THE RELATIONSHIP BETWEEN ENDOMETRIAL BIOPSY, CYTOLOGY, AND BACTERIAL PATHOGENS IN MARES

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Submitted to the Faculty of the Graduate College
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in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
July, 1984

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Thesis Approved:

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PREFACE

This study was designed to investigate the potential use of endometrial cytology in mares. The occurrence of cells and specific cell processes in cytology specimens was described and compared to cells and cell processes in the corresponding tissue specimens. These findings were correlated with endometrial pathogens reported from uterine cultures. The findings described could serve as a guide for evaluation of clinical specimens and as the basis for further research.

The author wishes to thank his major adviser, Dr. Jeffie F. Roszel, Dr. Kathy Peterson Freeman, and Marlene Castro for their assistance in this study.

A note of thanks is given to Mr. Jim Hellwege for his patience and excellent typing of this report.

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CHAPTER I

INTRODUCTION

History

Since the replacement of the horse for transportation by the internal combustion engine, the use of horses has changed. Today, the horse is used for pleasure, show, and sporting events. The reproductive efficiency of the horse has not kept pace with the increasing demand for quality horses. Unlike food animals, equine fertility has remained low. Within the total North American horse population, the number of foals delivered per number of broodmares seldom exceeds 60% in any given breeding season (34, 35, 38, 60, 65). Reasons for infertility are related to a seasonal breeding pattern of man-imposed breeding before initiation of the physiologic breeding season, lack of genetic selection of animals to increase fertility, failure to utilize artificial insemination techniques, limited reproduction research, and counterproductive management practices. This research endeavor was undertaken to increase knowledge of the equine uterus and, when combined with other efforts, should expand the overall knowledge of equine reproduction and hopefully increase fertility.

Endometrial Cytology

Veterinary cytology has evolved from human cytology. Cytology of the reproductive tract of women has contributed to early diagnosis

of cervical carcinoma. The squamocolumnar junction near the ectocervix in women has been the most prominent area from which carcinomas arise that may lead to death within five years if not detected and treated (33). Papinacolaou developed a simple cytologic technique to be performed on the human cervix that provided evidence of cellular changes indicative of in situ carcinoma and invasive carcinoma (33) which is referred to as the "Pap smear" and is routinely used today (33). His technique involved obtaining epithelial cells, fixation and staining, and examination for microscopic cellular changes. With appropriate genital smears, it has been possible to distinguish phases of the normal menstrual cycle, inflammatory alterations in the uterus or cervix, the presence of intrauterine birth control devices, hormonal aberration, and early cancerous changes or invasive carcinoma (33). Knowledge of these subtle pathologic changes prompted veterinary investigators to apply these techniques to cytologic diagnosis of cancer in dogs and cats (46, 47). Hence, reports of techniques for collection and staining of specimens from the reproductive tract and other body organs of dogs and cats have been made. The results of these interpretations of animal cytologic specimens have promoted the diagnosis of cancer and increased the chance of a cure in some pets (48).

Besides the use of exfoliative pathology in the female genital tract to be discussed in part in this thesis, exfoliative vaginal cytology from the bitch has been used to detect normal estrous cycle changes and predict optimum breeding time, indicate threatened abortion, determine fetal death or incomplete abortion, diagnose subinvolution of placental sites, and indicate rupture of fetal membranes (48).

The use of exfoliative cytology diagnostic techniques has been

applied to equine cerebrospinal fluid (7), peritoneal aspirates (1, 6, 37), thoracic aspirates (56), tracheal aspirates (8, 9), skin cysts (23), and vaginal or uterine samples (4, 15, 21, 32, 54, 57, 62, 70, 73).

In most instances, the thrust of the research in equine genital specimens has been to quantify inflammatory cells. Some workers felt that changes in reproductive tract epithelial cells may indicate pathological processes (32, 54, 57, 71) or that other cellular changes might indicate pathological processes (9, 23, 54). Cytology has been used to classify epithelial cellular changes during the phases of the equine estrous cycle (32). Protozoa from gastrointestinal tract contamination and intranuclear eosinophilic inclusion bodies compatible with viral infection have been reported in cytology specimens from the cervicovaginal junction (21).

Endometrial Culture

Early diagnosticians of mare infertility directed their efforts towards the effects of mechanical and anatomical problems as they related to bacterial or fungal pathogens in the reproductive tract. Infection in the mare's reproductive tract was thought to be acquired from the bloodstream, local spread from foci of deep-seated endometritis, or ascending infections from the external genitalia (67). Other sources of infection included contamination during breeding from the stallion harboring pathogens without clinical evidence of infection (29). In one study, a large number of mares were found to be infected within 24 hours of breeding yet failed to manifest infertility (16). In addition, anatomical or mechanical changes in the mare's reproductive

tract may promote bacterial infection (3, 40, 45). These defects include poor vulvar tone or conformation of the perineum permitting aspiration of air into the reproductive tract, urethral defects including urine pooling and rectovestibular or perineal lacerations, or vaginal and cervical scars and adhesions. The existence of these conditions promotes intermittent bacterial contamination of the reproductive organs and frustrates the clinician attempting to diagnose the primary cause of infertility.

Equine uterine infection has occupied considerable clinical attention because endometrial pathogens prevent conception (29), result in abortion at various stages of gestation (26, 29, 67), cause birth of weak or stillborn foals (17, 42, 67), lead to disease or retention of the placenta (42, 67), or create postpartum dam infertility (45).

Various methods have been used to detect reproductive tract pathogens in mares, the earliest of which were cultures of the vagina, external cervical os, or the cervical canal (45). It was subsequently found that vaginal or cervical pathogens may not indicate uterine infection (14, 51) or affect mare fertility (26, 29, 51, 67). Pathogens can exist in the mare's uterus and escape detection (59) or infertility exist undetectable with routine sampling techniques (36, 69, 72). A double-guarded swab technique has been developed to reduce contamination during sampling (12).

There does not appear to be a resident bacterial flora within the mare's uterus (51). A pathogen's ability to remain in the uterus depends on local uterine resistance (5, 68) and levels of gonadal hormones (13, 22, 29).

Bacterial infection in the mare's uterus stimulates an inflammatory

response evident in both the endometrial biopsy (12, 27, 28, 30, 41, 72) and the uterine cytology (45, 53, 62, 71, 73). It is also possible to isolate bacterial pathogens from the mare's uterus without histologic evidence of inflammation (73) or apparent loss of fertility (29). Conversely, endometritis may be evident on uterine biopsy but the endometrial cultures negative for pathogens (73). Cytology specimens have indicated endometritis as a cause for infertility when the biopsy and culture were inconclusive (4, 58, 72, 74).

Endometrial Biopsy

Genital cancer in women during child-bearing years often arises from the vaginocervical junction. Cytologic specimens from this location have been used to diagnose carcinoma and carcinoma in situ (18). Endometrial biopsy may be used for diagnosis of endometrial cancer in postmenopausal women (33). These tumors are often missed with routine cytology because the cells are not shed. Human uterine biopsies have been used to establish hormonal disorders through staging of the menstrual cycle (18, 19, 39, 60) and have also been used to determine viability of early pregnancy (60, 67).

Equine endometrial biopsy has evolved from the veterinary clinician's desire for more knowledge of mare infertility. The clinical use of the endometrial biopsy as an aid in mare fertility prediction has been reported (14, 20, 24, 30, 31, 43, 44, 49, 53). The representativeness of a single sample from the mare's uterus has been reported (11). Problems with correlation between the endometrial biopsy and culture have been considered (12, 14, 20, 24, 30, 49). The effect of the estrous cycle and anestrus on the histologic appearance of the

equine endometrial biopsy has been reported (14, 24, 30, 49, 65). Serial biopsy of the equine endometrium has also been used to monitor normal uterine involution (25).

CHAPTER II

MATERIALS AND METHODS

Collections

The endometrial cytology specimens were collected first, then the culture obtained, and the uterine biopsy collected last. This order was maintained in all cases.

Uterine Cytology

In each of the 163 clinical cases, a history was recorded and the external genitalia inspected. Each mare was restrained in a horse stocks and the tail wrapped and secured to prevent contamination or obstruction of examination of the genitalia. The reproductive organs were examined by rectal palpation then the vulva and perineum prepared using a three-minute scrub with a tamed iodine soap (a). The vestibule, vagina, and cervix were examined through a speculum.

A uterine aspirate was collected using sterilized shoulder-length gloves (b). A sterile mare infusion pipette (c), guarded by the gloved hand, was guided through the vagina into the uterus. Fifty milliliters (ml) of sterile, normal 0.9% sodium chloride (d) was rapidly injected into the uterus using a 60-ml syringe attached to the pipette. The collector's index finger remained through the mare's cervix and gently manipulated the tip of the pipette backward and forward and from side to side as negative pressure was applied. Manipulation was necessary

to prevent occlusion of the tip by endometrial folds. Usually, 1 to 5 ml of fluid was aspirated, despite the larger volume infused. Following guarded withdrawal, the aspirate in the syringe was injected into a test tube containing approximately 5 ml of 40% ethanol preservative. If the volume of aspirate was contained only in the pipette, the syringe was detached while the free end of the pipette was in the test tube, allowing the aspirate to flow into the alcohol preservative.

Between two and four smears were prepared from the centrifuge sediment. The cells were fixed and stained by a method previously described (49). All material on each slide was examined using overlapping horizontal fields. The slides were screened at 40X magnification and points of interest examined at 60X magnification. A specimen was considered unsatisfactory if there were virtually no cells or if they were cellular with no epithelial cells present. Notation was made of cellularity, number, types, and morphologic features of cells and extracellular material. The amount of material and number of cells were recorded according to a subjective evaluation. Findings were recorded on a standard form (Appendix I). Gomori's Methenamine Silver (GMS) was used on a few cases.

Uterine Culture

The method of collection of culture swab from the uterus involved passage of the sterile guarded culture swab (e) in the fashion described for the infusion pipette. The end of the culture pipette was passed into the uterus until resistance was felt; then, the culture swab was pushed ahead of the pipette against the wall of the endometrium and rotated several times to collect a sample. The culture swab then was

pulled back into the guarded pipette, withdrawn through the cervix into the collector's hand, and guarded during removal through the vagina. The culture swab was immediately transferred to a sterile culturette containing transport medium (f).

Uterine Biopsy

The method of collection of the uterine biopsy was as previously described (30) with some modifications. The biopsy forcep (g) was introduced through the vagina and cervix into the uterus near the junction of the right horn and the body. The basket of the instrument was rotated 90°, opened, and with the operator's hand inserted into the rectum a fold of the endometrium removed.

The tissues were immediately fixed in either Bouin's solution for 30 minutes to 1 hour or neutral buffered 10% formalin for 6 to 72 hours. After processing, the untrimmed tissue was paraffin-blocked and cut at 5 microns. At least two sections from each tissue were stained with hematoxylin and eosin. All clinical evaluations were made on hematoxylin and eosin. The form used in the evaluation of the clinical cases is Appendix II. For better cellular detail, a few tissue specimens were imbedded in glycomethacrylate plastic and cut at 1.5 microns prior to staining. Some tissues were also imbedded in hard paraffin and sectioned at 2 microns. In addition to hematoxylin and eosin staining, GMS and Alcian Blue/Periodic Acid Schiff-Hematoxylin, pH 2.5 (AL/BL-PAS-H), were used on selected cases.

Interpretation of Specimens

Cytology

While the uterine biopsy is a valuable predictor of fertility, the cytology specimens provide new methods to evaluate uterine pathology. These changes are subtle and represent focal or diffuse pathology. It does not require special cutting or staining techniques to see a wide range of cell changes in the cytology specimens. The cells collected from the lumen of the uterus have been allowed to float free from the uterine epithelial lining. These cells may be seen in groups or clusters, sheets, or individually, depending on the destructive process or irritation. These changes are not apparent in the tissue because during collection, fixation, and transport loose cells are lost.

Had a specimen contained insufficient cells for interpretation, it would have been graded 0. All other cytology specimens were graded 1, 2, 3, and 4 based on the following criteria. Grade 1 cytology specimens in addition to epithelial cells had a few lymphocytes and neutrophils with few destructive cellular patterns. Grade 2 cytology specimens frequently contained histiocytes and/or eosinophils with a few lymphocytes and neutrophils; epithelial cell cohesion often was poor; and mild destructive cellular patterns often were evident. Grade 3 cytology specimens contained more inflammatory cells, and cell cohesiveness was poor with focal necrosis evident. Grade 4 cytology specimens contained a significant number and variety of inflammatory cells that often were degenerated; the cellular cohesion was poor; and there was marked evidence of necrosis. Regenerative changes

(hyperplasia) were sometimes seen with necrosis. Abnormal regenerative changes (dysplasia) were seen in grade 4. The cytology grading was not intended to predict fertility as intended with biopsy specimens. Instead, it was used to indicate the amount of cell damage and inflammation found upon examination of the specimens.

Culture

Culture swabs were streaked on enriched blood agar plates and placed in thioglycolate broth within one to three hours. Inoculated plates and broth were incubated at 37° in an atmosphere containing 10% carbon dioxide. Media was observed for growth at 24, 48, and 72 hours and appropriate subculturing done to allow identification of bacterial or fungal pathogens. Significant pathogens were reported after 72 hours. All the interpretation was done by trained laboratory personnel.

Biopsy

Uterine biopsies were graded 1, 2, 3, and 4 based on criteria previously established (24, 30). The grades indicate predicted fertility of the mare. Grade 1 refers to a mare of normal expected fertility; grade 2 indicates abnormal expected fertility based primarily on inflammation; grade 3 denotes abnormal fertility based on a combination of inflammation and slight tissue fibrosis; and grade 4 typifies abnormal fertility due to advanced irreversible fibrosis and may or may not be associated with inflammation. When reporting grades 3 and 4, it is helpful to list the probable or expected chance for the mare to carry a foal to term pregnancy. The endometrial biopsy evaluation was recorded on a standard form used in all cases.

Eosinophil Study

A study was conducted to determine the effect of deliberate introduction of air into the uterus. Four mature Quarter Horse mares were used and randomly assigned as follows. Two mares were fitted with a newly described indwelling uterine catheter (56). The free end of the catheter in mare 1 was left open theoretically to increase air flow into the uterus. The catheter in mare 2 was inserted and remained closed to minimize air aspiration into the uterus. Cytology specimens in mares 1 and 2 were collected by inserting a pipette alongside rather than through the indwelling catheter. Mares 3 and 4 did not have indwelling catheters, and cytology specimens were collected in the manner described for the clinical cases.

Biopsy and cytology specimens were collected from all mares at the start (day 1) and conclusion (day 43) of the study. After the initial uterine specimens were collected on day 1, indwelling catheters were inserted in mares 1 and 2. Thereafter, cytology specimens were collected daily on mares 1, 2, and 3 through day 7; at which time, the indwelling catheters were removed, and biopsy specimens were collected from all three mares. Mare 4 (control) had cytology and biopsy collections on day 1 and day 43 only.

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a. Betadine Surgical Scrub, Purdue Frederick Company, Norwalk, Connecticut 06856.

b. Disposable Shoulder-Length Gloves, Catalogue Number 34590, Haver-Lockhart Laboratories, Box 390, Shawnee, Kansas 66201.

c. Sterile Fusettes, Catalogue Number 20570, Haver-Lockhart Laboratories, Box 390, Shawnee, Kansas 66201.

d. 0.9% Sodium Chloride Injection USP, American McGaw, Irvine, California 92714.

e. Teigland Uterine Swab (Modified), Catalogue Number 206400, Haver-Lockhart Laboratories, Box 390, Shawnee, Kansas 66201.

f. Culture Collection Transport Tube, Precision Dynamics Corporation, Burbank, California 91504.

g. Equine Uterine Biopsy Forceps, Catalogue Number 21-3040, Narco Pilling, Delaware Drive, Fort Washington, Pennsylvania 19034.

CHAPTER III

RESULTS

The clinically significant objectives of tissue specimen interpretation were to determine the degree of fibrosis and inflammation. Fibrosis was the primary feature of tissue grades 3 and 4 while inflammation was most significant in grades 2 and 3. Tissue grade 1 indicated that no significant inflammation or fibrosis was present while a grade of 4 indicated the presence of significant fibrosis to likely prevent the uterus from supporting a fetus to term.

The objectives of cytology specimen interpretation were to evaluate the amount of epithelial damage, the degree of regeneration, and the amount and type of inflammation. Ultimately, these factors were used to predict if the mare was ready for breeding.

Uterine cultures were used to isolate significant pathogens that might explain the changes noted in tissue and cytology specimens and help in the choice of therapeutic agents.

Cytology Specimens

The parameters for grading equine endometrial biopsy specimens have been explored previously and are clearly defined (24, 30). Reports on endometrial cytology specimens have been confined primarily to numerical evaluation of inflammatory cells (32, 58, 71, 72, 74). In this study, all material on the slides was examined.

Smears From Normal Mares

The five mares in this study with no history of reproductive problems were used to establish the range of smear patterns related to the uterine response to fluctuation of ovarian hormonal levels (Tables I, II, III, IV, V). The only pattern recognized as representative of a specific phase occurred during winter anestrus. Smears collected at this time contained virtually only stripped epithelial nuclei and noncohesive cuboidal epithelial cells (Figure 1). During the active phases of the cycle, there was an admixture of cells. There were very few neutrophils and a few lymphocytes with varying amounts of mucus. The ciliated and nonciliated epithelial cells were cuboidal to columnar and were distributed as single cells, loose groups (Figure 2), or in cohesive groups which when seen in pole view are called "honeycomb" or "mosaic" arrangements (Figure 3) or when in horizontal groups are called "picket fence" arrangements (Figure 4). The nonciliated epithelial cells often had foamy cytoplasm (Figure 5). Similar cells in corresponding tissue sections were pink with AL/BL-PAS-H, pH 2.5, indicating the presence of acid mucopolysaccharides (Figure 6).

Evidence of degenerative changes was frequently present. There were small collections of necrotic debris, often mixed with mucus (Figure 7). A few columnar ciliated epithelial cells had uniformly orange cytoplasm and homogenous dark nuclei, which are features of cell death with Pollak's trichrome stain. Often, the ciliated tip of these cells appeared to be separating (Figure 8), and ciliated cytoplasmic fragments (Figure 8) were present in smears containing these orange cells. Similar cells could be seen in corresponding tissue only when the tissue was fixed in hard paraffin, sectioned at 2 microns,

TABLE I

CYTOLOGY OF FIVE NORMAL CYCLING MARES

		Estrus	Diestrus	Anestrus
Lymphocytes:	none	0	3	3
	few	4	2	2
	moderate	1	0	0
	many	0	0	0
Neutrophils:	none	1	3	1
	few	3	2	1
	moderate	1	0	1
	many	0	0	2
Eosinophils:	none	4	5	4
	few	1	0	1
	moderate	0	0	0
	many	0	0	0
Histiocytes:	none	3	4	4
	few	2	0	0
	moderate	0	1	1
	many	0	0	0
Vacuoles:	none	4	5	5
	few	1	0	0
	moderate	0	0	0
	many	0	0	0
Loose Cell Groups:	none	4	5	5
	few	1	0	0
	moderate	0	0	0
	many	0	0	0
Mitotic Figures:	none	4	5	4
	few	1	0	1
	moderate	0	0	0
	many	0	0	0
Gland Casts:	none	5	5	1
	few	0	0	2
	moderate	0	0	1
	many	0	0	1
Hemosiderin		1	0	0
Columnar		4	4	3
Cuboidal		1	1	4

TABLE II

ENUMERATION OF CELLS IN CYTOLOGIC SPECIMENS
FROM FIVE NORMAL CYCLING MARES

	Estrus	Diestrus	Anestrus
Epithelial Cells:			
Detached ciliary tufts	0	2	2
Stripped nuclei	3	4	3
Columnar	4	5	3
Cuboidal	0	0	4
Loose groups	0	1	0
Mitotic figures	1	0	1
"Picket fence"	1	0	0
"Honeycombs"	3	0	0
Cells:			
Lymphocytes	5	2	2
Neutrophils	4	3	4
Eosinophils	1	0	0
Hemosiderin	1	0	0
Histiocytes	2	1	1
Inspissated gland casts	0	1	4
Orangophilic cells	0	2	0
Chlorocytes	0	0	1

TABLE III

EPITHELIAL CELLULAR PATTERNS IN THE CYTOLOGY OF FIVE NORMAL CYCLING MARES

	Estrus	Diestrus	Anestrus
Poor Cellular Cohesion	1	2	1
Moderate Cellular Cohesion	2	0	1
Good Cellular Cohesion	1	3	2
Regeneration	1	0	0
Degeneration	1	1	3
Hyperplasia	2	0	1
Necrosis	1	1	2
Mucus Clumps	1	0	0
Epithelial Atypia	0	0	1
No Pattern Reported	0	0	1

TABLE IV

TISSUE FEATURES NOTED ON THE ENDOMETRIAL BIOPSIES FROM FIVE NORMAL CYCLING MARES

		Estrus	Diestrus	Anestrus
Lymphocytes:	few	5	4	4
	moderate	0	1	1
	many	0	0	0
Neutrophils:	none	0	1	1
	few	4	4	3
	moderate	0	0	1
	many	0	0	0
Eosinophils:	none	1	2	5
	few	2	3	0
	moderate	2	0	0
	many	0	0	0
Edema:	none	1	1	5
	slight	4	4	0
	moderate	0	0	0
Luminal Epithelium Height:	low	0	0	5
	normal	2	3	0
	tall	3	2	0
Gland Epithelium Height:	low	0	0	2
	normal	3	1	3
	tall	2	4	0

TABLE V

CYTOLOGY FEATURES IN FIVE NORMAL CYCLING MARES NOT SEEN IN THE TISSUE

		Estrus	Diestrus	Anestrus
Chlorocytes:	none	5	5	4
	few	0	0	1
	moderate	0	0	0
	many	0	0	0
Honeycomb Arrangement:	none	2	5	5
	few	3	0	0
	moderate	0	0	0
	many	0	0	0
Picket-Fence Arrangement:	none	4	5	5
	few	1	0	0
	moderate	0	0	0
	many	0	0	0
Detached Ciliary Tufts:	none	5	3	2
	few	0	0	1
	moderate	0	2	2
	many	0	0	0
Stripped Nuclei:	none	2	1	2
	few	1	0	1
	moderate	2	2	0
	many	0	2	2
Orangophilic Cells:	none	5	2	5
	few	0	2	0
	moderate	0	1	0
	many	0	0	0

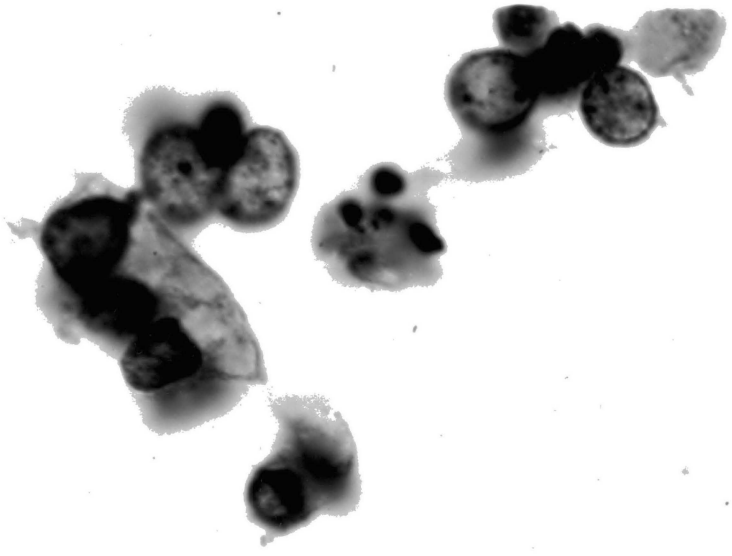

Figure 1. Endometrial smear with stripped nuclei and cuboidal epithelial cells typical of those seen during winter anestrus. (Pollak Trichrome X160)

Figure 2. Loosely arranged group of cuboidal and columnar epithelial cells in endometrial washing sediment smear. (Pollak Trichrome X160)

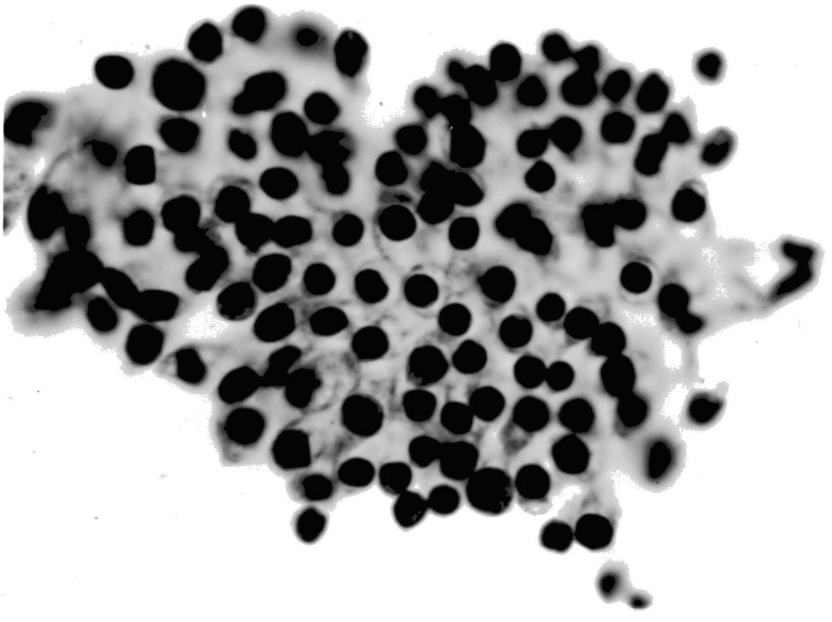

Figure 3. Pole view of endometrial epithelial cells in "honeycomb" or "mosaic" arrangement. Endometrial smear. (Pollak Trichrome X320)

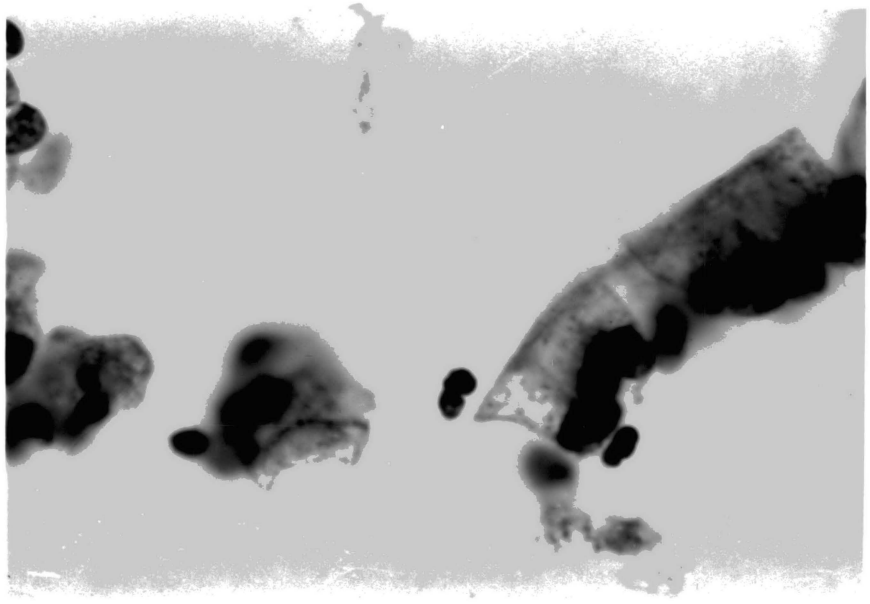

Figure 4. Row of nonciliated endometrial epithelial cells in a "picket fence" arrangement. Endometrial smear. (Pollak Trichrome X315)

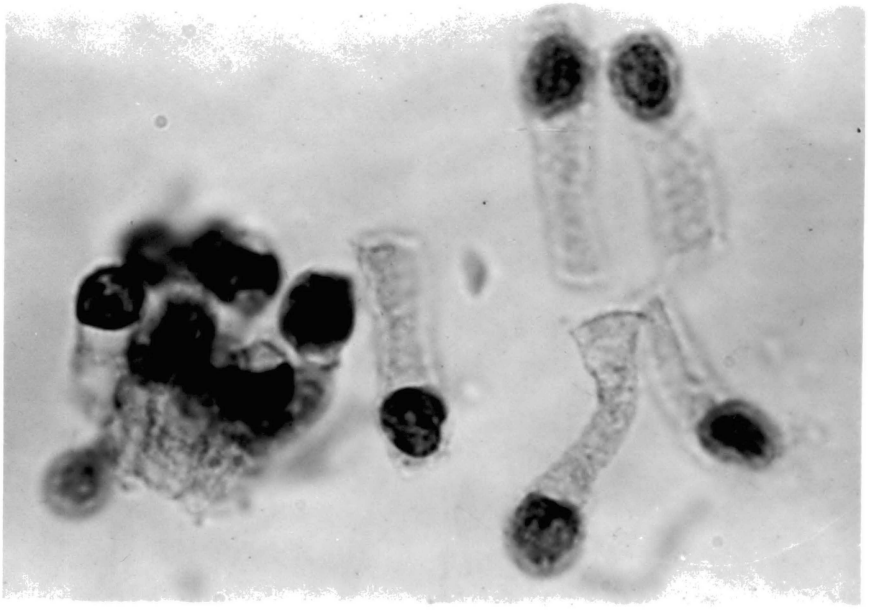

Figure 5. Tall nonciliated columnar endometrial epithelial cells with foamy cytoplasm. Endometrial smear. Compare cells with corresponding tissue in Figure 6. (Pollak Trichrome X400, oil immersion)

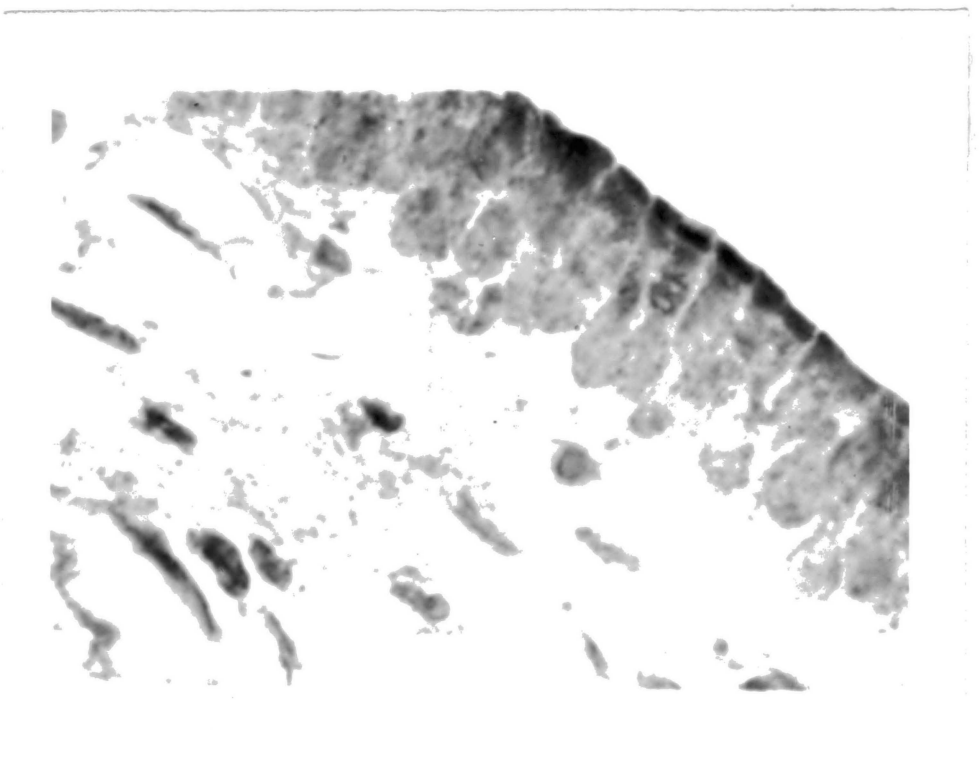

Figure 6. Uterine biopsy with PAS-positive granules in luminal epithelial cells. Compare with cells in corresponding smear (Figure 5). (AL/BL PAS/H, pH 2.5, X400)

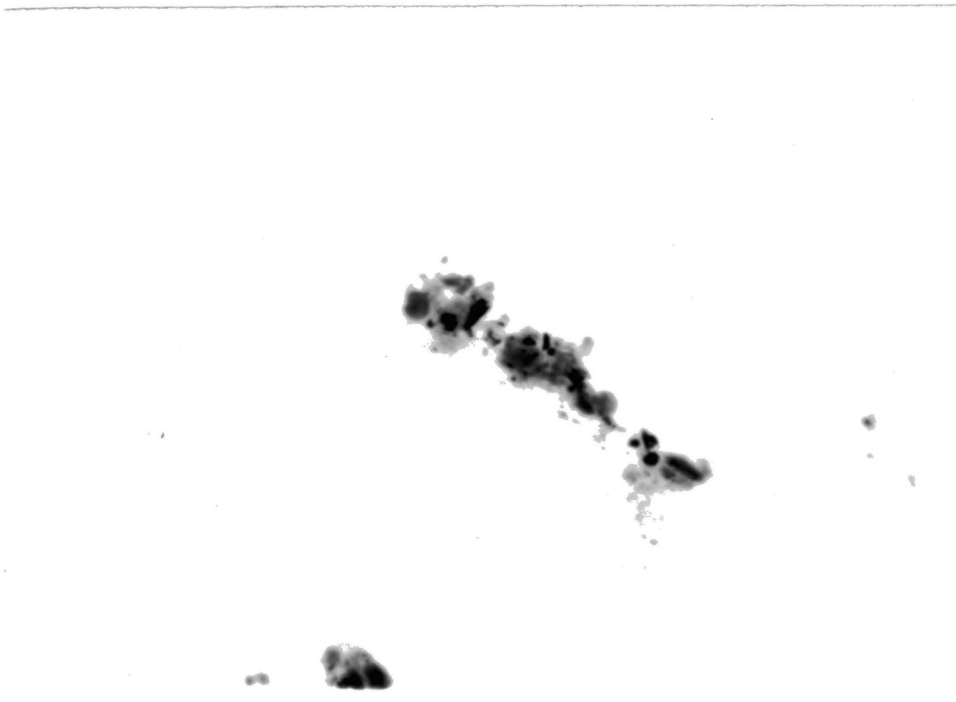

Figure 7. Mucus mixed with cellular debris in endometrial smear. (Pollak Trichrome X160)

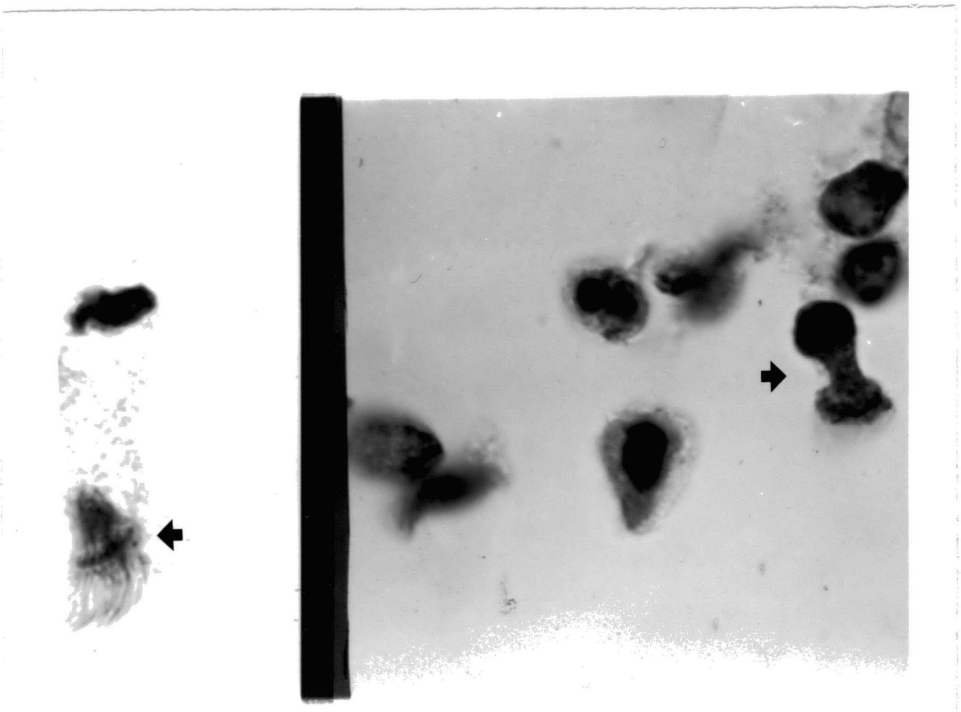

Figure 8. Endometrial smears. Orangophilic cell with thickening of luminal cytoplasm with cilia (right). To left is cytoplasmic tuft with cilia. (Pollak Trichrome X400)

and Pollak's trichrome stain used (Figure 9). These orange cells were mixed among normal-appearing luminal epithelial cells.

Smears From Clinical Cases

Twenty-five of the 63 smears from clinical cases were indistinguishable from those seen in animals studied as normal (Tables VI, VII, VIII, IX).

The smears considered abnormal always had one or more of the following features. (1) The most common abnormality seen was a large number of neutrophils. These were often enmeshed in copious amounts of mucus (Figure 10). (2) A small amount of precipitated protein was seen in some normal smears but was considered abnormal when it formed a consistent background pattern (Figure 11). Such a pattern corresponded to edema in the histologic sections. (3) Blood in a small quantity when erythrocytes were well formed was not considered abnormal because of the uncertainty of collection trauma. Pigmented aggregates presumed to be platelets or fragmented fibrin (Figure 12) were seen with degenerated erythrocytes. (4) Luminal glandular material (Figure 13) seen in tissue sections often appeared in corresponding cytology specimens as inspissated protein material (Figure 14), sometimes containing cellular debris. In one specimen, a Curschmann's spiral was seen (Figure 15). (5) A few isolated epithelial cells with enlarged nuclei and prominent nucleoli indicative of hyperplasia were seen in normal specimens. Large groups of hyperplastic cells (Figure 16) were seen in specimens from uteri following extensive epithelial destruction. Sometimes, corresponding biopsies contained similar cells (Figure 17). (6) Squamous metaplasia and dysplasia (Figure 18) were seen only in

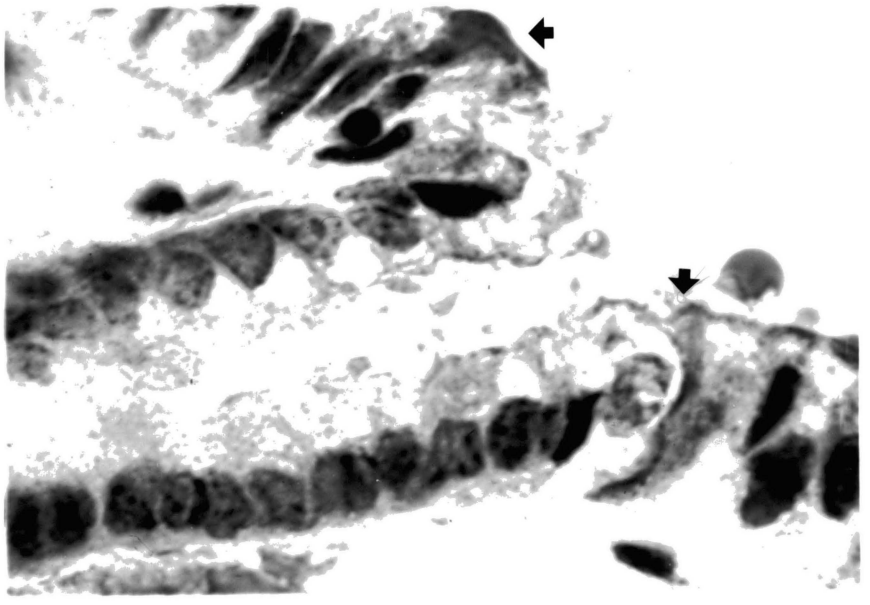

Figure 9. Long, slender orangophilic cells (arrow) in situ among luminal epithelial cells. Endometrial biopsy from same case shown in Figure 8. (Pollak Trichrome X400)

TABLE VI

COMPARISON OF BIOPSY GRADES AND CELLS FOUND WITH CYTOLOGY GRADES IN 163 MARES

	Grade			
	1	2	3	4
Biopsy Cells:				
Lymphocytes	7	94	29	7
Neutrophils	4	92	27	7
Eosinophils	1	48	16	0
Hemosiderophages	0	27	12	2
Histiocytes	0	11	8	0
Plasma cells	1	11	3	0
Mast cells	0	2	1	0
Cytology Grade:				
1	3	22	2	0
2	4	75	25	3
3	0	16	4	1
4	0	4	1	3
Total	7	117	32	7

TABLE VII

ENUMERATION OF CYTOLOGY CELLS AND EPITHELIAL CELL PATTERNS IN THE FOUR BIOPSY GRADES FROM 163 MARES

	Grade			
	1	2	3	4
Cytology Cell Patterns:				
Lymphocytes	3	74	19	4
Neutrophils	5	88	26	6
Eosinophils	1	15	9	4
Chlorocytes	2	37	13	2
Inspissated gland casts	2	32	17	4
Loose gland casts	0	9	4	1
Detached ciliary tufts	0	21	5	1
Histiocytes	2	23	7	2
Orangophilic cells	0	17	3	0
Stripped nuclei	0	6	1	0
Cytoplasmic fragments	0	8	7	2
Hemosiderophages	0	6	1	0
Mast cells	0	0	1	0
Urine crystals	0	2	1	0
Muscle fragments	0	1	0	0
No cells recorded	0	3	0	0
Cytology Epithelial Cell Patterns:				
Poor cell cohesion	2	27	8	1
Good cell cohesion	1	22	8	2
Degeneration	1	35	4	0
Necrosis	2	29	6	0
Squamous metaplasia	0	3	0	2
Hyperplasia and regeneration	0	24	10	4
Epithelial atypia and dysplasia	0	4	2	1
No patterns recorded	2	25	9	3

TABLE VIII

COMPARISON OF CYTOLOGY GRADES WITH BIOPSY GRADES AND CELLS FOUND IN 163 MARES

	Grade			
	1	2	3	4
Biopsy Grade:				
1	3	4	0	0
2	20	77	16	4
3	2	25	4	1
4	0	3	1	3
Total	25	109	21	8
Biopsy Cells:				
Lymphocytes	25	87	17	8
Neutrophils	21	82	19	8
Eosinophils	9	44	11	1
Hemosiderophages	0	32	6	3
Histiocytes	2	10	5	2
Plasma cells	2	11	1	1
Mast cells	0	2	1	0

TABLE IX

DISTRIBUTION OF CELLS AND EPITHELIAL CELLULAR PATTERNS AMONG THE FOUR CYTOLOGY GRADES IN 163 MARES

	Grade			
	1	2	3	4
Cellular Patterns:				
Lymphocytes	12	71	11	6
Neutrophils	13	84	20	8
Eosinophils	0	16	8	5
Chlorocytes	5	39	7	3
Inspissated gland casts	3	35	12	6
Loose gland casts	1	11	3	0
Detached ciliary tufts	5	16	4	2
Histiocytes	3	21	5	5
Orangophilic cells	8	9	2	1
Stripped nuclei	0	4	1	2
Cytoplasmic fragments	3	11	2	1
Hemosiderophages	0	6	1	0
Mast cells	0	1	0	0
Urine crystals	0	1	1	1
Muscle fragments	0	1	0	0
No cells recorded	2	0	1	0
Yeast spores	0	0	1	0
Epithelial Cellular Patterns:				
Poor cohesion	4	27	5	2
Good cohesion	12	17	2	2
Degeneration	4	29	6	1
Necrosis	5	20	8	4
Squamous metaplasia	0	1	2	2
Hyperplasia and regeneration	2	13	18	5
Epithelial atypia and dysplasia	0	1	1	5
No patterns recorded	3	33	3	0

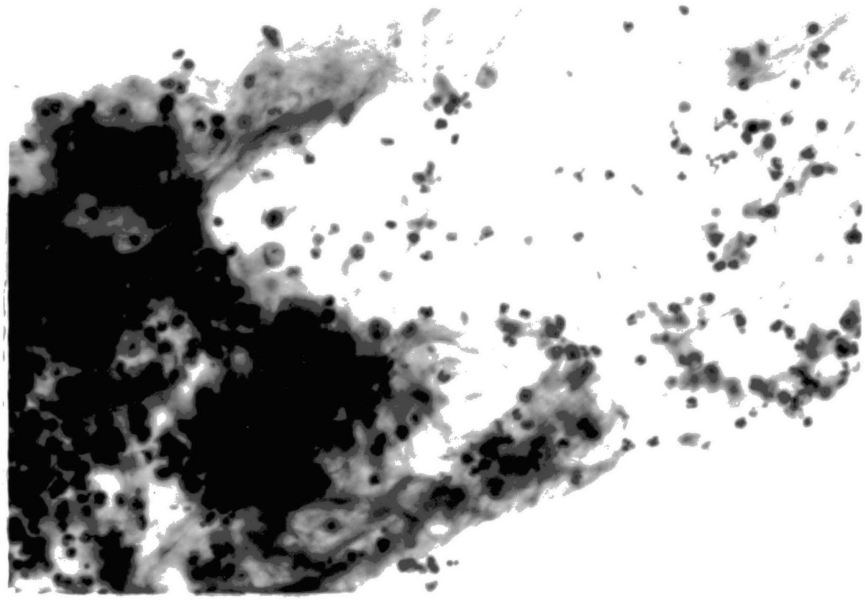

Figure 10. Endometrial smear with large number of neutrophils mixed with abundant mucus. (Pollak Trichrome X128)

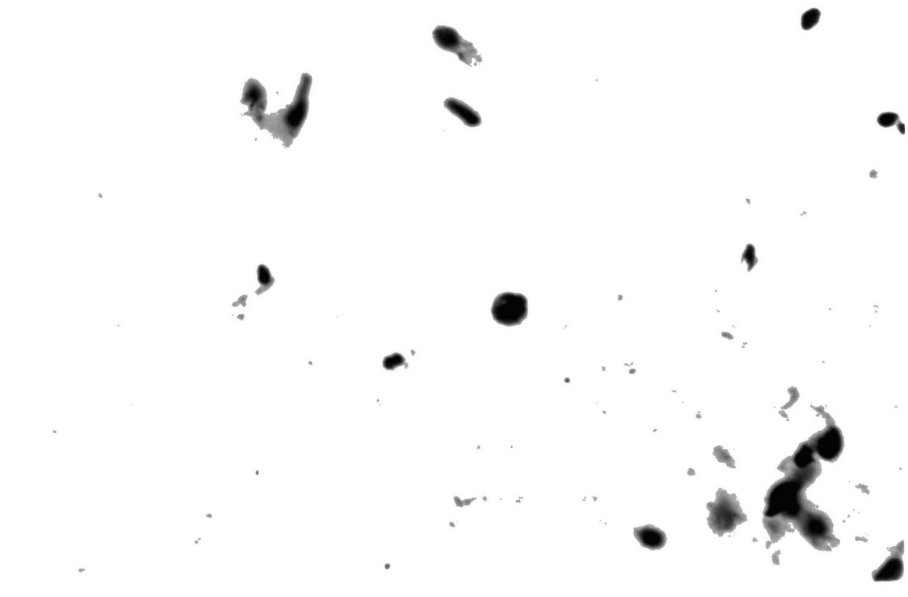

Figure 11. Mixture of epithelial and inflammatory cells in endometrial smear. Background of faintly stained granular protein is consistent with edema. (Pollak Trichrome X128)

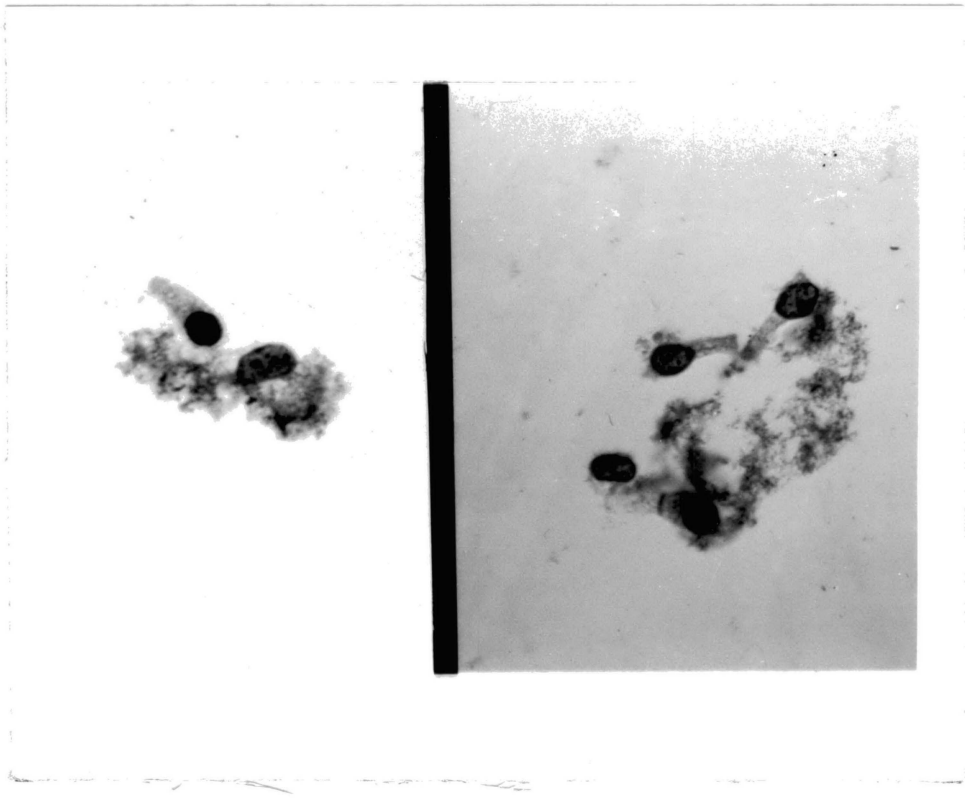

Figure 12. Endometrial smears. Composite of granular, pigmented material seen with decomposed blood. (Pollak Trichrome X320)

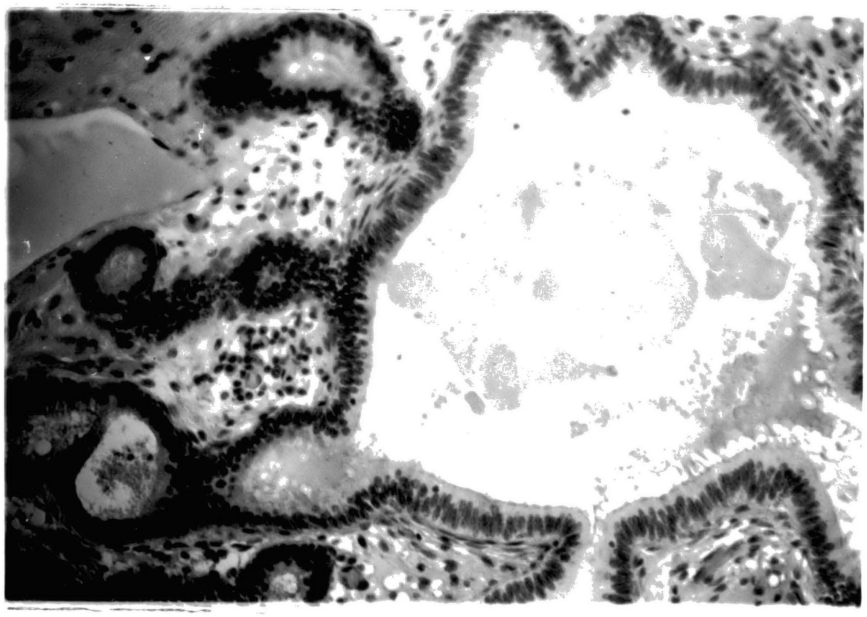

Figure 13. Enlarged endometrial gland with inspissated luminal material. (H&E X64)

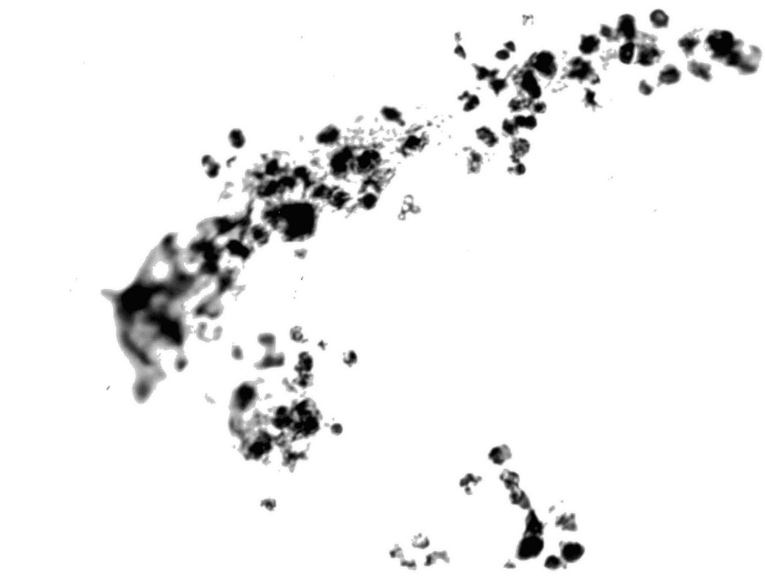

Figure 14. Glandular cast of inspissated material and cell debris in endometrial smear. (Pollak Trichrome X260)

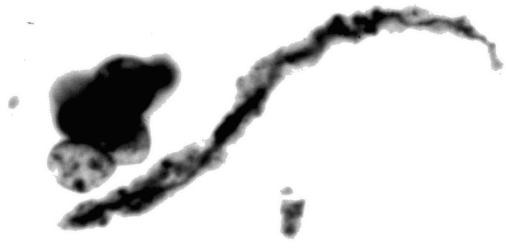

Figure 15. Curschmann's spiral with dense, undulating core encased in protein material. Endometrial smear. (Pollak Trichrome X320)

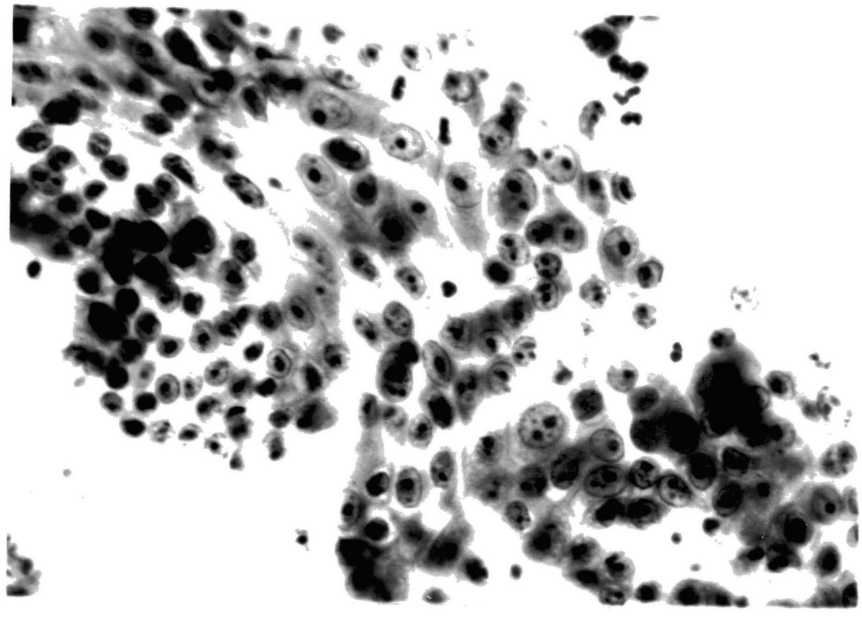

Figure 16. Endometrial smear. Large group of hyperplastic epithelial cells. Elongated squamous metaplastic cells in center of group. (Pollak Trichrome X320)

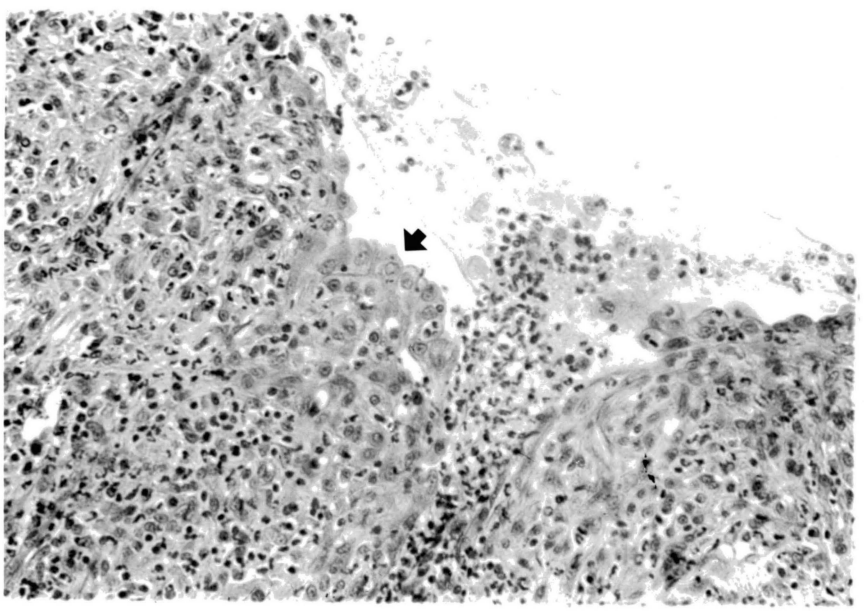

Figure 17. Endometrial biopsy with hyperplastic and squamous metaplastic luminal epithelium. Compare with cells in smear from same case in Figure 16. (H&E X64)

Figure 18. Large group of disorganized cells that vary in size and shape characteristic of epithelial dysplasia. Endometrial smear. (Pollak Trichrome X160)

abnormal smears usually associated with chronic irritation of the endometrial epithelium.

Diagnostic material was seen histologically in some of the clinical specimens. Urinary crystals were seen in one case (Figure 19) and not evident in the corresponding tissue, nor was this a clinical consideration. One specimen with a marked inflammatory reaction contained black yeast (Figure 20). With routine hematoxylin and eosin-stained specimens (Figure 21), black yeast were not visible microscopically. Black yeast were recognized microscopically in the tissue only with GMS (Figure 22).

Small histiocytes were seen in a few normal smears, but large and/or multinucleated histiocytes were seen only in abnormal smears (Figure 23).

An unusual cell in normal but in larger numbers in abnormal smears were small cells usually single with dense green cytoplasm. The nuclear-cytoplasmic ratio was high. They sometimes strongly resembled squamous cells (Figure 24). The cytoplasmic boundaries were sometimes angular (Figure 24), and the cells were sometimes cuboidal (Figure 24). There was condensation of the chromatin in some of these cells. The origin of these cells has not been identified in tissue sections. The significance of eosinophilic leukocytes is included in the following results on the eosinophil study.

Eosinophil Study

Eosinophils were found in 33 specimens from 27 of the 81 clinical cases (Table X). Clinical conditions associated with eosinophils in uterine specimens included pneumovagina, urovagina, poor vulvar or perineal conformation, poor vulvar or perineal muscle tone, and

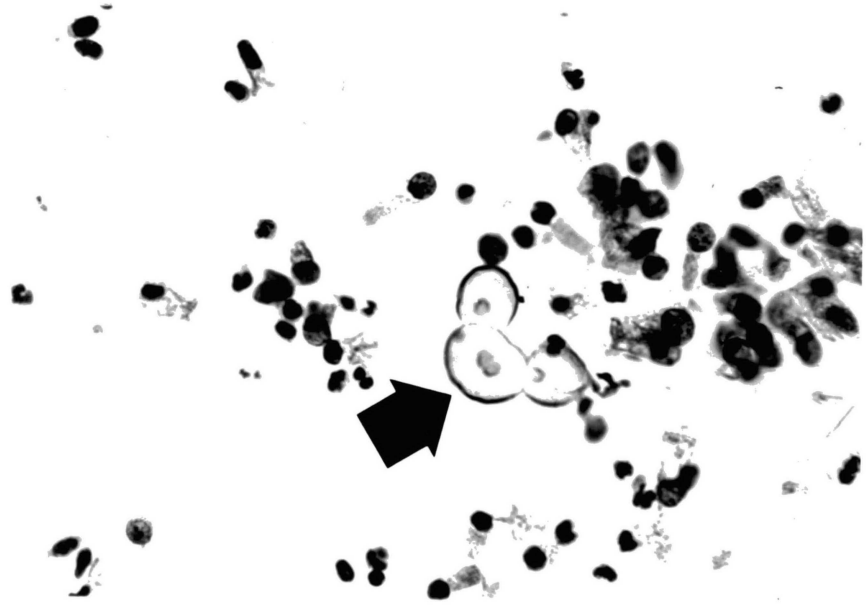

Figure 19. Mixture of epithelial and inflammatory cells with urine crystals (arrow). Endometrial smear. (Pollak Trichrome X180)

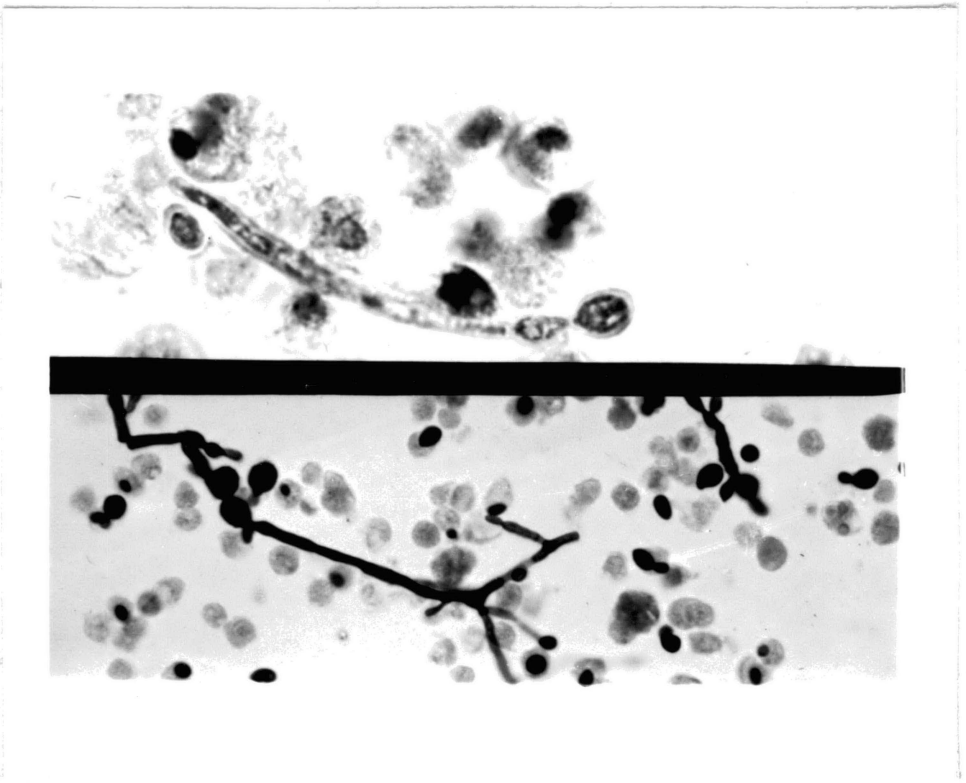

Figure 20. Endometrial smears. Hyphae with a budding yeast at one end in picture at top. Bottom picture has branched hyphae. (Top--Pollak Trichrome X400, bottom--GMS X160)

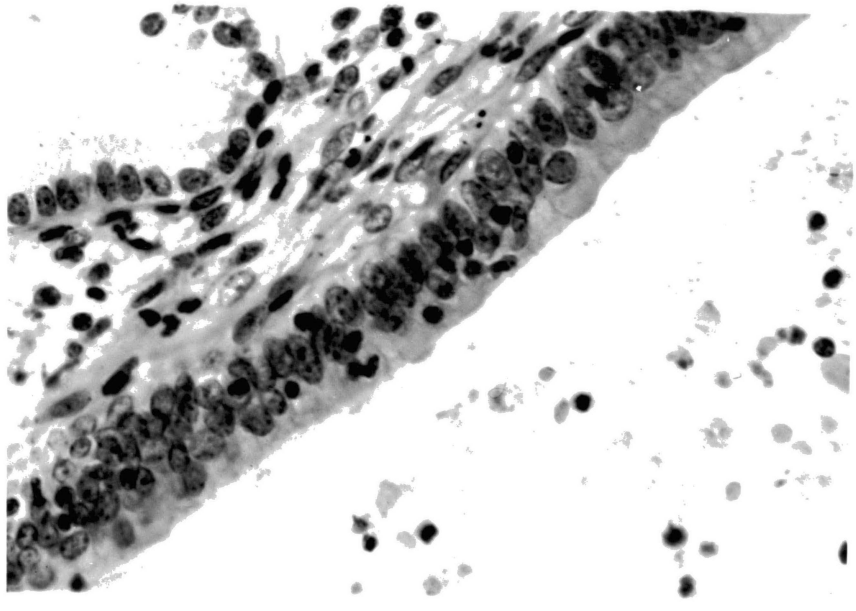

Figure 21. Uterine biopsy of case in Figure 20. Fungal elements not evident. (H&E X160)

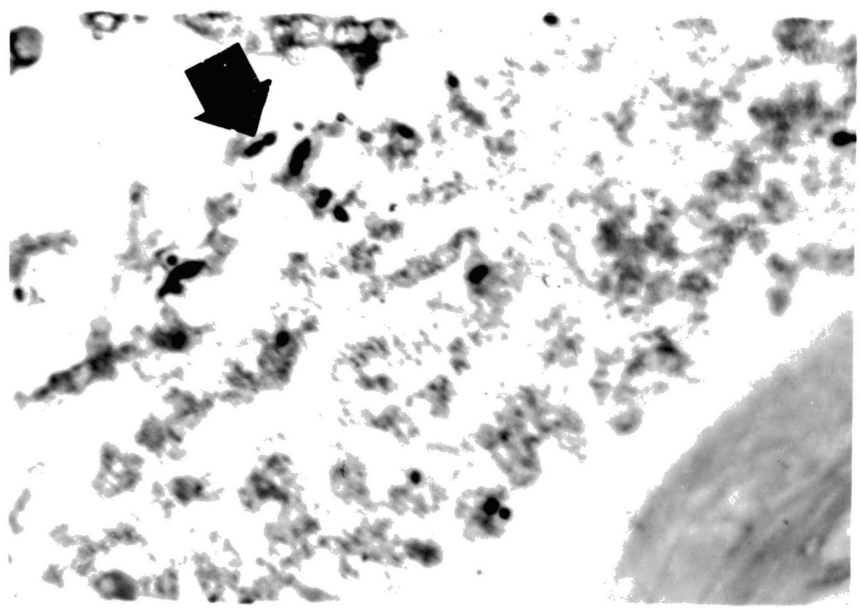

Figure 22. Same tissue as in Figure 21 with budding yeast (arrow) in luminal material. (GMS X130)

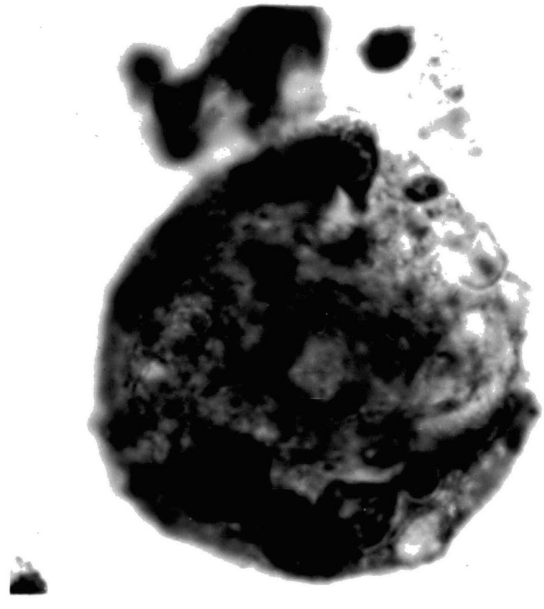

Figure 23. Large multinucleated histiocyte in endometrial smear. (Pollak Trichrome X400)

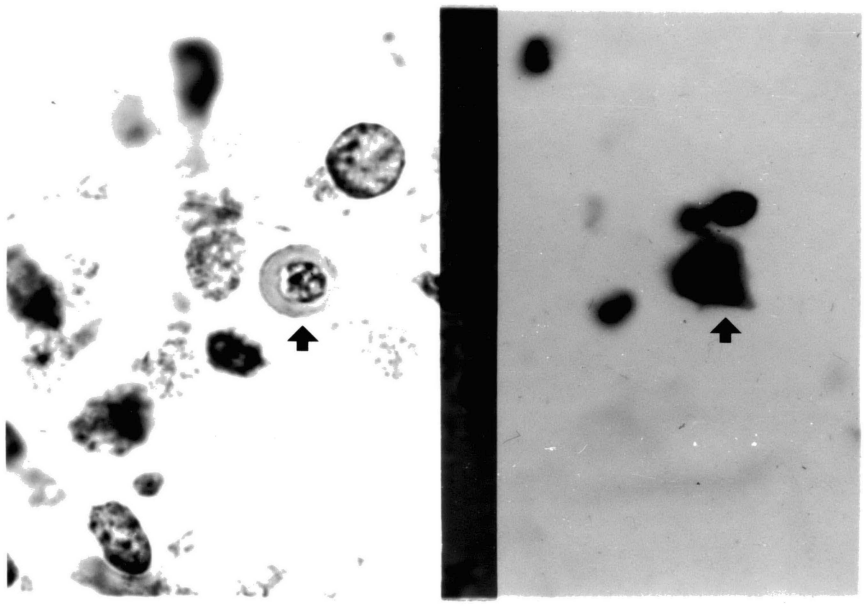

Figure 24. Composite of chlorocytes (arrow) in endometrial smears. (Pollak Trichrome X400)

TABLE X

EOSINOPHILS IN 33 CORRESPONDING CYTOLOGY AND/OR HISTOLOGY UTERINE SPECIMENS FROM 27 MARES

Clinical Diagnosis	Number Animals	Total Number Specimens	Eosinophils in Cytology	Eosinophils in Biopsy
Pneumovagina (Windsucker):				
1. Physical Exam	3	3	0	3
2. Tranquilizer Test Simulating Estrus	1	1	1	1
Urovagina	3	3	2	3
Abnormal Perineal Conformation and/or Vulvar Closure	15	19	9	13
Good Conformation and Vulvar Closure	2	2	1	2
Perineal Conformation and Vulvar Closure Not Recorded	1	1	1	1
Previous Perineoplasty	1	1	1	0
Postabortion (3 Days After Induction)	1	1	1	1
Episioplasty in Place (a)	-	2	0	2
Totals	27	33	16	26

a. These specimens represent additional collections following episioplasty on animals included in the abnormal conformation group.

post-abortion. Two mares with good vulvar conformation and tone had eosinophils in uterine specimens. One of these mares, which had not foaled for two years, was evaluated as a prospective breeder and had eosinophils in the tissue only. The second was a maiden eight-year-old Welsh pony-Arabian cross from which specimens were obtained to gain additional information on uterine cells and tissue from mares without known reproductive problems.

In the 33 concurrently collected specimens, 26 had eosinophils in uterine tissue specimens, and 16 had eosinophils in uterine cytology specimens. Fifteen corresponding tissue and cytology specimens had eosinophils in both (Table X). One mare had eosinophils in both specimens on initial collection and in specimens collected following corrective surgery for urovagina and uterine therapy, using an indwelling uterine catheter. Specimens taken 86 days after treatment still had eosinophils in both tissue and cytology specimens.

In another mare, eosinophils were initially present in the tissue specimen only, and an episioplasty was performed. Approximately nine months later and after periodic uterine therapy involving opening and closing of the episioplasty, eosinophils were present only in the cytology specimen.

A mare in the urovagina category of Table X, without clinically apparent poor conformation or genital pooling of urine, had eosinophils in both histology and cytology specimens. The diagnosis of pooling of urine in the uterus was possible only by the urinary crystals seen in the cytology specimen.

To test the hypothesis that eosinophils may appear in the uterus related to the estrous cycle, all mares with specimens containing

eosinophils were tabulated according to stage of the cycle at the time of specimen collection (Table XI).

In 32 of the 81 clinical cases, there were conditions predisposing to pneumouterus, but neither uterine cytology nor tissue specimens contained eosinophils. Twenty-seven mares with poor conformation did not have eosinophils in either cytology or histology specimens. Uterine specimens from three mares with clinical diagnoses of pneumovagina, one mare with urovagina and one mare with a perineal tear of unknown duration, did not contain eosinophils. Eosinophils were not present in uterine specimens from 13 mares with good perineal conformation and vulvar closure nor in specimens from five mares that already had an episioplasty in place at the time of reproductive examination. The case records of four mares without uterine eosinophils did not contain information regarding perineal conformation and vulvar closure.

In the eosinophil study, the four mares used to determine the effect of air on the uterus, eosinophils first appeared in the cytology smears of mares 1, 2, and 3 on day 2 of the study and were consistently present for the following 6 days except for the specimen collected from mare 1 on day 4. The biopsy specimens of day 7 from these three mares also contained eosinophils. On the 43rd day, there were no eosinophils in the tissue from mares 1, 2, and 3. Mares 1 and 2 had eosinophils in the cytology specimens on this day, but there were none in the smears from mare 3. When eosinophils were in the tissue or smears, they always were accompanied by neutrophils. Eosinophils were not seen in the tissue or cytology specimens from control mare 4 collected only on day 1 and day 43.

Distribution of eosinophils in uterine tissue specimens included

TABLE XI

THE OCCURRENCE OF EOSINOPHILS
DURING STAGES OF THE EQUINE
ESTROUS CYCLE

Stage of Cycle	Total Number of Specimens	Number of Uterine Specimens With Eosinophils
Estrus	3	1
Early Estrus or Proestrus	8	3
Diestrus	13	5
Seasonal Transition (a)	40	12
Winter Anestrus	30	11
Precycling (Immature)	1	0
Ovariectomized Mare	1	0
Totals	96	32

a. Both fall and spring transitional periods.

intraluminal, intraepithelial, and in both strata of the lamina propria either diffuse, focal, or around glands (Figure 25).

The identification of eosinophils in the tissue was not always straightforward when the granules were refractile or when the cells were amidst hemorrhage or hemosiderophages. Occasionally, degranulated eosinophils were seen in the tissue. The extruded pink granules were nearby. Similar degranulated eosinophils often were seen in cytology preparations (Figure 26).

Hyperplasia was seen in the exfoliated epithelial cells in specimens from mares 1 and 2, beginning on day 3 and continuing through the last day of daily collections (day 7). Collections of degenerated epithelial cells were mixed with granular protein material and nuclear debris. On day 43 (39 days after removal of the catheters), the epithelial cells appeared normal. No abnormality was seen in the two biopsy specimens or two cytology specimens from control mare 4 collected at the initiation and conclusion of the experiment.

This mixed necrotic and epithelial proliferative smear pattern was also seen in eight clinical cases. These specimens were collected immediately after removal of indwelling catheters in three mares. In one mare, the smears were collected following induced abortion. Similar changes were seen in four mares with urine pooled in the uterus. In the latter group, the hyperplastic groups of cells had smaller nuclei and nucleoli.

In all cases, the hyperplastic changes were more apparent in the cytologic (Figure 16) than histologic specimens. Plastic-imbedded tissue specimens demonstrated the diversity of the cells seen in the smears which was not seen in routine paraffin-imbedded tissue

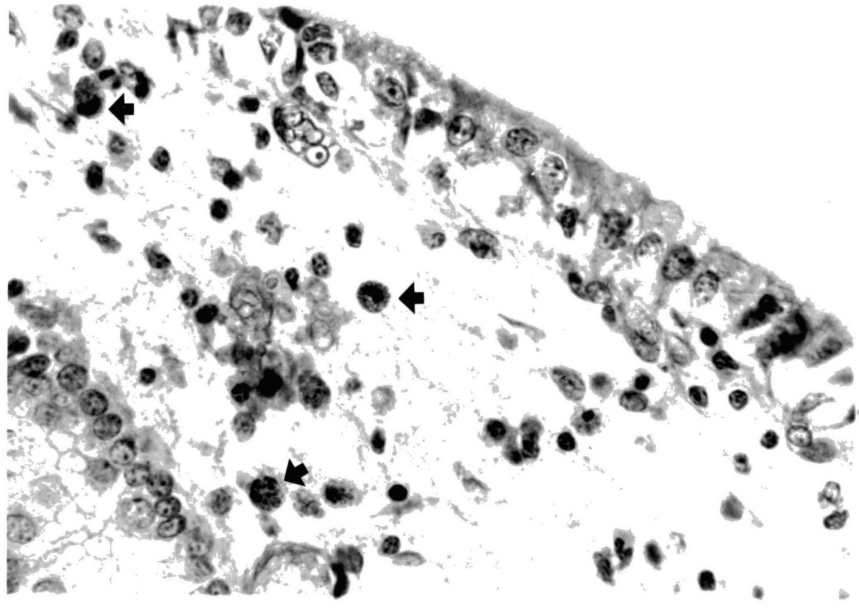

Figure 25. Multiple eosinophilic leukocytes (arrow) in endometrial biopsy. (H&E X160)

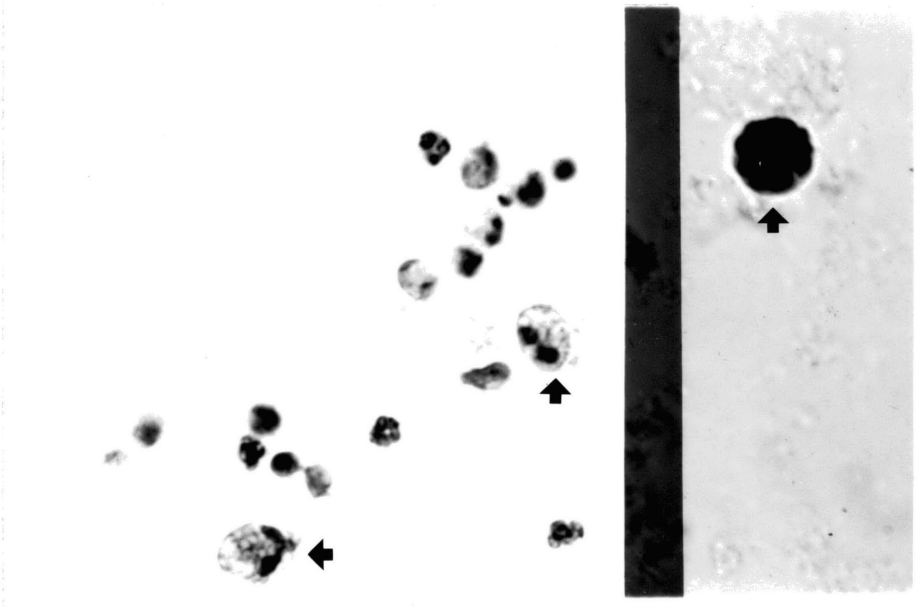

Figure 26. Composite of granulated and degranulated eosinophils (arrows) in endometrial smears. (Left--Pollak Trichrome X260, right--Pollak Trichrome X400)

(Figures 27, 28, 29).

Endometrial Grading

Seven mares had grade 1 endometrial biopsy specimens, three of which had a cytology specimen grade 1 and four with grade 2 (Table VII). There were 117 cases in which the endometrial biopsy was graded 2 (Table VII). The cytology specimens from this group were graded 1 in 20 cases, 2 in 77 cases, 3 in 16 cases, and 4 in 4 cases (Table VII). Thirty-two of the 163 mares' endometrial biopsies were grade 3; two cytology specimens were grade 1; 25 specimens were grade 2; four specimens were grade 3; and one specimen was grade 4 (Table VII). Seven of the 163 mares in this study had grade 4 uterine biopsies (Table VII). Three of the cytology specimens were grade 2; one was grade 3; and three were grade 4 (Table VII).

Cytology Grading

A grade 1 was recorded for the cytology specimens of 25 mares (Table VIII). Tissue specimens from these mares were graded 1 in 3 cases, 2 in 20 cases, and 3 in 2 cases (Table VIII). There were 109 of the 163 mares with grade 2 cytology specimens (Table VIII). Four of the 109 cases had tissue grades 1; 75 had grades 2; 25 had grades 3; and three had grades 4 (Table VIII). Of the 163 cases, 21 grade-3 cytology specimens were recorded (Table VIII). Sixteen of these 21 had tissue specimens of grade 2, four of grade 3, and one of grade 4 (Table VIII).

Eight of the 163 endometrial cytology specimens were grade 4 (Table VIII). Four of the eight simultaneously collected tissue

Figure 27. Luminal epithelial cells with no evidence of foamy cytoplasm. Endometrial biopsy. Compare with Figures 28, 29. (H&E X160)

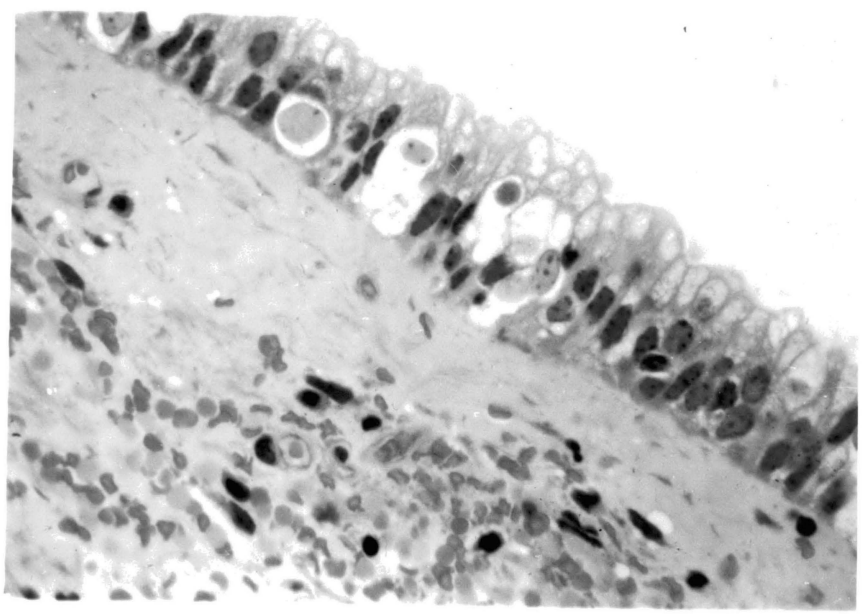

Figure 28. Uterine biopsy using plastic embedding. Vacules in luminal epithelium are evident. Compare with specimens from same case in Figures 27, 29. (H&E X160)

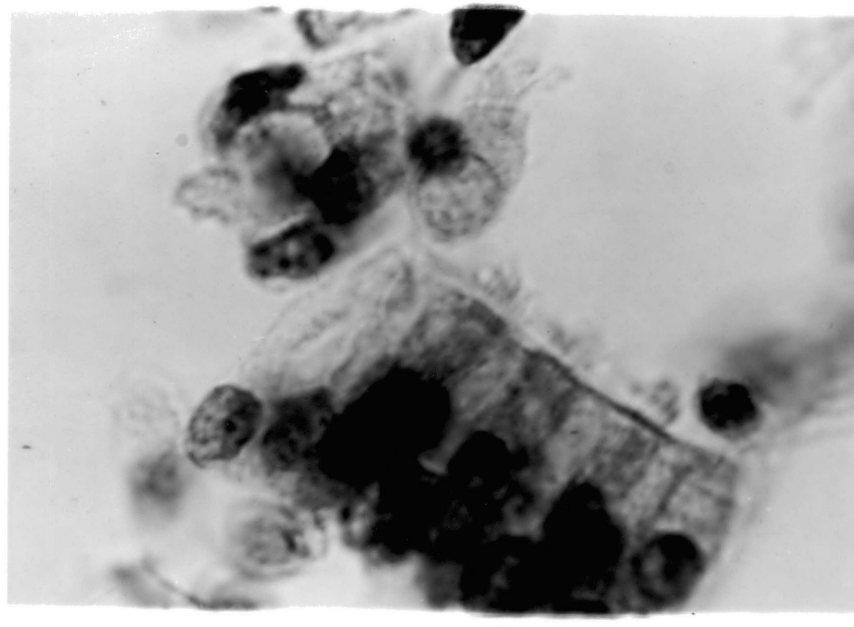

Figure 29. Endometrial smear. Epithelial cells with foamy cytoplasm. Compare with specimens from same case in Figures 27, 28. (Pollak Trichrome X400)

specimens were grade 2; one was grade 3; and three were grade 4 (Table VIII).

Uterine Cultures

There were 118 cases with simultaneously collected tissue, cytology, and uterine culture specimens. Seventy-seven of these cases had positive uterine cultures, and 41 were negative for uterine pathogens (Table XII). Of the positive uterine cultures, 57 were identified when the tissue specimen was a grade 2, 15 when the tissue specimen was grade 3, 4 when the tissue specimen was grade 4, and 1 when the tissue specimen was grade 1 (Table XII). Positive uterine cultures were identified in 50 grade-2 cytology specimens, 11 each in grade-1 and grade-3 cytology specimens, and 5 in grade-4 cytology specimens (Table XII).

TABLE XII

UTERINE TISSUE AND CYTOLOGY GRADES VERSUS CULTURES

Biopsy Grade	Number Cases	Cytology Grade 1	2	3	4	
		Number Cases 25	109	21	8	163
1	7	(3) (a)	1/1 (b) (2)	0/0	0/0	
2	117	10/4 (6)	35/19 (23)	10/3 (3)	2/0 (2)	
3	32	1/1	12/10 (3)	1/1 (2)	1/0	
4	7	0/0	2/0 (1)	0/1	2/1	
	163					

a. (Indicates number mares not cultured.)
b. Positive culture/negative culture.

CHAPTER IV

DISCUSSION

Grading of the tissue specimens is aimed to predict the probability of successful conception and delivery of a live foal. Periglandular fibrosis is important due to the significance of the uterine glands as they related to placentation in the mare. Profound fibrosis appears to interfere with placentation by excluding glands from accepting placental attachment.

Cytology is unable to detect features that would indicate loss of fertility but seems to be a reflection of uterine surface phenomena and in some cases registers the current inflammatory state of the uterus.

The tissue specimens provide information about fibrosis while cytology specimens are unable to detect the deeply located fibrosis (Figure 30). This explains why there is not a good correlation between tissue and cytology grades in some cases. Likewise, significant inflammation seen in the cytology specimen may not be apparent in the tissue specimen. One biopsy specimen is representative of the entire uterus (11); however, the cytology specimen represents a pooling of all available material in the uterine lumen. It must be remembered that in cytology cells are collected from a wide uterine area, preventing the differentiation of focal and diffuse changes. It is possible there may be an area of inflammation missed with a single tissue specimen,

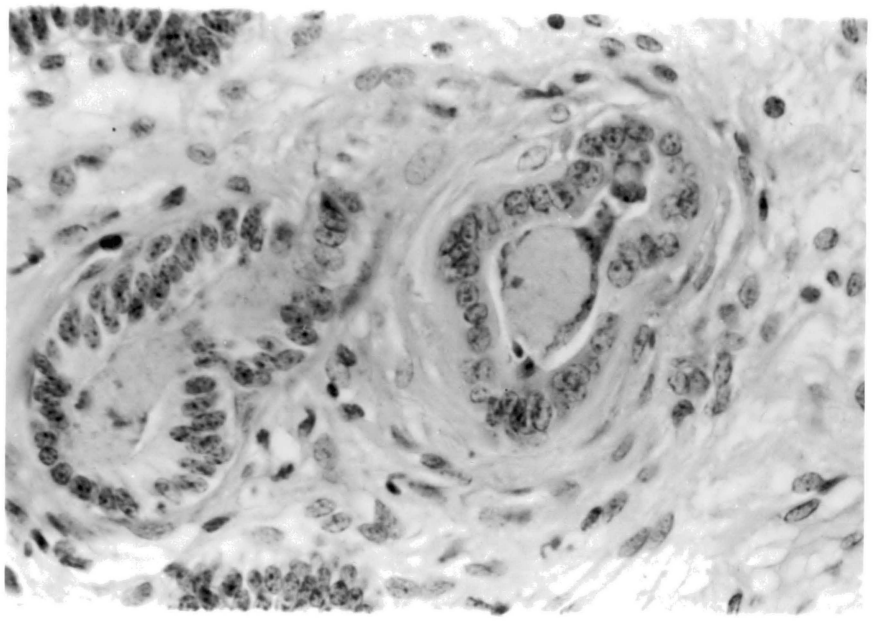

Figure 30. Endometrial biopsy with periglandular fibrosis. (H&E X135)

but this would not raise the overall grade more than one grade point, with the changes still being reflected in the cytology.

One phenomenon that cannot be appreciated in the tissue specimens but is readily seen in the cytology is the manner in which the epithelial cells break free from the uterine lining. Many abnormal cellular processes occur in the cytology specimens because of the susceptibility of the uterine epithelial lining to minute physiologic changes causing the cells to break away singly or in groups. These processes include cohesion, degeneration, necrosis, squamous metaplasia, hyperplasia, dysplasia, regeneration, and epithelial atypia. These cellular processes may be focal or generalized throughout the uterus and will necessitate correlation with multiple postmortem specimens in future studies. The cell processes may be a reflection of hormonal influences, represent alterations in uterine resistance factors, indicate uterine pathogens are present, or result from mechanical defects of the reproductive tract.

With normal "wear and tear", epithelial cells are removed from the endometrial surface in flat cohesive groups called "honeycombs" and "picket fences" (Figures 3, 4). Lack of cohesion occurs during winter anestrus, to a minor degree while cycling, and is pronounced during disease conditions. This is seen as epithelial cells in small loose groups, as individual cells with intact nuclei, or become stripped nuclei (Figure 1).

Degenerative changes of the epithelial cells in the endometrial cytology specimens result from cell death. The cells may appear red with Sano's modification of Pollak trichrome stain and contain swollen, dark nuclei (Figure 8). A perinuclear halo may be present with

degeneration as well as other processes. Orangophilic cells are those degenerating epithelial cells that are elongated, that have pyknotic nuclei, and where the ciliary tuft is detaching (Figure 8). They stain orange with Sano's modification of Pollak trichrome stain. Degenerative changes including orangophilic cells were identified during all phases of the estrous cycle and during anestrus. Mild proliferation frequently accompanies these degenerative changes. They are associated with many types of uterine pathology.

Cytoplasmic fragments (Figure 1) were seen in cytology specimens also containing degeneration or necrosis. Cytoplasmic fragments result from epithelial-cell destruction, leaving the nucleus unattached. The unattached nuclei are termed stripped nuclei and may be broken into small pieces. Stripped nuclei, nuclear pieces, other pyknotic debris, and detached ciliary tufts were associated with necrosis or degeneration.

Epithelial dysplasia is an early form of degeneration. Some of the cells in groups were noted to be poorly oriented toward one another, and some nuclei lose polarity and appear to lie in different planes (Figure 18). Nuclear size varied within the cell group. Epithelial dysplasia was seen following uterine therapy with indwelling catheter placement.

Hyperplasia and regenerative changes noted in the uterine cytology specimens are closely related. Hyperplastic cells have enlarged nuclei with large prominent nucleoli and foamy cytoplasm (Figure 16). They usually occur in tight groups or "honeycomb" arrangements. Hyperplasia was seen in groups of cells; however, regenerative changes were noted in individual cells. Cells with regenerative changes were variable in

size, had prominent nucleoli, and were the result of cell proliferation. Hyperplasia and regeneration were found in mares following uterine therapy with indwelling uterine catheters.

Squamous metaplasia occurred with irritation to the columnar epithelial cells lining the uterus. The vertically oriented epithelial cell begins to elongate horizontally, folding over the neighboring cells (Figure 16). By folding over, they form a protective shield for other cells.

The epithelial cells are dynamic and react rapidly to change and return to normal quickly. Conversely, the endometrial stroma reacts slowly to change and requires longer healing time than the epithelium. Inflammation in the stroma may persist for several estrous cycles and require repeated tissue collections to determine return to normal. The cytologic specimen may indicate return to normal within two cycles if proper therapy is used. Pathologic changes, as noted from uterine cytologic specimens, may prevent normal spermatozoa passage to the ovum awaiting fertilization. Implantation of the equine embryo occurs several days following fertilization, and this may be sufficient time for adequate stromal healing, permitting normal pregnancy establishment.

It is possible to stage the estrous cycle in women with the endometrial biopsy (39). The uterine tissue and cytologic samples taken from five mares during estrus, diestrus, and anestrus emphasized the inability to stage the equine estrous cycle with these specimens. The height of the luminal and glandular epithelium and the amount of stromal edema were assessed, and no significant difference could be seen between the tissue specimens from mares in estrus versus diestrus (Table IV). No increase in inflammatory cells was seen in the tissue during estrus

(Table IV).

Equine anestrus was recognizable by examination of uterine tissue specimens. The luminal epithelium was short to cuboidal, the stromal edema reduced significantly, and gland diameter decreased. Taking these seasonal changes into account, it was possible to accurately recognize winter anestrus in equine uterine biopsies as well as cytology.

The two techniques were very helpful, both with limitations. The tissue can not only show the deviation from normal but also locate the problem in the uterus. In some instances, the cytology provided valuable information not available in the tissue. For example, black yeast was detected in the cytologic specimen from a mare's uterus but was only apparent in the tissue with special staining (Figures 20, 21, 22). This special stain would not have been done if the organism had not been recognized in the cytology.

The use of uterine culture is not to be minimized, but the demonstration of an organism is significant only when supported by either biopsy or cytology. The use of sensitivity is a valuable tool helping the clinician choose appropriate therapy when an infectious process is known to be present.

The study of the significance of eosinophils yielded some interesting results. Eosinophils were associated with many processes, some of which are not completely understood. They have been described in uterine biopsy specimens from the mare, but no hypothesis has been offered regarding their significance. Eosinophils have been reported in the human uterus under circumstances of undetermined cause as well as during the follicular and postovulatory phases of the cycle in

increasing numbers (10). In rats, the gravid uterus does not contain eosinophils although they are present in all phases of the estrous cycle, with the highest number present during estrus (10). Estrogen receptors have been reported on eosinophils as well as uterine cells, and there is speculation that the eosinophil may be a carrier of estrogen to the uterus (10). Based on the eosinophil and cycling-mare studies, there was no correlation between the presence of eosinophils in the tissue or cytology specimens and the stage of the estrous cycle, seasonal transition, or winter anestrus.

The process of degranulation or exocytosis of eosinophils has been described in human patients and is reported to involve the eosinophil's role in modulation of inflammatory processes (10). The role of the eosinophil as a mediator in type I hypersensitivity reactions has been established (64). Urogenital secretions are rich in immunoglobulin E, which is an important component of type I reactions (64). This raises a question as to the role of the eosinophil in uterine immune responses and the antigenicity of components introduced by the presence of air in the uterus, a tissue in which air must be considered as foreign. Degranulated eosinophils were noted in equine specimens in this study and have been pictured in equine peritoneal specimens (6).

Air in the equine reproductive tract is an important cause of inflammation and infection (46). Some obvious causes of air aspiration into the reproductive tract are perineal lacerations and indwelling uterine catheters. Other conditions such as poor vulvar conformation and muscle tone are difficult to evaluate and are considered causes of air aspiration. Clinically inapparent changes in vulvar tone may allow air aspiration only during estrus.

Within the eosinophil study, 42 of 81 mares had poor vulvar conformation, and only 15 had eosinophils in the uterine specimens. Eosinophils may not have been present in the mares' reproductive tracts, or it was due to the limitations of specimen collection or preparation. Eosinophils in the specimens presented in this paper comprised a relatively small number in comparison with neutrophils. It may be possible for a single biopsy to be void of eosinophils when they are present in other sites in the uterus. This was substantiated by those cases in which eosinophils were present in the cytologic smears but absent in the concurrently collected tissue preparations.

In cytologic evaluation, the temptation to quantify cells in such specimens must be tempered when such results have limited value. The sites of the endometrial collections were unknown except that they were within the uterine lumen. The collection of cells represents a sampling of many cells in the uterine lumen, and the smear is prepared from a random collection of those cells present in a centrifuged sediment. As in all such cases, negative findings, be it cancer cells or eosinophils, are inconclusive results.

There seemed to be no correlation between location of eosinophils in the tissue and their presence in cytologic specimens. They may be present throughout the tissue and within the lumen and not be present in the corresponding cytologic specimens.

The epithelial changes first were observed in clinical cases following intrauterine antibiotic therapy using an indwelling catheter and were thought to represent a favorable response to the instilled agent by shedding of necrotic epithelium and replacement with healthy tissue. The results of the eosinophil study suggest that similar

changes may occur in the absence of medication. It is possible, therefore, that the trauma of an indwelling catheter is itself therapeutic. It is known that cyclic rejuvenation may eliminate some infectious agents from the uterus (2, 51). The infusion of dilute caustic agents also has been used clinically to achieve this effect (62). The indwelling catheter may function in a similar, though less aggressive manner.

In the eosinophil study, all mares except the control had eosinophils. In these three mares, the uterus was presumed to have been exposed to air through daily sampling or an indwelling open or closed catheter. These procedures also could traumatize the uterus. The role trauma may play in the eosinophilic reaction is unclear. Although it may be possible to get eosinophils without neutrophils, every uterine specimen in the experimental and clinical cases that contained eosinophils had neutrophils. Many conditions can induce infiltration of neutrophils, but exposure to air appears to enhance the probability that eosinophils will be in the uterus.

With induced pneumouterus, there was a neutrophilic and eosinophilic response of the uterus within 24 hours following a single exposure to air during initial biopsy and lavage for cytologic specimens. This suggests that only a small volume of air may be required to induce acute uterine inflammation with eosinophils. An initial histologic or cytologic evaluation may reflect the actual state of the uterus, but specimens obtained later are likely to include changes associated with iatrogenic pneumouterus.

This study has indicated that pneumouterus results in acute inflammation with eosinophils. The use of both cytologic and histologic specimens increased the number of mares detected with this condition.

Initial specimens containing this acute eosinophilic inflammatory response should alert the clinician to the possibility of pneumouterus so that attention can be directed to corrective measures.

This study was directed towards evaluating exfoliative cytology in diagnosis, prognosis, and selection of therapeutic choices for mare infertility. Equine uterine cytologic parameters were defined and correlated with the established diagnostic tools such as endometrial biopsy and culture. The results of this study indicate that cytology is not a substitute for culture or biopsy but enhances clinical management of a case by supporting results of the other two techniques and in some instances providing information not available through the other techniques. The place for endometrial cytology in general practice is dependent on personnel trained to interpret samples, which is also true for tissue specimens.

A SELECTED BIBLIOGRAPHY

(1) Adams, S.B.; Fessler, J.F.; Rebar, A.H.: Cytologic interpretation of peritoneal fluid in the evaluation of equine abdominal crisis. Cornell Vet 70:232-246, 1980

(2) Andrews, F.N.; McKenzie, F.F.: Estrus, ovulation, and related phenomena in the mare. In: Research Bulletin Missouri Agriculture Experiment Station 329:5-117, 1941

(3) Ansari, M.M.: The Caslick operation in mares. Compend Cont Ed 5:S107-S111, 1983

(4) Asbury, A.C.: The reproductive system. In: Equine Medicine and Surgery, ed. Mansmann and McAllister, pp. 1305-1365, American Veterinary Publications, Santa Barbara, California, 1982

(5) Asbury, A.C.; Halliwell, R.E.W.; Foster, G.W.; Lungino, S.J.: Immunoglobulins in uterine secretions of mares with differing resistance to endometritis. Therio 14:299-308, 1980

(6) Bach, L.G.; Ricketts, S.W.: Paracentesis as an aid to the diagnosis of abdominal disease in the horse. Equine Vet J 6:116-121, 1974

(7) Beech, Jill: Cytology of equine cerebrospinal fluid. Vet Pathol 20:553-562, 1983

(8) Beech, Jill: Evaluation of the horse with pulmonary disease. Vet Clin North Am, Large Animal Practice, Symposium on Equine Respiratory Disease 1:43-58, 1979

(9) Beech, Jill: Cytology of tracheobronchial aspirates in horses. Vet Pathol 12:157-164, 1975

(10) Beeson, P.B.; Bass, D.A.: Volume 14 in the series Major Problems in Internal Medicine, ed. L.H. Smith, Jr., pp. 7, 47, 154-157, W.B. Saunders, Philadelphia, 1977

(11) Bergman, R.V.; Kenney, R.M.: Representativeness of a uterine biopsy in the mare. Proc Annu Convention Am Assoc Equine Pract 21:355-362, 1975

(12) Blanchard, T.L.; Cummings, M.R.; Garcia, M.C.; Hurtgen, J.P.; Kenney, R.M.: Comparison between two techniques for endometrial swab culture and between biopsy and culture in barren mares. Therio 16:541-552, 1981

(13) Blue, M.G.; Brady, A.A.; Davidson, J.N.; Kenney, R.M.: Studies on the composition and antibacterial activity of uterine fluid from mares. J Reprod Fertil, Suppl 32:143-149, 1982

(14) Brandt, G.W.: The significance and interpretation of uterine biopsy in the mare. Proc Annu Convention Am Assoc Equine Pract 16:279-294, 1970

(15) Britton, B.A.: Endometrial change in the annual reproductive cycle of the mare. J Reprod Fertil, Suppl 32:175-180, 1982

(16) Bryans, J.T.: Research on bacterial diseases of horses. Lectures to Stud Manager's Course, Lexington, Kentucky, 1962

(17) Crouch, J.R.F.; Atherton, J.G.; Platt, H.: Venereal transmission of Klebsiella aerogenes in a Thoroughbred stud from a persistently infected stallion. Vet Rec 90:21, 1972

(18) Crow, J.; Gordon, H.; Hudson, E.: An assessment of the Mi-Mark endometrial sampling technique. J Clin Pathol 33:72-80, 1980

(19) Daly, D.C.; Walters, C.A.; Soto-Albors, C.E.; Riddick, D.H.: Endometrial biopsy during treatment of luteal phase defects is predictive of therapeutic outcome. Fertil Steril 40:305-310, 1983

(20) Doig, P.A.; McKnight, J.D.; Miller, R.B.: The use of endometrial biopsy in the infertile mare. Can Vet J 22:72-76, 1981

(21) Gadd, J.D.; Jensen, B.: The cytology of the cervicovaginal junction in the broodmare. Handout material, Annu Meet Am Assoc Equine Pract, 1982

(22) Ganjam, V.K.; McLeod, C.; Klesius, P.H.; Washburn, S.M.; Kwapien, R.; Brown, B.; Fazeli, M.H.: Effect of ovarian hormone on the phagocytic response of ovariectomized mares. J Reprod Fertil, Suppl 32:169-174, 1982

(23) Gordon, L.R.: The cytology and histology of epidermal inclusion cysts in the horse. J Equine Med Surg 2:371-374, 1978

(24) Gordon, L.R.; Sartin, E.M.: Endometrial biopsy as an aid to diagnosis and prognosis in equine infertility. J Equine Med Surg 2:328-336, 1978

(25) Gygax, A.P.; Ganjam, V.K.; Kenney, R.M.: Clinical, microbiological, and histological changes associated with uterine involution in the mare. J Reprod Fertil, Suppl 27:571-578, 1979

(26) Hughes, J.P.; Loy, R.G.; Asbury, A.C.; Burd, H.E.: The occurrence of Pseudomonas in the reproductive tract of mares and its effect on fertility. Cornell Vet 56:595, 1966

(27) Hughes, J.P.: Contagious equine metritis: a review. Therio 11:209-216, 1979

(28) Hughes, J.P.; Stabenfeldt, G.H.; Kindahl, H.; Kennedy, P.C.; Edquist, L.E.; Neely, D.P.; Schalm, O.W.: Pyometra in the mare. J Reprod Fertil, Suppl 27:321-329, 1979

(29) Hughes, J.P.; Loy, R.G.: The relation of infection to infertility in the mare and stallion. Equine Vet J 7:155-159, 1975

(30) Kenney, R.M.: Cyclic and pathologic changes of the mare endometrium as detected by biopsy, with a note on early embryonic death. J Am Vet Med Assoc 172:241-262, 1978

(31) Kenney, R.M.: Prognostic value of endometrial biopsy in the mare. J Reprod Fertil, Suppl 23:347-348, 1975

(32) Knudsen, Odd: Endometrial cytology as a diagnostic aid in mares. Cornell Vet 54:415-422, 1964

(33) Koss, L.G.: Diagnostic Cytology and Its Histologic Bases, pp. 412-455, Volume II. J.B. Lippincott, Philadelphia, 1979

(34) Laing, J.A.; Leech, F.B.: The frequency of infertility in Thoroughbreds. J Reprod Fertil, Suppl 23:307-310, 1975

(35) Laing, J.A.: Fertility and Infertility in Domestic Animals, pp. 1-4, Balliere Tindall, London, 1979

(36) Mather, E.C.; Refsal, K.R.; Gustafsson, K.K.; Seguin, B.E.; Whitmore, H.L.: The use of fiberoptic techniques in clinical diagnosis and visual assessment of experimental intrauterine therapy in mares. J Reprod Fertil, Suppl 27:293-297, 1979

(37) McGrath, J.P.: Exfoliative cytology of equine peritoneal fluid--an adjunct to hematologic examination. In: Proceedings First International Symposium on Equine Hematology, pp. 408-416, American Association of Equine Practitioners, Golden, Colorado, 1975

(38) Merkt, H.; Gunzel, Anne-Rose: A survey of early pregnancy losses in West German Thoroughbred mares. Equine Vet J 11:256-258, 1979

(39) Noyes, R.W.; Hertig, A.T.; Rock, J.: Dating the endometrial biopsy. Fertil Steril 1:3-25, 1950

(40) Pascoe, R.R.: Observations on the length of angle of declination of the vulva and its relation to fertility in the mare. J Reprod Fertil, Suppl 27:299-305, 1979

(41) Peterson, F.B.; McFeely, R.H.; David, J.S.E.: Studies on the pathogenesis of endometritis in the mare. Proc Annu Convention Am Assoc Equine Pract 15:279-287, 1969

(42) Platt, H.: Infection in the horse fetus. J Reprod Fertil, Suppl 23:605-610, 1975

(43) Ricketts, S.W.: The technique and clinical application of endometrial biopsy in the mare. Equine Vet J 7:102-107, 1975

(44) Ricketts, S.W.: Endometrial biopsy as a guide to diagnosis of endometrial pathology in the mare. J Reprod Fertil, Suppl 23: 341-345, 1975

(45) Ricketts, S.W.; Wingfield-Digby, N.J.: Fertility evaluation of the mare. J Reprod Fertil, Suppl 32:635-645, 1982

(46) Roberts, S.J.: Veterinary Obstetrics and Genital Diseases (Theriogenology), p. 350, Edwards Brothers Inc., Ann Arbor, Michigan, 1971

(47) Roszel, J.F.: Cytologic procedures. J Am Anim Hosp Assoc 17: 903-910, 1981

(48) Roszel, J.F.: Exfoliative cytology in diagnosis of malignant canine neoplasms. Vet Scope 12:14-20, 1967

(49) Roszel, J.F.: Genital cytology of the bitch. Vet Scope 19:2-15, 1975

(50) Sartin, E.M.: A study of the Use of the Endometrial Biopsy in the Evaluation of Equine Infertility. Paper for completion of Masters Degree in Veterinary Pathology, Oklahoma State University, 1978

(51) Seaborn, E.: The oestrous cycle in the mare and some related phenomena. Anat Rec 31:277-287, 1925

(52) Scott, P.; Daley, P.; Gridley, G.; Baird, Sturgess S.; Frost, A.J.: The aerobic bacterial flora of the reproductive tract of the mare. Vet Rec 88:58, 1971

(53) Shideler, R.K.; McChesney, A.E.; Morrow, G.L.; Voss, J.L.; Nash, J.G.: Endometrial biopsy in the mare. Proc Annu Convention Am Assoc Equine Pract 23:97-103, 1977

(54) Shin, S.J.; Lein, D.H.; Aronson, A.L.; Nusbaum, S.R.: The bacteriological culture of equine uterine contents, in vitro sensitivity of organisms isolated, and interpretation. J Reprod Fertil, Suppl 27:307-315, 1979

(55) Slusher, S.H.; Freeman, K.P.; Roszel, J.F.: Eosinophils in equine uterine cytology and histology specimens. J Am Vet Med Assoc 184:665-670, 1984

(56) Slusher, S.H.: Equine indwelling uterine catheter. VM/SAC 78: 114-117, 1983

(57) Smith, B.P.: Diseases of the pleura. Vet Clin North Am, Large Animal Practice, Symposium on Equine Respiratory Disease 1:197-204, 1979

(58) Solomon, W.J.; Schultz, R.H.; Fahning, M.L.: A study of chronic infertility in the mare utilizing uterine biopsy, cytology, and culture methods. Proc Annu Convention Am Assoc Equine Pract 18:55-68, 1972

(59) Stratton, L.G.: Personal communication. Stillwater, Oklahoma, May 1, 1980

(60) Sulewski, J.M.; Ward, S.P.; McGraffic, W.: Endometrial biopsy during a cycle of conception. Fertil Steril 34:548-551, 1980

(61) Sullivan, J.J.; Turner, P.C.; Self, L.C.; Gutteridge, H.B.; Bartlett, D.E.: Survey of reproductive efficiency in the Quarter Horse and Thoroughbred. J Reprod Fertil, Suppl 23:315-318, 1975

(62) Threlfall, W.R.: Broodmare uterine therapy. Compend Cont Ed 11:S246-S252, 1980

(63) Timoney, P.J.; O'Reilley, P.J.; McArdle, J.F.; Ward, J.; Harrington, A.M.; McCormich, R.: Response of pony mares to the agent of contagious equine metritis. J Reprod Fertil, Suppl 27:367-375, 1979

(64) Tizard, I.: An Introduction to Veterinary Immunology, Chapters X and XVII. W.B. Saunders, Philadelphia, 1982

(65) Ven Niekerk, C.H.; Ferneke, W.H.; Van Heerden, J.S.: Anatomical and histological observations on the reproductive tract of mares with abnormal oestrous cycles. J South Africa Vet Assoc 44: 141-152, 1973

(66) Von Lepel, J.: Control of fertility in Thoroughbred horses in West Germany. J Reprod Fertil, Suppl 23:311-313, 1975

(67) Wentz, Anne Colston: Endometrial biopsy in the evaluation of infertility. Fertil Steril 33:121-124, 1980

(68) Whitwell, K.E.: Investigations into fetal and neonatal losses in the horse. Vet Clin North Am, Large Animal Practice, Symposium on Equine Reproduction 2:313-331, 1980

(69) Williamson, P.; Dunning, A.; O'Connor, J.; Penhale, W.J.: Immunoglobulin levels, protein concentration, and alkaline phosphatase ability in uterine flushings from mares with endometritis. Therio 19:441-448, 1983

(70) Wilson, Gary L.: Equine hysteroscopy: a window to the internal reproductive tract. VM/SAC 78:1455-1466, 1983

(71) Wingfield-Digby, N.J.; Ricketts, S.W.: Results of concurrent bacteriological and cytological examination of the endometrium of mares in routine stud farm practice 1978-1981. J Reprod Fertil, Suppl 32:181-185, 1982

(72) Wingfield-Digby, N.J.: The technique and clinical application of endometrial cytology in mares. Equine Vet J 10:167-170, 1978

(73) Witherspoon, D.M.; Goldstein, R.T.; Adsit, M.E.: Uterine culture and biopsy in the mare. J Am Vet Med Assoc 161:1365-1366, 1972

(74) Woolcock, J.B.: Equine bacterial endometritis: diagnosis, interpretation, and treatment. Vet Clin North Am, Large Animal Practice, Symposium on Equine Reproduction 2:244-245, 1980

APPENDIX A

EQUINE ENDOMETRIAL WASHING

EQUINE ENDOMETRIAL WASHING

ANIMAL ID BLOCK

CYTOLOGY GRADE:
BASED ON:

SIGNATURE:

DATE:

PHASE OF CYCLE: estrus___ diestrus___ transition___ anestrus___
INITIAL___ REPEAT___ VISIT NUMBER___ CONCURRENT BIOPSY___
CELLULARITY: insufficient___ abundant___
EPITHELIAL CELLS:
NUMBER: many___ moderate___ few___
COHESIVENESS: good___ poor___ moderate___
CELL TYPES:
Cuboidal: ciliated___ secretory___ nonsecretory___
Columnar: ciliated___ secretory___ nonsecretory___
Tall Columnar: ciliated___ secretory___ nonsecretory___
STRIPPED NUCLEI: few___ moderate___ many___
CHLOROCYTES: squamous___ cuboidal___ nuclear change___ spindle___
HYPERPLASIA___ NECROSIS: focal___ diffuse___
METAPLASIA___ DYSPLASIA___
HYPOPLASIA___ CCP___
ORANGOPHILIC: Slender: few___ moderate___ many___
CILIATED TUFTS: few___ moderate___ many___
CYTOPLASMIC FRAGMENTS: few___ moderate___ many___
CELLS WITH INCLUSIONS: Cytoplasmic: few___ moderate___ many___
Nuclear: few___ moderate___ many___
INFLAMMATORY CELLS:
NEUTROPHILS: few___ moderate___ many___ degenerated___
LYMPHOCYTES: few___ moderate___ many___
EOSINOPHILS: few___ moderate___ many___ degranulated___
HISTIOCYTES: few___ moderate___ many___ multinucleated___
HEMOSIDEROCYTES: few___ moderate___ many___
ERYTHROCYTES: few___ moderate___ many___ degenerated___
GLANDULAR CONTENTS:
CASTS: few___ moderate___ many___
CHARACTER: inspissated___ nuclear material___ loose___
SIZE: small___ medium___ large___
BACKGROUND MATERIAL:
PROTEIN: edema___ blood___
FIBRIN___ MUCUS___

APPENDIX B

EQUINE ENDOMETRIAL BIOPSY

EQUINE ENDOMETRIAL BIOPSY

ANIMAL ID BLOCK

BIOPSY GRADE:

RECOMMENDATIONS:

SIGNATURE:

DATE: Initial___ Repeat___
Concurrent Cytology: Yes No
PHASE OF CYCLE: Anestrus Transition (early, late) Estrus Diestrus
LUMINAL EPITHELIUM:
Columnar: normal low tall hyperplastic metaplastic dysplastic
Vacuolations: none few moderate many large small
Squamous Metaplasia: occasional frequent none
LUMINAL CONTENTS: none mucus debris cells (give type)_____
GLANDS: adequate inadequate questionable abnormal dilations
Casts: none few moderate many cellular protein inspissated
Periglandular Fibrosis: none slight moderate severe
Fibrosed Nests: none few moderate many
Periglandular Inflammation: none slight moderate severe
cell type_____
GLANDULAR EPITHELIUM:
Columnar: normal low tall
Vacuolations: none few moderate many
Metaplasia: absent present
STRATUM COMPACTUM:
Edema: none slight moderate loose solid
Inflammatory Cells: none
neutrophils: none Focal: few mod many Diffuse: few mod many
eosinophils: none Focal: few mod many Diffuse: few mod many
lymphocytes: none Focal: few mod many Diffuse: few mod many
plasma cells: none Focal: few mod many Diffuse: few mod many
histiocytes: none Focal: few mod many Diffuse: few mod many
erythrocytes: none Focal: few mod many Diffuse: few mod many
hemosiderophages: none few moderate many
Fibrosis: none slight moderate severe
Hyperemia: absent present
STRATUM SPONGIOSUM:
Edema: none slight moderate loose solid
Inflammatory Cells: none
neutrophils: none Focal: few mod many Diffuse: few mod many pv
lymphocytes: none Focal: few mod many Diffuse: few mod many pv
eosinophils: none Focal: few mod many Diffuse: few mod many pv
plasma cells: none Focal: few mod many Diffuse: few mod many pv
histiocytes: none Focal: few mod many Diffuse: few mod many pv
hemosiderophages: none Focal: few mod many Diffuse: few mod many pv
erythrocytes: none Focal: few mod many Diffuse: few mod many pv
Fibrosis: none slight moderate severe

VITA

Steven Harold Slusher

Candidate for the Degree of

Master of Science

Thesis: THE RELATIONSHIP BETWEEN ENDOMETRIAL BIOPSY, CYTOLOGY, AND BACTERIAL PATHOGENS IN MARES

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