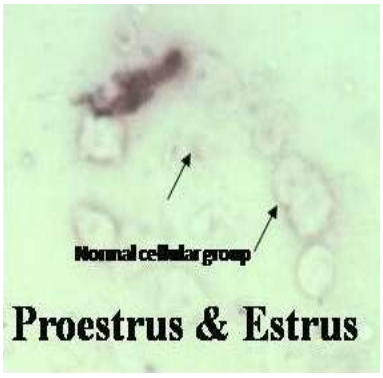

Plate 1: Proestrus and estrus - showing presence of single, normal cellular nests of cells with uniformly stained cytoplasm and regularly shaped nucleus

Plate 2: Diestrus showing presence of both normal and degenerated endometrial epithelial cells with cytoplasm filled with large lipid droplets ('foamy' appearance)

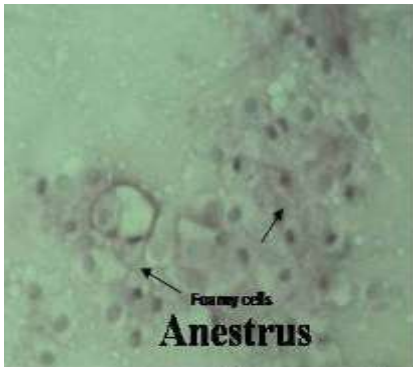

Plate 3: Anestrus showing presence of intact cytoplasm and degenerated nuclei and the cells appear foamy

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The epithelial cells showed a typical 'foamy' appearance during diestrum of estrous cycle in bitches. Neutrophils and erythrocytes were also seen during diestrum of bitches (Plate 2). Anestrus was differentiated from other stages of estrus cycle with endometrial epithelial cells containing intact cytoplasm and degenerated nuclei. Sometimes cells appeared foamy along with other type of cells like macrophages, neutrophils and erythrocytes (Plate 3). This is in agreement with the studies of Groppetti *et al.* (2010) in different stages of reproductive cycle in dogs and Nishikawa (1985) in pyometra.

CEH was characterized by the presence of degenerated endometrial epithelial cells with cytoplasmic droplets and pyknotic nuclei. Sometimes amorphous materials surround the cells which may be due to presence of cystic conditions. Rarely neutrophils and erythrocytes were also incident (Plate 4). Predominance of neutrophils with few endometrial epithelial cells in degenerative process were present in pyometra with few incidence of plasma cells and binucleate cells (Plate 5). Neutrophils are present in large amounts indirectly represents the presence of numerous chemoattractant producing organisms whereas, plasma cells produce antibodies and is indicative of chronic inflammatory condition (Groppetti *et al.*, 2010).

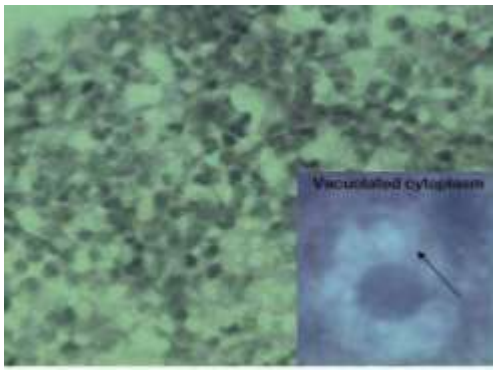

Plate 4: Presence of degenerated endometrial epithelial cells with cytoplasmic droplets and pyknotic nucleiindicative of CEH

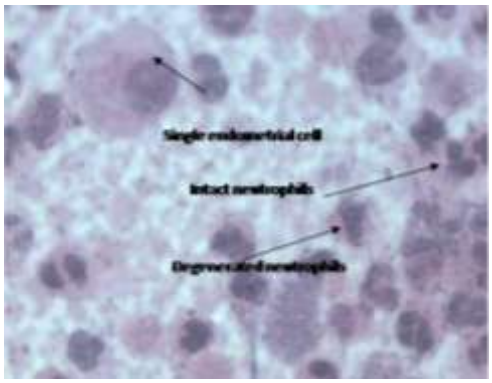

Plate 5: Predominance of neutrophils few endometrial epithelial cells in a degenerative process indicative of pyometra

The endometrial cytology of Case 1, 2 and 3 revealed the presence of degenerated endometrial epithelial cells with cytoplasmic droplets and pyknotic nuclei and other cell types including erythrocytes and neutrophils. The statistical data confirmed the correlation of these three cases with CEH with respect to cellular population in endometrial cytology. Similarly, Case 4 and 5 showed predominance of neutrophils with plasma cells and macrophages. Few degenerated endometrial epithelial cells and single cells were also encountered in these cases which concurred with the studies of Riddle *et al.* (2007). Though there was no evidence of clinical signs and visible diagnostic evidence of uterine pathology through ultrasongraphy and X-ray in the cases of 1, 2, 3, 4 and 5 in group IV, the endometrial cytology shows correlation of the first 3 cases in group IV towards CEH and cases 4 and 5 in group IV towards pyometra, respectively. These cases may undergo either cystic endometrial hyperplasia or pyometra or CEH-Pyometra complex in due course

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of time with clinical incidences. This might be one of the reasons for the bitches to be infertile prior to clinical onset of pathological conditions and thus endometrial cytology may help to detect the uterine cause of infertility in bitches.

The Case 7, 8 and 10 of endometrial cytology revealed the endometrial epithelial cells containing intact cytoplasm and degenerated nuclei and cells appear foamy along with other type of cells like few macrophages, neutrophils and erythrocytes. The cellular population of Case 7, 8 and 10 in group IV is similar to anestrus stage of estrus cycle in bitches. Similarly the case 6 and 9 of endometrial cytology evidenced the presence of both normal and degenerated endometrial epithelial cells with cytoplasm filled with large lipid droplets, pyknotic nuclei and cells with 'foamy' appearance. The case 6 and 9 are more in resemblance with diestrum stage with respect to cellular population of endometrial cytology. There was no significant difference ($P>0.05$) between case 1, 2, 3 and CEH group; case 4, 5 and pyometra group; case 6, 9 and diestrum and case 7, 8, 10 and anestrum in group IV and group I, respectively. Diagnostics of canine infertility depends upon the etiological factors of each cause. Ultrasonography and radiographic evaluation has been used for diagnosis of pyometra and cystic endometrial hyperplasia since many years, but useful only after the onset of the diseases. Hemogram and biochemical analyses help in ruling out basic deficient phases whereas appropriate genital tract abnormalities go unnoticed many times. This technique is a non-invasive, non-surgical and performed in non-sedated animals. Advantage of endometrial cytology lies in the fact of diagnosing the abnormality causing infertility before the onset of clinical signs so that appropriate measures are taken in prior.

Conclusion

Endometrial cytology revealed the difference between the type of cellular population among various stages of reproductive cycle in bitches and pathology conditions like pyometra and cystic endometrial hyperplasia and helpful in correlating with the physiological and pathological status in normal bitches and with bitches with reproductive failure prior to the onset of clinical signs. Thus, the present study concludes that, endometrial cytology can be employed as one of the non-invasive diagnostic aid to detect infertility due to uterine pathology in bitches even before the onset of clinical signs.

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