A STUDY OF THE USE OF THE ENDOMETRIAL BIOPSY IN THE EVALUATION OF EQUINE INFERTILITY

By

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CHAPTER I

INTRODUCTION

The mare is classified as a seasonal breeder as are the dog (Asdell, 1964), cat (Scott and Lloyd-Jacobs, 1955; and McDonald, 1969), and some sheep (Dutt and Bush, 1955; and Godley et al., 1965). But under conditions of good nutrition, shelter, and proper light exposure, mares can be bred and conceive during a normally nonproductive season. The duration of the estrous cycle is about twenty-one days, with estrus lasting about five days. During the last day or two of estrus ovulation usually occurs (Roberts, 1971). The breeding season is related to the duration of light exposure the mare receives (Osborn, 1966; Kenny et al., 1975). Kenny et al. (1975) transposed Osborne's data (1966) to illustrate that most ovulations occurred during June, July, and August (the months of longest duration of light) in the northern hemisphere. Unfortunately, the operational breeding season begins February 15 and continues until early July, for breeds that use the universal birthday of January 1. Therefore, one major cause of equine infertility is breeding during a period when most mares are not physiologically able to conceive. Other noninfectious causes of infertility include: infertile semen, irregular estrous cycles, stress, poor nutrition, ovarian hypoplasia, tumors of the reproductive tract, and rarely pituitary and hypothalamic tumors (Roberts, 1971).

Poor perineal conformation (Caslick, 1937a), foaling accidents, dystocia, improper sterilization of instruments prior to vaginal examination or artificial breeding, and poor hygiene during breeding can predispose to infections of the reproductive tract and subsequent infertility.

As a result of these factors the horse is considered to be of low reproductive performance when compared with other classes of livestock. Special light barns, hormone therapy, and carefully kept teasing records can greatly decrease noninfectious breeding problems. Infectious causes of infertility can often be prevented through corrective surgery (Caslick, 1937a; Gadd, 1975; Vaughan, 1970). Treatment of genital tract infections should be with an effective antibiotic for an adequate time.

Because of the many interacting causes of equine infertility, it is often difficult to determine the primary or inciting cause. Rectal, vaginal, and bacteriologic findings are unreliable evidence of infection because of normal physical and cultural variations (Brandt, 1970).

The endometrial biopsy has gained increased use as a diagnostic and prognostic aid in equine reproductive problems. It allows simple, direct, visual evaluations of the endometrium in contrast to other techniques used in the living animal. Bergman and Kenny (1975) have shown that one biopsy is representative of the entire uterus. The endometrial biopsy has been used to determine the stage of the estrous cycle cycle (Hammond and Wodzicki, 1941; Andrews and McKenzie, 1941; Brandt and Manning, 1969; and Brandt, 1970) to detect endometrial disease (Brandt and Manning, 1969; Brandt, 1970; Solomon et al., 1972; Ricketts, 1975; and Kenny, 1978), and to determine if the uterus is capable of carrying a fetus to term (Kenny, 1975 and 1978).

The purpose of this study was to accomplish three main objectives. The first was to establish for endometrial biopsies a uniform grading system from which a diagnosis could be formed. The second was to place mares in prognosis groups based on the diagnosis. The third was to measure the accuracy of the diagnosis and prognosis by obtaining information on each mare's reproductive performance after the biopsy interpretation.

CHAPTER II

LITERATURE REVIEW

The Estrous Cycle

Studies on the reproductive cycle of the mare began primarily in the nineteen hundreds. Differences in the sexual cycle were noted between mares under different conditions and parts of the world. Heape (1900) observed that the mare was a polyestrus animal with a tendency toward monestrum. The seasonal tendency of the estrous cycle was described by Ewart (1915). He noted that estrous cycles were shorter later in the season and ceased when availability and quality of food decreased. Under favorable conditions, however, mares could become pregnant in the winter. Weather conditions and plane of nutrition had no effect on the breeding season or length of estrous periods in a study of normal Iowa mares (Aitken, 1927).

Seaborn (1925), stated that the estrous cycle had a duration of 24 days and consisted of four periods which were: rest, 8 days; proestrus, 3 days; estrus, 3 days; and metestrus, about 10 days. He divided what he called the heat period into two parts. The first period was proestrus in which external signs of heat (relaxed vulva, reddened mucous membranes, mucous discharge from the vulva) are present, but the mare is unwilling to accept the male. The second period was estrus. In estrus Seaborn noted decreased discharge from

the vulva and willingness to accept the male. Ovulation occurred at the middle or end of estrus. Aitkens (1927) did not commonly observe proestral signs of rejection of the stallion.

It was found from the mid-twenties to the mid-thirties that heat and ovulation did not always occur concurrently (Aitken, 1926, 1927; Satoh and Hoshi, 1932-1933; Caslick, 1937b; and McKenzie and Andrews, 1937). In 1928, KUpfer noted that mares may show estrus for one or two days, have a period of nonreceptivity for about two days and then return to heat. This phenomenon was also observed by Andrews and McKenzie (1941) and termed "split estrus." The period from foaling to the first heat ("foal heat") was of variable duration in different areas of the world. McKenzie and Andrews (USA, 1937) observed foal heat about two days after parturition while Sato and Hoshi (Korea, 1934) observed foal heat from 3 to 100 days after parturition. In a 1941 study of draft and light mares in Missouri, foal heat occurred 2 - 30 days after parturition with a mean of 11.4 days (Andrews and McKenzie, 1941).

The range of the estrous cycle is 4 to 83 days (Caslick, 1937b), but in most mares it is 19 - 24 days. Light mares usually have a longer average length of cycle than draft mares (Andrews and McKenzie, 1941).

Anatomical Responses to Hormonal Stimulation

Ovary

The ovary of the mare is ovoid with a depression at the ovulation fossa. It attains its largest size during estrus (Seaborn, 1925), just before ovulation (Aitken, 1927). Aitken states that this is primarily

due to the mature follicle or follicles (4-7 cm diameter), and smaller follicles (1-3 cm diameter), and congestion and infiltration of fluid which enlarges and softens the ovary. The ovary is at its smallest at the end of estrus in the nonovulating ovary (Aitken, 1927).

Ovulation occurs only at the ovulation fossa (Aitken, 1927). Although more than one follicle may mature during estrus, usually only one ruptures (Kupfer, 1928; and Satoh and Hoshi, 1933). Estrus usually ends one to two days after ovulation (Aitken, 1926-1927; Sato and Hoshi, 1933). The mare's ovary continues to form large follicles during early pregnancy (Aitken, 1926, 1927). Cole et al. (1931) observed the corpus luteum of pregnancy persisted, and several follicles of various sizes were present in each ovary from conception to the 40th day of pregnancy. They noted continued follicular development, and corpora lutea from the 40th to the 150th day of pregnancy. Some of the follicles ovulated. Large follicles were absent from the end of the fifth month to late pregnancy and the corpora lutea repressed. At the end of pregnancy, only a few vestiges of corpora lutea remained. Follicles greater than one centimeter in diameter were present.

Vulva, Vagina, Cervix, and Their Secretions

Roberts (1971) has described the changes of the genital tract in response to hormonal influence of the estrous cycle. Congestion and hyperemia of the mucosa of the vulva, vagina and cervix characterize the signs of estrum in the genital tract. The congestion and hyperemia increase in intensity from proestrus to ovulation and then decrease to a pale quiescent state during late metestrum and diestrum. Increased

secretion of the vaginal mucus makes the mucosa appear shiny. The mucus is greatest in amount and lowest in viscosity during estrum. In late metestrum, diestrum, and proestrum the mucus is scant and sticky. During gestation the mucus is copious, sticky and viscid.

Stockard and Papanicolaou (1917) have observed that the appearance of the vaginal smear, in guinea pigs, correlated consistently with the stage of the estrous cycle. In mares, however, there is little correlation in the appearance of the vaginal smear and the stage of the estrous cycle (Andrews and McKenzie, 1941).

The stratified squamous vaginal epithelium was at its greatest thickness (5-10 cell layers) during the proestrus and estrus periods and thinnest during the fifth to fifteenth days of the interestrous period and early and late pregnancy (Andrews and McKenzie, 1941). These findings were similar to findings in other species (McKenzie, 1926; McKenzie and Terrill, 1937). With some exceptions, Andrews and McKenzie (1941) observed the greatest number of leukocytes in the stroma and epithelium during estrus. They were least numerous during the fifth to tenth days of the interestrous period.

During the estrous cycle, the cervix undergoes definite changes, which were illustrated by Lieux (1963, 1970). In the early part of the heat period the cervix is enlarged and congested and the folds are thickened. As the estrous period progresses to its late stages (i.e., just before ovulation) the cervix is so relaxed that it is flaccid and no longer protrudes into the vaginal lumen. The cervical canal is dilated and three to four fingers can be introduced without resistance. Because the cervix has no definite outline during proestrus and estrus, it is not well defined by rectal palpation. The cervix becomes

constricted and the congestion gradually decreases during metestrum. In diestrum the cervix is tightly closed, it may protrude into the vaginal lumen, and the folds are thin. It can be easily outlined by rectal palpation (Roberts, 1971).

Uterus

The uterus, when palpated per rectum is flaccid and soft during estrus. Before estrus and during pregnancy it is tonic and tubular (Roberts, 1971; and Hammond and Wodzicki, 1941). Seaborn (1925) noted that the uterine mucosa at the onset of proestrus was a rosy color, and became moister than during the rest period. The uterine folds thickened and increased in size. The congestion causing the rosy color could be influenced by aspiration of air into the genital tract (Caslick, 1937a). As estrus subsided the mucosa became less moist, thinner, and the mucosal folds decreased in size. There was also a reduction of the size of blood vessels and glandular ducts (Seaborn, 1925).

The endometrium consists of the mucosal epithelium and the lamina propria divided into the stratum compactum and stratum spongiosum (where the glands are located). The mucosal and glandular epithelium is cuboidal to tall columnar, depending on the stage of the cycle. Less than one-half of the epithelium is ciliated. The uterine glands are long and tubular and are more numerous in the uterine horns than elsewhere in the uterus (Hammond and Wodzicki, 1941). In a study of mares killed at various stages of the estrous cycle (Seaborn, 1925) the general microscopic changes were described. During proestrus there was increased growth of epithelial cells and many mitotic figures. After the proliferation of cells, superficial epithelial degeneration

occurred sometimes on the first day of the estrus. Leucocytes were present in large numbers during estrus (Seaborn, 1925; Andrews and McKenzie, 1941). The mucosal and glandular epithelial cells were tallest during late estrus and the early part of the interestrous period in the study by Andrews and McKenzie (1941).

Seasonal and Hormonal Regulation

Most information concerning the hormonal regulation of the estrous cycle in the mare has been extrapolated from other species. One of the most important factors in understanding the reproductive patterns in mares is relating them to the season of the year. The seasonal influences are related to the length of daylight, nutrition, and climatic factors (especially temperature) (Loy, 1970). Quantitative estimations of monthly ovulations were made from 6763 slaughter mares in Australia (Osborne, 1966). The peak ovulation of 91.5% of the mares was in the summer (January) while the low point was 18.5% in the winter (August). The estrous cycle is divided clinically into the follicular phase or estrus and the luteal phase or diestrus. During the follicular phase there is follicular growth, secretion of estrogen, and sexual receptivity. In a study of 11 mares by Stabenfeldt et al. (1972), the duration of estrus remained relatively constant at about 5 to 6 days from April to October. They found no significant difference between mares with single or multiple ovulations in regards to length of estrus and the estrous cycle, life span of the corpus luteum, and plasma progestin during April, May and June. Active resistance to the stallion occurs during diestrus. During this phase of the cycle there was formation of the corpus luteum with its secretion of progesterone.

The peripheral plasma progestin increased abruptly from less than 1 ng/ml during estrus to a maximum of 10 ng/ml six days after ovulation.

Causes of Infertility

The Breeding Season

In 1833 the universal birthday of Thoroughbreds and Standardbreds was changed from May 1 to January 1. Mares which were bred starting June 15, during the normal physiologic breeding season, were now being bred beginning February 15 (Osborne, 1968). This is called the operational breeding season. Therefore many of the low conception rates of mares may be because, when the breeding season begins, mares are not ovulating.

Breeding on the First Postpartum Estrus

Mares are often bred during foal heat to produce as many foals as possible early in the breeding season. The uterine epithelium, however, seldom has returned to its normal state (involution) by foal heat (Andrews and McKenzie, 1941). Moreover, studies have shown that mares bred on foal heat have a lower than normal conception rate (Jennings, 1941; Crowhurst and Caslick, 1946).

Infection of the Genital Tract

Poor perineal conformation such as high tail head, flat croup, sunken anus, and small underdeveloped vulva lips that are pulled nearly horizontal by a sunken anus predispose to pneumovagina (Roberts, 1971). Pneumovagina (aspiration of air into the genital tract) is

also caused by injuries that occur at the time of foaling. Pneumovagina is also called "windsucking" and results in infection of the vagina, cervix, and sometimes the uterus. Caslick (1937a) noted that pneumovagina occurs more often in mares during estrus when the perineal region is more relaxed.

Sunken vagina is mainly a problem in aged mares, whose genital tract is pulled cranioventrad and lacks normal muscle tone. Urine and vaginal secretions are retained in the floor of the vagina ("urine pooling") and may accumulate around the external os of the cervix, thus predisposing to cervicitis and metritis. Urine pooling also may accompany pneumovagina.

Dystocia, dead or diseased foal, retained placenta, or trauma to the genital tract, are major causes of delayed or incomplete involution and uterine inertia. These conditions can result in temporary or permanent sterility, because of secondary infections (Day, 1939).

Lack of proper hygienic procedures during natural and artificial breeding and in routine examination and treatment can result in introduction of many infectious organisms into the genital tract. The most current example of this is the spread of contagious equine metritis in Kentucky (Swerczek, 1978).

Irregularity of the Estrous Cycle

Irregularity of the estrous cycle is common before or early in the physiological breeding season. Anestrum can occur when mares are diseased or emaciated. Anestrus rarely occurs with ovarian hypoplasia or pituitary, hypothalamic and ovarian tumors (Roberts, 1971). Caslick (1937b) felt that many irregular cycles are caused by imbalances

between the various endocrine glands or hormones and the nervous system. Although it is commonly believed that follicular and luteal cysts occur in mares, McEntee (1970) and others have stated that follicular or luteal cysts associated with nymphomania or anestrum do not occur in mares.

Other Factors in Equine Infertility

Other possibile causes of infertility in the mare include: infertile semen, lesions of the oviduct, uterine cysts, uterine anomalies, mucometra, lesions of the cervix and vagina, persistence of a portion of the hymen (Harris, 1956), and congenital anomalies.

Methods of Correcting Infertility

Artificial Lighting

Artificial lighting is an effective method of hastening the onset of the estrus cycle and subsequent ovulation in mares during months when they are normally in late seasonal anestrus or early estrus. Loy (1968) found that by increasing the amount of exposure to artificial light by 30 to 60 minutes per week beginning in December, most mares will be cycling and ovulating normally within 40 to 100 days, when total daily light exposure reaches 15 to 19 hours. Two hundred watt incandescent light bulbs that provide 7 to 15 foot candles of light, five and one-half feet above the floor are satisfactory sources of artificial light.

Hormone Therapy

Many hormones have been used at different times in the estrous cycle to cause estrus during periods other than the normal physiological breeding season in an attempt to correct some problems caused by the operational breeding season.

Mares injected with prostaglandin F2α (PGF2 α) on the 4th to 12th day of diestrus returned to estrus within 3-4 days and ovulated normally (Hughes, 1973). Intramuscular or subcutaneous injection of 1.25 mg is effective in returning mares that are in prolonged diestrus to estrus. PGF2 α has also been used to return mares with endometritis to estrus so that treatment can be more beneficial. The PGF2α causes lysis of the mature corpus luteum and consequent decline of serum progesterone to less than 1 ng/ml (Kenny et al., 1975). Kenny et al. (1975) suggests that the low serum progesterone then releases the inhibition of the hypothalamic-pituitary axis resulting in activation of the follicular system. The corpus luteum through three days is resistant to lysis by PGF2α or saline infusion of the uterus. Hurtgen and Whitmore (1978) recently conducted a study which indicates that manipulation of the cervix and uterus by any uterine infusions, by taking cervical or uterine cultures, or by taking endometrial biopsy during diestrum may cause rapid return to estrus with shortened diestrus.

Human chorionic gonadotropin (HCG) has been used as a lutenizing hormone and is of value in inducing ovulation of existing follicles at a predictable time during estrus. Mares injected with 2000 IU HCG intraveneously or intramuscularly on the second day of estrus usually

ovulated within 48 hours (Loy and Hughes, 1966; Sullivan et al., 1973).

Follicle stimulating hormone (FSH) has been used to stimulate follicle growth, but there is no evidence that it is efficacious (Hughes, 1973). Pregnant mare serum gonadotropin (PMSG), a FSH-like compound, has also been used to stimulate follicle growth, and induce ovulation. Hughes (1973) stated that this has not been shown to occur and therefore may not be useful in treatment of anestrus mares.

Diethylstilbesterol may initiate lutenizing hormone (LH) release and subsequent ovulation. If the doses are too high, estrogen will inhibit follicle growth and may have deleterious effects on reproductive function (Burkhardt, 1946).

Progesterone has been used to prevent early embryonic death due to a progesterone deficiency, but Hughes (1973) states there is little evidence to support this use of progesterone. Progesterone can be used to treat mares in prolonged estrus or diestrus as often occur early in the breeding season (Kenny et al., 1975).

Infection of the Reproductive Tract

Infections of the reproductive tract are determined by cervical and uterine culture. Dimmock and Edwards (1928) have described appropriate methods of bacteriologic culture. In their study noninfected mares consistently had negative cultures (no bacterial growth) and infected mares consistently had positive cultures (bacterial growth). A report of Lord Porchester's Veterinary Committee (1965) defined that "positive swabs" are those which yielded pathogenic organisms. This did not mean that mares had an established infection and would be

barren. Often these infections subsided spontaneously while others could be treated successfully. Rasenbeck (1965) made several prophylactic recommendations for prevention of genital infections. These recommendations dealt with care of the mare and stallion during service, care of the mare during gestation, during and after foaling and after an abortion. He also emphasized when cultures should be taken, proper nutrition, pen sanitation, and helminth control.

Brandt (1970) illustrated variance of cervical and uterine cultures in the mare. He found that two consecutive cervical cultures from the same mare at the same examination period differed 30% of the time in normal mares and 23% of the time in infected mares. Results of uterine cultures revealed that the microbial population of the cervix was not necessarily representative of that of the uterus. Uterine cultures are therefore more reliable than cervical cultures, in revealing the etiologic agent of uterine infections. Another method of determining the prescence of uterine infection is by endometrial cytology (Knudsen, 1964). Cytology is useful in the diagnosis of chronic uterine disease when there is no cultural evidence of infection (Solomon et al., 1972).

Treatment of Uterine Infections

When the causal agent is isolated the drug sensitivity of the organism should be determined and the mare should be treated with an effective drug at an effective dose for a sufficient view. Too often mares are treated for infections with an inappropriate drug or with a good, effective drug for too short a duration (Davis and Abbitt, 1977).

Surgical correction of conformational defects of the reproductive tract can be effective in preventing infection. Caslick's surgery is useful in prevention of "windsucking" (Gaslick, 1937a). Surgery to prevent urine pooling is also efficacious in preventing concentration and establishment of organisms in the genital tract. Surgical procedures include: submucosal resection (Lieux, 1963) and urethroplasty (Twisselmann, 1971).

Endometrial Biopsy

Endometrial biopsy provides a more direct means of examining the reproductive capacity of the uterus. The increased reliability of endometrial biopsy over cervical and uterine culture has been shown (Brandt, 1970). Three instruments for uterine biopsy and fluid collection from the mare and cow have been described by Tobler (1966). These were the first practical and safe instruments for use in the mare. The technique of endometrial biopsy has been described by many workers (Tobler, 1966; Brandt and Manning, 1969; Brandt, 1970; and Ricketts, 1975). The Olympus biopsy gastrocamera fiberscope is useful for more intricate intrauterine studies (Brandt and Manning, 1969). Solomon et al. (1972) conducted a study in which reproductive history was compared with uterine histologic and cytologic findings. They observed that several mares that had previously had poor reproductive performance had histologic and cytologic evidence of chronic uterine disease with no cultural evidence of infection. Bergman and Kenny (1975) described the histologic changes of the endometrium in response to infection and the effect of these changes on the reproductive performance of 60 mares. Ricketts (1975) classified mares based on the

histology of the endomotrial biopsy. Based on this classification, certain histologic changes were positively correlated with subfertility. Interpretation of the histology required a thorough reproductive history as well as clinical and cultural findings. Kenny (1975, 1978) has shown that the endometrial biopsy allows the examiner to determine if the uterus is capable of carrying a fetus to term. He placed mares into three categories (I, II, III) based on clinical findings (rectal palpation and culture) history, and seriousness of the endometrial changes. The categories: I, II, and III served as prognostic categories to indicate the necessity of treatment and the ability of the endometrium to carry a fetus to term. Kenny conducted a prognostic study of 244 mares placed in these three categories. His findings demonstrated the effectiveness of the endometrial biopsy as a prognostic tool.

The endometrial biopsy is probably the most practical, safe, and reliable means of detecting abnormalities of the equine endometrium. It is used to its greatest advantage when its interpretation is correlated with history, clinical, and cultural findings. This can be a great aid to the horse owner. Much expenditure of money, time, and work can be saved by determining early in the breeding season that a mare is not capable of supporting a fetus to term.

The purpose of this study was to establish a uniform grading system for endometrial biopsies from which a diagnosis could be made. The diagnosis was used to determine to which of 4 prognostic groups the mare could be assigned. The prognostic group was to predict the mare's future reproductive performance. The final step of the study

was to test the efficacy of the grading and prognostic system by comparing the reproductive performance of these mares after the biopsy to the prognostic group in which they were placed.

CHAPTER III

MATERIALS AND METHODS

Collection of Biopsies

Some 400 endometrial biopsies were sent to the Department of Pathology at Oklahoma State University and to the Oklahoma Disease and Diagnostic Laboratory from 1974 to 1978. The biopsies had been taken from Thoroughbred, Arabian, Appaloosa and Morgan mares, most of which had been barren for several years. Some biopsies were taken as a part of a routine reproductive examination of mares with good reproduction histories. Many of these biopsies were used as the normal controls. The biopsies were taken at all phases of the estrous cycle.

Preparation of the Biopsy

The tissue was fixed in 10% buffered formalin. The completed uterine biopsy report (Figure 1) was sent with the biopsy to the histology laboratory. The uterine biopsy report provided information concerning the mare's reproductive history, behavior, cultural and physical findings. The average size of the biopsy was 0.8 cm x 0.5 cm x 0.3 cm. The tissue was cut lengthwise to provide a flat surface for orientation of sectioning. The biopsy was then prepared (in the usual way) for histology. Two sections were stained, one with hematoxylin and eosin, and the other with Pollack's trichrome (Sano,

UTERINE BIOPSY REPORT
Department of Veterinary Pathology
Oklahoma State University
Stillwater, Oklahoma 74074

Date Collected_____ Date Received in Lab_____ Pathology No._____

Clinician(s)_____ Address of Clinician_____

Owner_____ I.D. of horse_____

Species_____ Breed_____ Age_____ Culture Results_____ Date Taken_____

OVARIES_____

UTERUS_____

CERVIX_____ VAGINA_____

BEHAVIOR_____

HISTORY:

MACROSCOPIC:

MICROSCOPIC:

HISTOLOGIC DIAGNOSIS:

PROGNOSIS:

Figure 1. Uterine Biopsy Report. The physical, clinical, cultural, and behavioral findings and history were supplied by the clinician submitting the biopsy to the study.

1949). The stained sections were then graded on histologic findings by the proposed grading system.

Grading Criteria

The following grading criteria were used to determine the diagnosis (Figure 2, Tables I and II). Superficial inflammation was based on the number of inflammatory cells (neutrophils, plasma cells, lymphocytes) in the mucosal and glandular epithelium (Figure 3) per high power field (hpf, 400x). If the leucocytes were only seen in a few foci it was classified as focal. If they were distributed uniformly in the epithelium, it was classified as diffuse. The subclasses, light, moderate, and severe, indicated the density of the leucocytes. One to three leucocytes per hpf was light, 4 to 10 was moderate, and more than 10 was severe. Deep inflammation was based on the approximate number of leucocytes in both layers of the lamina propria (the stratum compactum and stratum spongiosum) (Figures 4 and 5). If there were 4 to 5 cells per hpf the inflammation was light. If there were 6-15 cells it was moderate. If there were greater than 15 cells, it was severe.

Fibrosis of glands was elevated on the change in gland structure and arrangement, and on the severity of the change (Table II). The descriptive categories were: (a) fibrosis without clumping of glands (Figure 6); (b) fibrosis with clumping of glands; and (c) fibrosis with clumping and dilation of glands (Figures 7, 8, 9, and 10). Gland dilation without fibrosis was also recorded. The criteria for the severity of fibrosis (Table II) were based on Kenny's observations (1975). One to three layers of fibrocytes encircling most glands in

Dr.
Owner:
Date:
Horse ID:
Path. No.
Score:

EQUINE ENDOMETRIAL BIOPSIES

A. INFLAMMATION

1. Superficial (mucosal and glandular epithelium)
   F - l m s
   D - l m s
   Epithelial vacuoles

2. Deep (both layers of lamina propria)
   neutrophils
      F - l m s
      D - l m s
   plasma cells and lymphocytes
      F - l m s
      D - l m s
   eosinophils
      F - l m s
      D - l m s

B. LAMINA PROPRIA

1. Fibrosis
   a. Without clumping of glands - l m s

   b. With clumping of glands - l m s

   c. With clumping and dilation of glands - l m s

2. Glands dilated without fibrosis

3. Hemosiderophages

4. Edema - l m s

C. GLANDS - NUMBERS

1. Normal

2. Too few:
   atrophy-senile/gonadal
   degenerative-2° inflammation
   hypoplasia

3. Too many

4. Gland contents

Diagnosis:

Prognosis:

F = focal
D = diffuse
l = light
m = moderate
s = severe

Figure 2. Endometrial Biopsy Grading Criteria. These criteria were used in the evaluation of all biopsies.

TABLE I

CLASSIFICATION OF INFLAMMATION IN THE EQUINE ENDOMETRIAL BIOPSY

	Light	Moderate	Severe
Superficial Inflammation	1-3 cells/hpf*	4-10 cells/hpf	> 10 cells/hpf
Deep Inflammation	4-5 cells/hpf	6-15 cells/hpf	> 15 cells/hpf

*cells/hpf: Indicates the number of inflammatory cells per high power microscopic field (400x).

TABLE II

CLASSIFICATION OF PERIGLANDULAR FIBROSIS
IN THE EQUINE ENDOMETRIAL BIOPSY

	Light	Moderate	Severe
Focal	< 10 layers*	10-15 layers	
Diffuse	1-3 layers	4-10 layers	> 10 layers

*layers indicates layers of fibrocytes encircling glands (periglandular fibrosis).

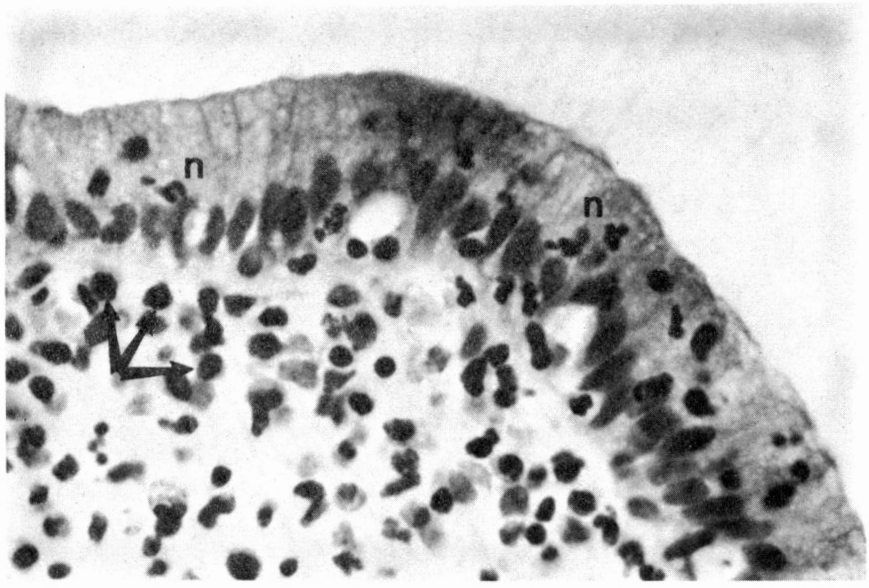

Figure 3. Histologic Section of Endometrium with Superficial Inflammation. There is a moderate infiltrate of neutrophils (n) in the luminal epithelial cell cytoplasm. A light infiltrate of lymphocytes and plasma cells (arrows) is seen in the lamina propria (deep inflammation).

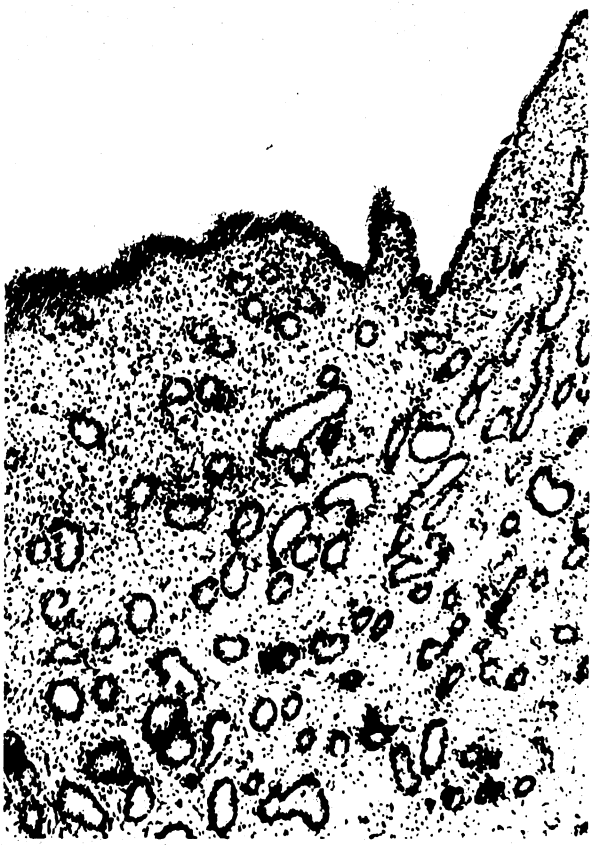

Figure 4. Histologic Section of Endometrium with Deep Inflammation. Moderate diffuse inflammatory cell infiltrate of the lamina propria. Prognosis Group II.

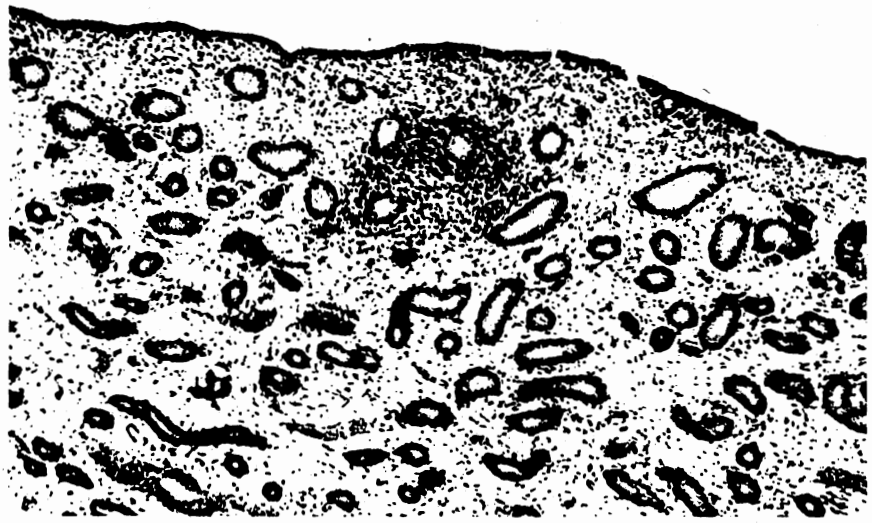

Figure 5. Histologic Section of Endometrium with Deep Inflammation. There is a severe focal inflammatory cell infiltrate and no fibrosis. Prognosis Group II.

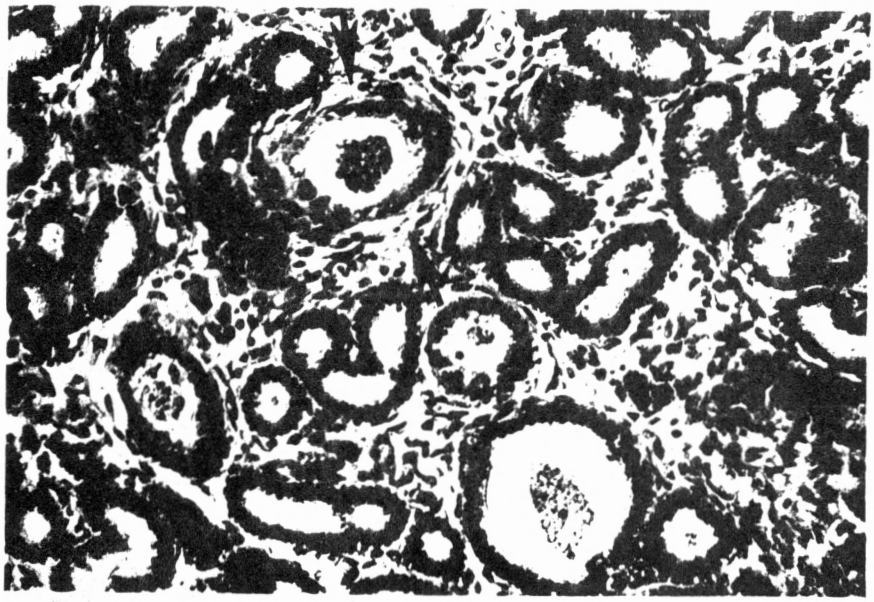

Figure 6. Histologic Section of Endometrium with Light Fibrosis Without Clumping of Glands. Light focal fibrosis (arrows) is seen around a dilated gland containing cellular debris in its lumen. Prognosis Group II.

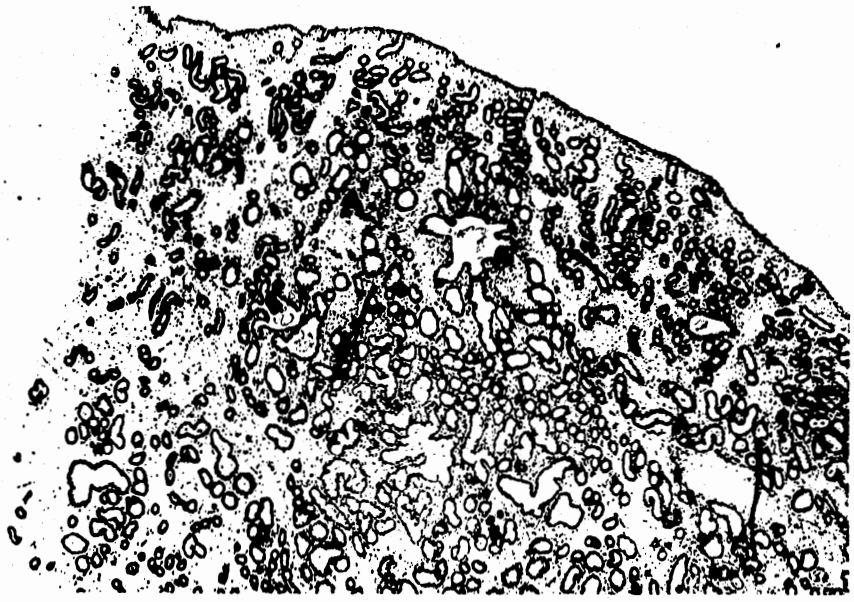

Figure 7. Histologic Section of Endometrium with Moderate Fibrosis and Dilation of Glands. Prognosis II.

Figure 8. Histologic Section of Endometrium with Moderate Fibrosis, Dilation and Clumping of Glands. The glands are clumped into two distinct zones. Prognosis Group III.

Figure 9. Histologic Section and Endometrium with Severe Fibrosis and Dilation of Glands. Prognosis Group IV.

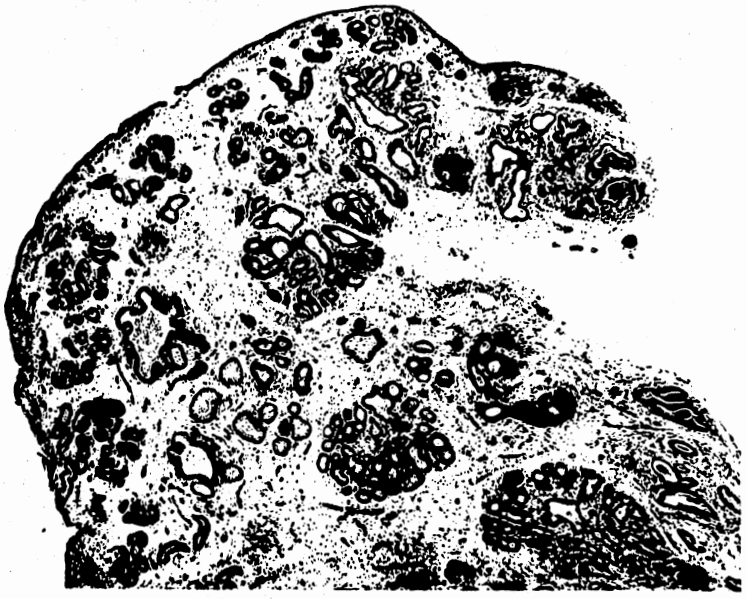

Figure 10. Histologic Section of Endometrium with Severe Fibrosis, Dilation, and Clumping of Glands. Prognosis IV.

the section (40x), or up to 10 layers surrounding glands in a few foci was light fibrosis (Figure 6). Four to ten layers of fibrocytes surrounding most of the glands, or 10-15 layers surrounding a few glands, were moderate fibrosis (Figures 7 and 8). When there were greater than 10 fibrocytes surrounding most of the glands, it was severe fibrosis (Figures 9 and 10).

The number of hemosiderophages (Figure 11) was roughly estimated as 0 to 4. Zero was none, and 4 was many hemosiderphages distributed regularly in the lamina propria and glandular epithelium. The amount of edema was estimated as light, moderate, or severe, and focal or diffuse. Later in the study, edema was found to be inappropriate to the study of endometrial biopsies.

The number of glands was graded subjectively through a low power objective (40 magnification) as normal, too few or too many (Figures 12, 13, and 14). If it appeared that there were too few or too many glands, the mare's age and history were reviewed to determine the cause.

Diagnosis and Prognosis

The diagnosis was made from data on the grade sheet (Figure 2). For example, if there was moderate inflammation composed of lympocytes and plasma cells along with light fibrosis and adequate number of glands, the diagnosis would be moderate or moderately severe, chronic endometritis with light fibrosis.

Four prognostic categories were formed (Table III). If the diagnosis was normal, Prognosis I was given. Prognosis I was translated as: nothing seen in the endometrium that would impair fertility.

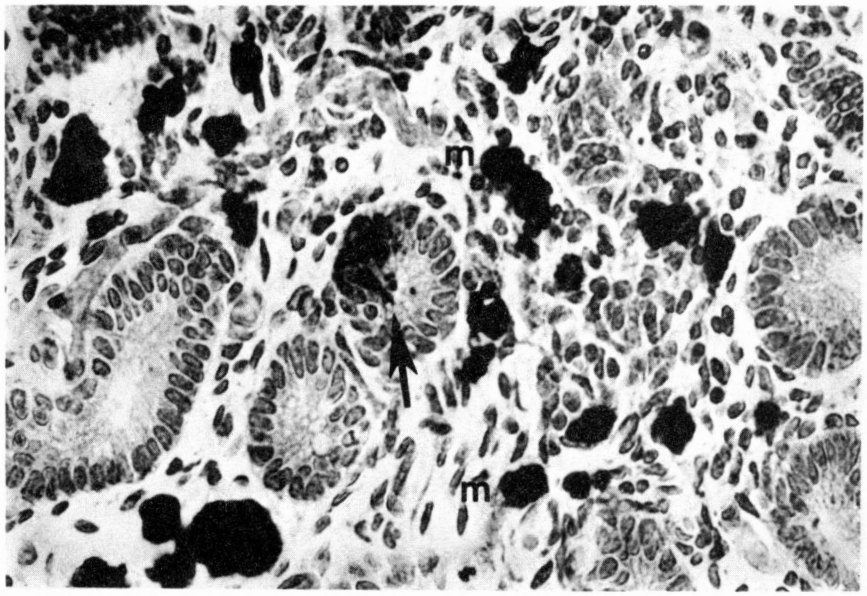

Figure 11. Histologic Section of Endometrium Stained with Prussian Blue to Show the Iron in Hemosiderin. Hemosiderin is in macrophages (m) and in glandular epithelium (arrow).

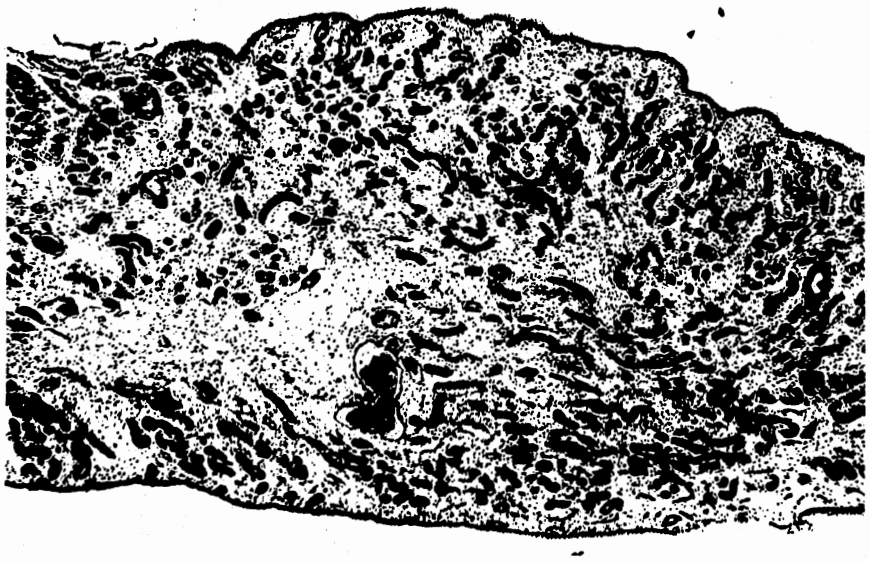

Figure 12. Histologic Section of Normal Estrual Endometrium. The glands are uniform in size and evenly spaced. Prognosis Group I.

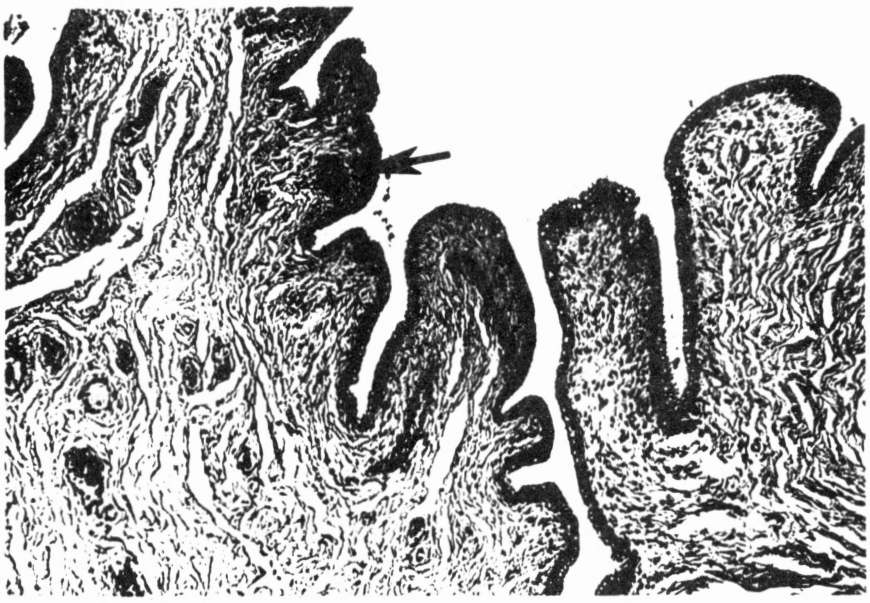

Figure 13. Histologic Section of Endometrium with Glandular Hypoplasia. Only an occasional gland is present (arrow). Prognosis Group IV.

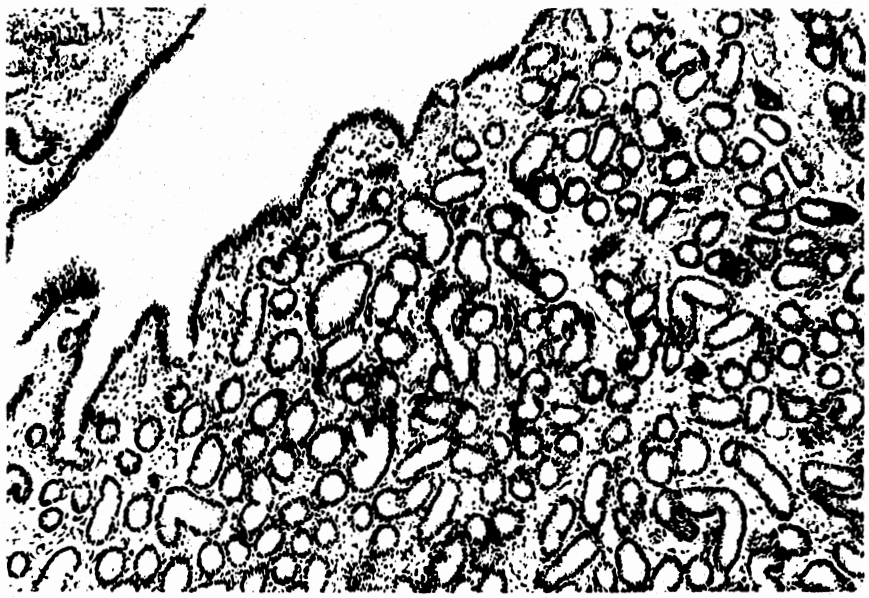

Figure 14. Histologic Section of Endometrium with the Appearance of Increased Number of Glands. The mare had a granulosa cell tumor of the ovary. Prognosis Group II.

TABLE III

CRITERIA FOR DETERMINING THE PROGNOSIS GROUP FOR THE MARE ON THE BASIS OF ENDOMETRIAL BIOPSY

Prognosis Group	Inflammation	Fibrosis	Glands
I	None	None to Light	Normal
II	Light to Severe	None to Moderate	Normal or More Than Normal
III	None to Severe	Moderate to Severe	Normal
IV	None to Severe	Severe	Normal or Less Than Normal

Prognosis II was given when there was light to severe inflammation, absent to moderate fibrosis and adequate or possibly too many glands. Prognosis II was translated as: the change was not permanent and the uterus should have the ability to conceive and carry to term after treatment. Prognosis III was given if there was absent to severe inflammation and moderate to severe fibrosis. Prognosis III was translated as: the mare probably could conceive, but it was not likely that she could carry the fetus to term. Prognosis IV was given if there was absent to severe inflammation and were too few glands or severe fibrosis, or both. Prognosis group IV was translated as: the mare had little chance of conception, and it was very unlikely she would be able to carry a fetus to term.

Retrospective and Current Data

The veterinarians, owners, or ranch managers were contacted at least one year after the biopsy results had been recorded. They were asked specific questions about their mare's reproductive performance after the biopsy (Figure 15). Pregnancy was diagnosed by rectal palpation by the attending veterinarian. The information obtained was correlated with the diagnosis and prognosis to determine the accuracy of the grading system. Comparisons were made with history, age, perineal conformation, prognosis group, and reproductive performance.

Analysis of Data

The four prognosis groups were compared by using a Z-test to determine if the assignment of a mare to a prognosis group, based on the

Dr._____
Owner_____
Horse ID_____

RETROSPECTIVE ANALYSIS
OF
EQUINE ENDOMETRIAL BIOPSIES

1. Has the mare ever carried a foal to term? Yes No
2. When did she have her last foal? _____years ago
3. Has she ever aborted a fetus? Yes No
   a. How many? _____(number)
   b. How long ago? _____years
   c. What was the cause of abortion? _____
4. Is she barren now? Yes No
   a. How many attempts at breeding were made since biopsy _____.
   b. How long has she been barren? _____years
5. Culture results _____ (what)
   a. When cultured. (dates)
   b. How many times cultures? _____
6. Conformation
   a. perineal conformation
   b. windsucker
   c. does she have a caslick?
   d. does she pool urine?
7. Bred since the biopsy? Yes No
   a. Did she conceive? Yes No
   b. Did she carry to term? Yes No
   c. If not to term - stage of abortion - cause of abortion.
   d. Criteria used for original pregnancy diangosis. (How long was the mare followed?)
8. If not rebred, why?
9. Where is mare now? name and address
10. Comments:

Figure 15. List of Questions Asked Owners, Veterinarians and Farm Managers After the Biopsies were Graded and Placed in Prognosis Groups

uterine biopsy, was indicative of the mare's ability to carry a foal to term. Age groups were also analyzed by the Z-test to determine whether age could be related to a particular prognosis group.

CHAPTER IV

RESULTS

The Biopsy Report

The requirement of physical, clinical and cultural findings and history on the biopsy report form (Figure 1) permitted observation of many factors that might affect reproductive ability. Although this information was not available on about 50 mares, it was useful in identifying asynchronies of the estrous cycle and even management problems. In the beginning and at the end of the breeding season there were many examples of asynchronies. Behavioral, ovarian and uterine aspects of the estrous cycle were considered in the individual mare. Some mares had clinical signs of estrus without ovulatory follicular development. In these mares, the uterus was in anestrus (Figure 16), rather than in estrus (Figures 12 and 17). Also, some had estrual behavior and ovulatory follicles and a uterus that was still in anestrus. These mares were put in group II because it was assumed the mare would not conceive if the uterus was not in estrus. For these mares no specific therapy was prescribed because it was thought the problem would be solved by time. Branched glands, a feature of progesterone response, was seen along with estrual behavior. This occurred in 2 mares with granulosa cell tumors of the ovary. Sometimes biopsies confirmed that the uterus was in estrus even though the mare did not show estrual behavior (silent heat).

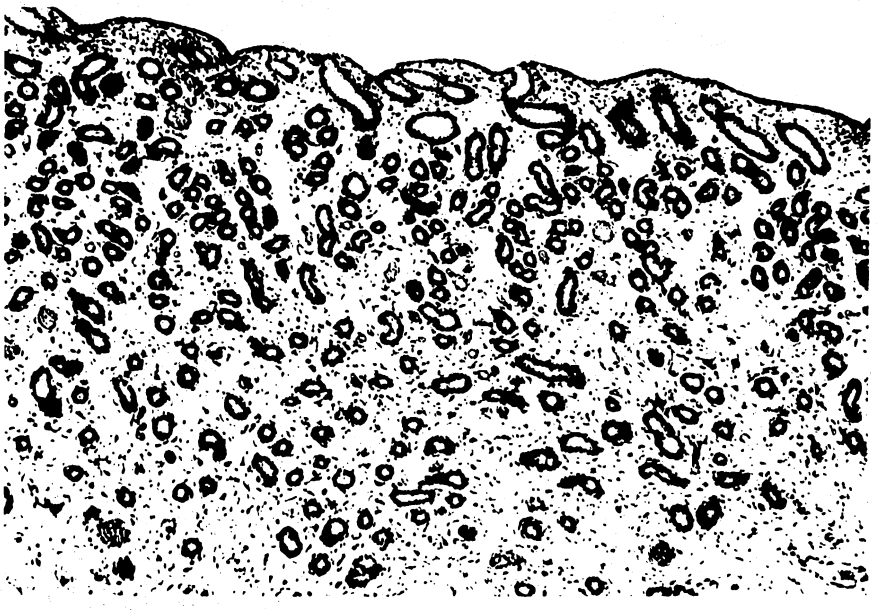

Figure 16. Histologic Section of Anestrual Endometrium. The epithelium is short and the glands are small and straight. Prognosis Group II.

Figure 17. Histologic Section of Normal Estrual Endometrium. The epithelium is tall columnar and the glands are separated by edema.

The Biopsy Grading System

About 10 percent of the 400 biopsies were not acceptable because samples were too small, incorrectly taken, or not properly fixed. The grading system was based, therefore, on the remaining biopsies.

The purpose of the three subclasses of fibrosis (Figure 2) was to determine if there was any differences when compared with prognosis. Because there were no significant differences in fibrosis with either clumped or dilated glands, fibrosis was evaluated as light, moderate, or severe (Table II).

Some mares had dilated glands without fibrosis of the lamina propria. Uniform dilation of glands was characteristic of recent pregnancy. Occasionally, anestrus mares had glands that were small but uniformly dilated and filled with dense pink inspissated protein. Nonuniform dilation of glands was found in some cases to correlate with fibrosis of the lamina propria in serial sections of the same biopsy.

Eosinophils were a common finding. They were sometimes very dense and migrated through the luminal epithelium along with neutrophils (Figure 18). In other mares there were many eosinophils in the lamina propria with no migration through the epithelium. Eosinophils were also seen as occasional companions of plasma cells and lymphocytes.

Hemosiderophages were most numerous in the lamina propria right after foaling. At foal heat, hemosiderin was also seen in the cytoplasma of glandular epithelial cells (Figure 11).

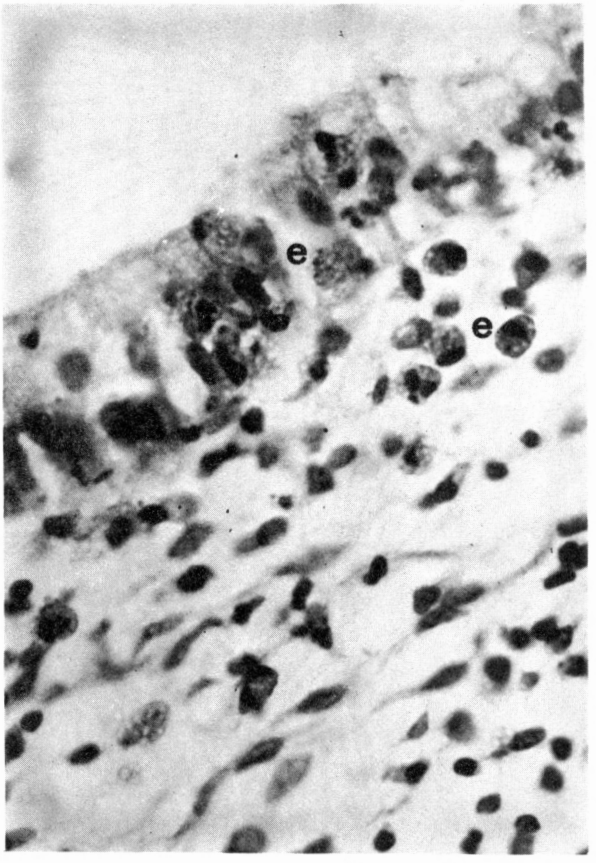

Figure 18. Histologic Section of Endometrium with Eosinophils in the Luminal Epithelium (e) and Lamina Propria (e)

The number of glands was judged subjectively by scanning the section with a low power objective. When the biopsy was collected in anestrus (Figure 16), a judgment of too few glands was never made. In anestrus the glands appeared few, straight and small (seasonal atrophy). When there was a question of too few glands in an anestrual uterus, it was recommended that another biopsy be collected when the mare had an ovulatory follicle as well as behavioral estrus. It was decided that biopsies should be collected at the end of the nonproductive season while the mare is still cycling. This was preferred over the beginning of the next breeding season when the uterus is likely to still be in anestrus.

No counts were made of the number of glands per microscopic field. Occasionally, it was believed that too many glands were present. This occurred when the glands filled the section of endometrium and were closely packed, even in the presence of edema (Figure 14). This was seen in 2 mares with ovarian granulosa cell tumors. Two other mares, on which this judgment was made, had normal ovaries on palpation. Subsequent reproductive performance was not available. In 2 other mares, this judgment was also made. These mares had been pregnant recently, however, and there was uniform growth and dilation of glands.

In one mare a diagnosis of endometrial hypoplasia was made (Figure 13). She had never had a foal but cycled regularly. Karyotyping was done on a buffy coat preparation from the mare, but no chromosomal abnormalities were seen. Giemsa-banding of the chromosomes was not done. A second biopsy from this mare looked the same as the first.

Testing the Diagnosis and Prognosis

Complete data were available on 118 mares (Figures 19 and 20). Almost 200 had to be removed from the study because of incomplete follow-up data or because they were still in foal. The performance of mares in each prognosis group follows: group I: 26 mares were bred, 17 conceived, 8 foaled, and 8 were still in foal at the time of the study; group II: 69 were bred, 47 conceived, and 43 foaled; group III: 18 were bred, 6 conceived, and none foaled; group IV: 5 were bred, one conceived, and none foaled. The Z-test was used to determine if there were significant differences in the conception or foaling rates of mares in the prognosis groups. No significant differences were found between groups I and II or between groups III and IV. More mares conceived and foaled in group I than in groups III or IV ($p < .05$). Also, more mares in group II conceived and foaled than in groups III or IV ($p < .05$).

Age and Prognosis Group

The ages of 105 mares that conceived and had time to foal were available. The age range was similar for all four prognosis groups. The respective age ranges were--group I: 5 to 23 years; group II: 2½ to 22 years; group III: 7 to 27 years; group IV: 7 to 28 years. The conception and foaling rates of mares in 5-year age groups are shown in Figure 21. There were four mares in the 1 to 5 year age range; one was in group I and three were in group II. Three of the four mares conceived, and 2 foaled. No mares were assigned to group III or IV. Thirty-one mares were 6 to 10 years old: 5 in group I, 22 in group II, 3 in group III, and one in group IV. Twenty-two of

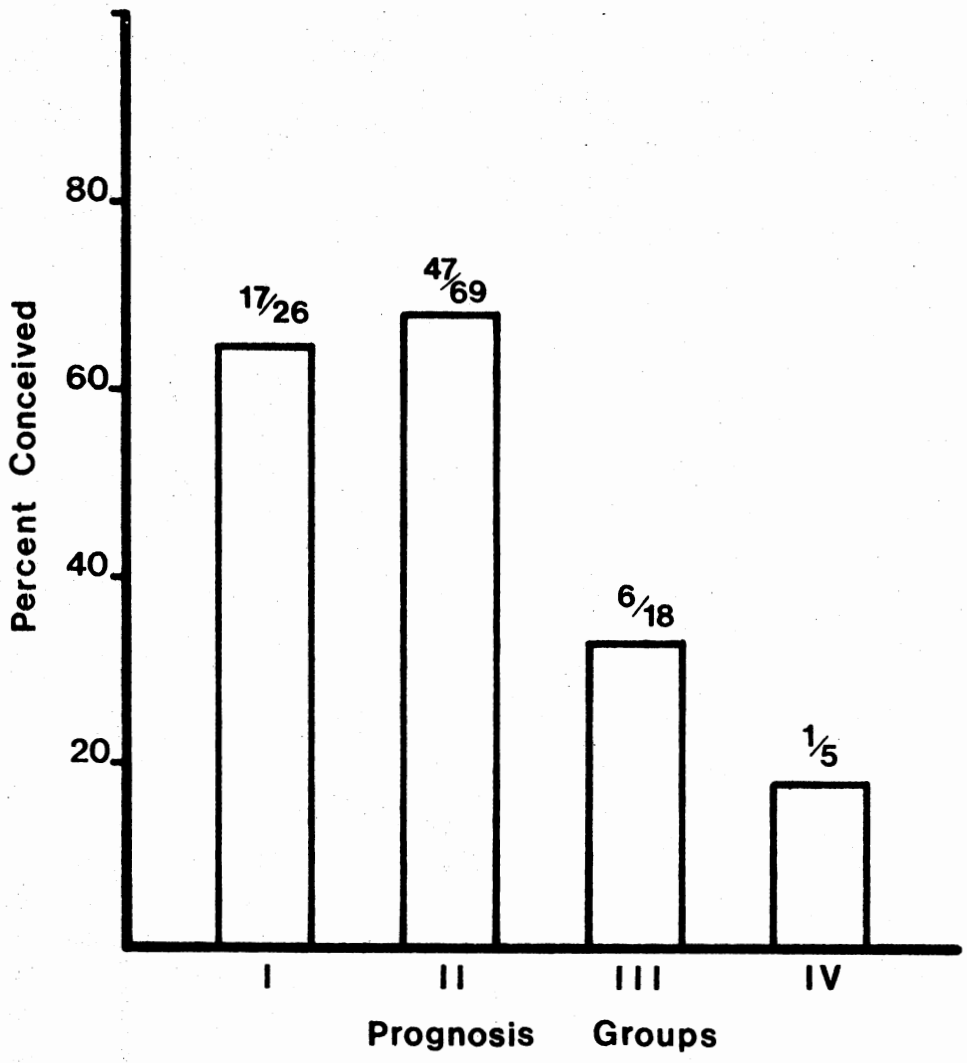

Figure 19. Conception Rate of the Four Prognosis Groups. Numbers above bars indicate number of mares that conceived (numerator) over the number that were bred (denominator).

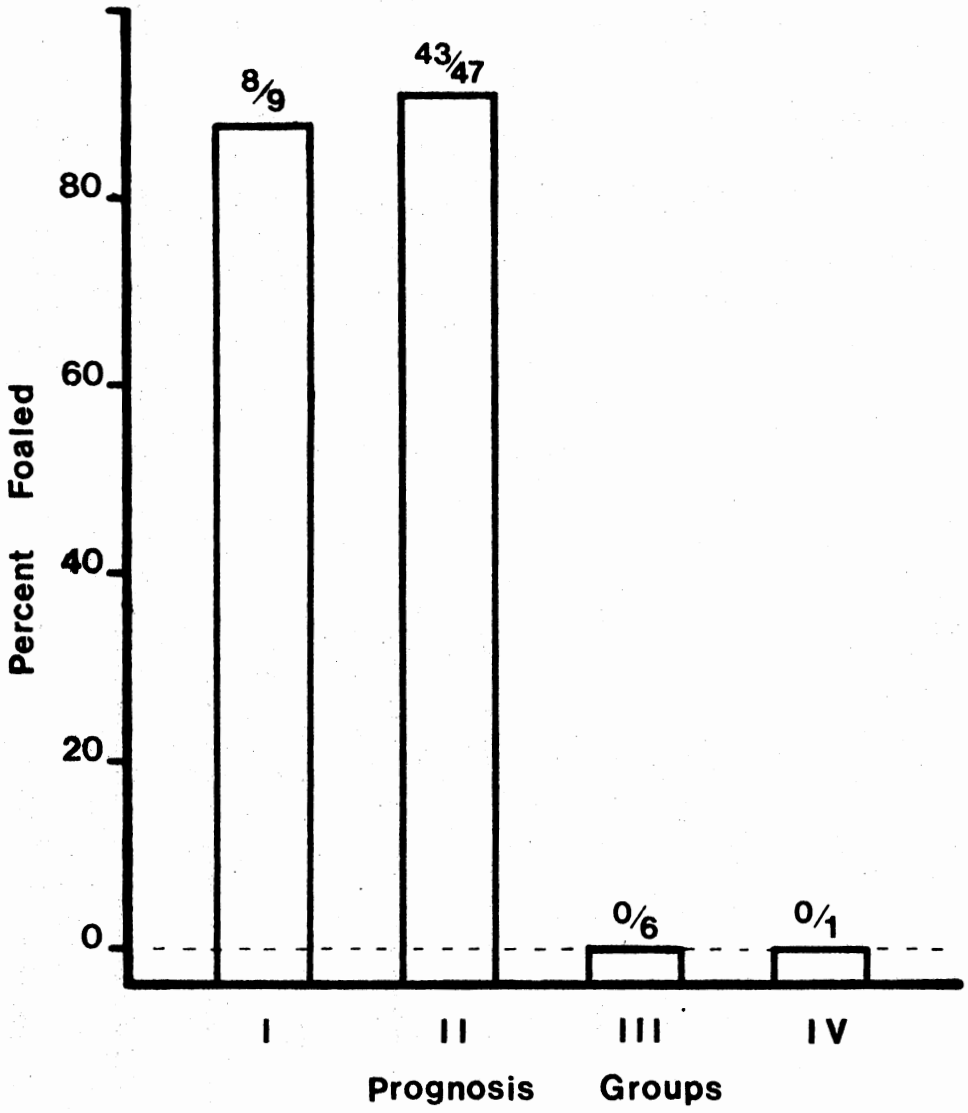

Figure 20. Foaling Rate of the Four Prognosis Groups. Numbers above bars indicate number of mares that foaled (numerator) over the number that conceived (denominator).

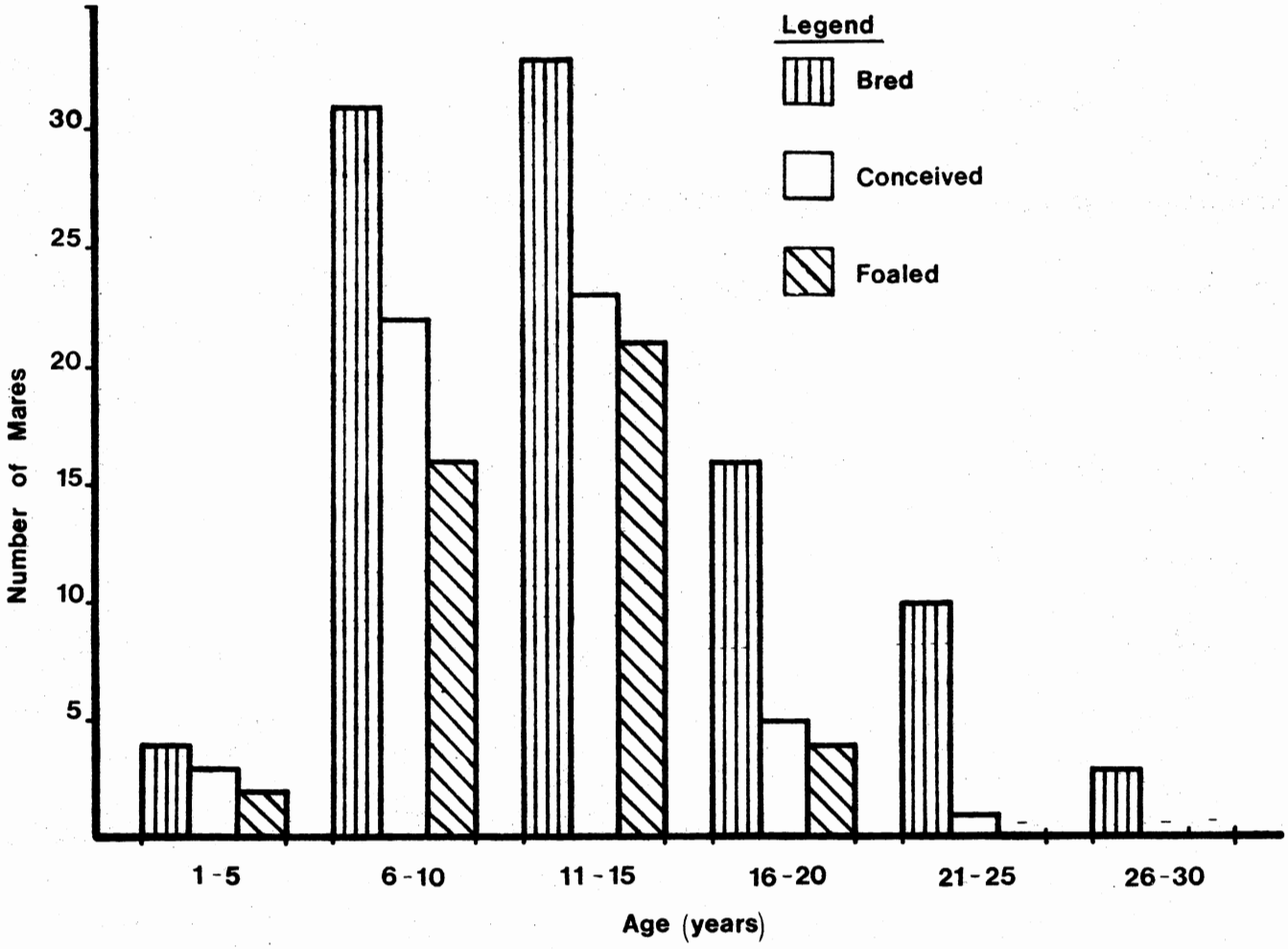

Figure 21. Conception and Foaling Rates of Mares in Five Year Age Ranges. The number of mares that were bred, conceived, and foaled are indicated.

the thirty-one mares conceived, and 16 foaled. Thirty-three mares were 11 to 15 years old: 8 were in group I, 22 were in group II, 3 were in group III, and none were in group IV. Of the thirty-three mares, twenty-three conceived, and twenty-one foaled. Sixteen mares were 16 to 20 years old. No mares were in group I, 12 were in group II, 3 were in group III, and 1 was in prognosis group IV. Only five of the sixteen conceived, and four foaled. Ten mares were in the 21 to 25 age range. One was in group I, 4 were in group II, 3 were in group III, and 2 were in group IV. One of the ten mares conceived, and none foaled. Three mares were 26 to 30 years of age: none were assigned to groups I or II, 2 were in group III, and 1 was in group IV. None of the mares in this age group conceived. There were no statistical differences, based on the Z-test in either the 1 to 5 or 6 to 10 age ranges. In the 11 to 15 year age range, more mares were in group I than in groups III or IV ($p < .05$). In the 16 to 20 year age range there were significantly more ($p < .05$) mares in each of the single groups II, III and IV than in I. In the 21 to 25 age group there were significantly more ($p < .05$) mares in group IV than in groups II or I, and significantly more ($p < .05$) mares in group III than in group II. The same was true of the 26 to 30 year group.

Bacteria, Conformation, and Prognosis Group

Based on the cultural results reported by the clinicians, no relationship was observed between prognosis group and species of bacteria isolated from the uterus. Also, there was no association between perineal conformation and species of bacteria. Common bacteria isolated from the uterus were β-hemolytic Streptococcus, Klebsiella,

Pseudomonas and E. coli. Three mares that were infected with Klebsiella conceived and aborted within 3 months. Just as many other mares with Klebsiella infections responded to therapy and subsequently conceived and foaled. Isolates of β-hemolytic Streptococcus commonly were associated with a history of abortion, although Klebsiella was isolated from 3 to 6 mares that aborted during this study.

Where recorded, there was a positive relationship between perineal conformation and prognosis group. In group I, 5 out of 7 mares had good conformation. In group II, 36 out of 65 mares had good conformation and in group III, only 2 out of 12 mares had good conformation. None of the mares in group IV had good conformation.

CHAPTER V

DISCUSSION

The Biopsy Report

It was difficult at times to get the practitioner submitting the biopsy to supply the information requested in the biopsy report form (Figure 1). As indicated in the results, this information was beneficial in detecting asynchronies, mismanagement and other problems. Often asynchronies probably occur because the physiological responses of the uterus tend to follow the phychological responses in the onset of the breeding season, and the physiological responses of the uterus supercede the psychological responses to the end of the breeding season. The first effect of the plasma hormones may be on the behavior of mares prior to physiological effects. This could be a natural mechanism to bring mare and stallion together in plenty of time prior to ovulation. Asynchronies may also result from endocrine imbalances, irregular cycles or stress conditions.

The Biopsy Grading System

Fibrosis was considered the most important factor in assigning the prognosis groups. Occasionally, nonuniform dilation of glands corresponded with gland fibrosis in serial sections of the same biopsy. This indicates that, although Bergman and Kenny (1975) found that one

biopsy was usually representative of the entire uterus, exceptions exist.

The real significance of eosinophils in the mare endometrium is not known. The findings of this study indicate they have a function in acute inflammatory reactions (Figure 18).

Hemosiderophages, often present after pregnancy (Figure 11) appear to have significance only in the fact that they occur after periods of congestion of the endometrium. One therefore may commonly see hemosiderophages after estrus also.

The amount of edema in the lamina propria changes in relation to the stage of the cycle. Since the biopsy technique can distort the appearance of edema in the tissue, no interpretations were based on the amount of edema. The number of glands per low power field could also change with respect to the biopsy technique. If glands were spread farther apart, fewer would be counted per field. But, if they were compressed, more would be counted. The number of glands, therefore, was subjectively estimated. Extreme degrees of change are required before any change of gland numbers can be regarded as pathologic change.

The Diagnosis and Prognosis

In contrast to three prognostic categories used by Kenny (1978), this study is based on four prognostic groups. The purpose of 4 groups was to place a more precise interpretation on the changes in the uterus and to determine how these changes influence the normal function of the uterus to support fetal life. As a result of these differences the two systems cannot be directly compared. Kenny's

category I (1978) and prognosis group I in this study are similar. Category II (Kenny, 1978) allows for more extensive fibrotic changes of endometrial glands than group II in this study. In group II it is postulated that the mare should be capable of normal reproductive performance (group I) if she is properly treated for the inciting cause. Therefore group II in this study performs similarly to group I. Category II is probably intermediate to group II and III in this study. In group III the endometrial changes are permanent and severe to the degree that they inhibit the ability of the endometrium to support the fetus to term. If conception does occur there is a high possibility that early embryonic death will occur. The conception rate was lower in group III than in groups I and II. This may indicate that the criteria of group III are of such a diffuse degree that they in fact lower conception rate. Before this can be definitely stated, more data should be collected in group III. Group IV was established as what was considered to be a hopeless prognostic group. The changes in group IV mares were to represent the degree of change required to greatly inhibit conception and prevent any chance of carrying a foal to term. The conception rate was lower than seen in group I and II but not significantly different than group III. Groups III and IV actually performed similarly: lower conception rate and zero foaling rate. This may suggest that the criteria for these two groups may be too closely related. However both groups are extremely small for completely objective comparisons. More mares in both groups will be necessary before proper evaluations of groups III and IV can be made. The significance of the lympathetic lacunae as discussed by Kenny (1978) was not appreciated as such structures were rarely seen.

Although twenty-six mares were placed in group I (Normal), these mares were not entirely normal. Mares that were biopsied as a part of a routine reproductive examination were likely to be normal. Other "normal" mares biopsied, however, were biopsied for a reason relating to a history of decreased fertility. So even though the uterine endometrium of these mares was normal, they often had some other problem. They may have had irregular estrous cycles and were subsequently difficult to breed when in estrus. The "irregular cycles" may have been a real problem of the mare or a product of a poor management program. If the mares were not teased by the stallion regularly and records kept, estrus, (although it occurs), may not be observed. Irregular estrous cycles can also occur in mares that are in poor physical condition, under stress, have a poor nutritional status or have hormone imbalances. Hormonal imbalances may be very difficult to detect clinically. Other factors causing infertility that may go undetected are lesions of the oviduct, uterine cysts, uterine anomalities, mucometra, lesions of the cervix (cervical adhesions) and vagina, persistence of a part of the hymen or congenital anomalies. Therefore the results in group I are probably not the results of entirely normal mares. Real control (normal mares) was difficult to obtain. No management control could be established in the population. But because some of the abnormalties of apparently normal mares are so difficult to detect, one may never be sure of absolute normals. Large numbers of mares should be sampled to help eliminate the built-in prejudice of "abnormal-normal" mares. In spite of the prejudice against normals which may be built into this study, the conception and foaling rate of these mares compares well with the work of others.

A wide range of inflammatory changes (mild focal to severe diffuse) was used in the criteria of prognosis group II. It was felt that if no permanent changes, such as severe fibrosis were present, the endometrium should be able to resolve the infection with appropriate treatment. In the cases of a more chronic cellular infiltrate, plasma cells and lymphocytes, the resolving process was expected to take longer than for acute inflammatory processes. The major concern in the time taken to clear up the infection is that permanent damage (periglandular fibrosis) may occur before resolution of the inflammatory process. As in group I, mares in group II may have reproductive problems other than those in the uterus.

Due to the fact that mares in groups III and IV were given a poor prognosis, few owners continued to attempt breeding these mares. The consequence of this is that there was very little data available on the two groups. If this study was redone with no limitation of funds, it would be beneficial to breed enough mares in groups I, III, and IV to yield equal numbers of mares in all 4 groups.

Prognostic categories were designed so that there could be a definite evaluation of the mare's reproductive potential. If prognosis III or IV was given it was suggested that the mare be biopsied again for a second judgment. This was because the first biopsy may have been taken from a focal area of atrophy, or fibrosis, and the remainder of the uterus may be capable of supporting a fetus to term. When a prognosis III or IV is given veterinarians commonly advise owners that there is little chance that the mare can ever reproduce again. Therefore a very good mare with only focal disease of the endometrium may be

destroyed. To prevent such a mistake a second biopsy is always indicated in mares given a prognosis of III or IV.

Age and Prognosis Group

The literature review revealed little on the effect of age on the reproductive performance in mares. This study indicates that age directly or indirectly has a negative influence on reproductive ability. The effect may only be due to the fact that older mares frequently have poor perineal conformation and have a greater duration of time to suffer from endometrial damage of varying degrees of severity. Studies in rats and mice (Lu et al., 1977; Parkening et al., 1978), however, indicate that there is a decreased or slowed response to circulating hormones with increasing age. One might argue that these mares were placed in poorer prognosis groups on the basis of endometrial damage. Lack of adequate response of the endometrium to circulating hormones, however, may also result in endometrial atrophy.

Bacteria, Conformation, and Prognosis Group

The results of uterine swab cultures indicate that there is no specific relationship between species of bacteria and prognosis group. The longer the infection exists (bacterial or mycotic), however, the poorer the prognosis may become with the prolonged endometrial inflammation. The prognosis group was often directly related to perineal conformation. Poor perineal conformation lowers one of the defense mechanisms of the reproductive tract to infection. Therefore, poor perineal conformation left uncorrected allows more bacteria to enter

the uterus and inhibits the ability of the mare to rid herself of this infection. Correction of these conditions by surgery is frequently beneficial in restoring fertility if serious damage has not already occurred (Caslick, 1937a).

CHAPTER VI

SUMMARY AND CONCLUSIONS

The grading system established is useful in assessing the condition of the equine endometrium and in reaching an accurate diagnosis. Based on the diagnosis the mare can be placed in a prognosis group. The prognosis group is valuable in predicting the probable reproductive performance of mares. Low fertility mares with a normal reproductive tract based on physical, clinical, and endometrial histology findings (prognosis group I) often have less obvious causes for their poor reproductive ability. These causes include: infertile semen, poor program of teasing mare with stallion, and subsequent lack of detection of estrus, endocrine imbalances, poor nutrition, and stress. Lesions of the oviduct, uterine cysts, uterine anomalies, mucometra, lesions of the cervix, or vagina and various congenital anomalies of the reproductive tract may also be obscure causes of decreased fertility.

Mares that have an inflammatory cell response in their endometrium and little or no permanent change, such as with fibrosis around glands (prognogis II), can often increase their reproductive performance if properly treated for the inciting cause. These mares sometimes may have the less obvious problems mentioned above for prognosis group I.

Permanent endometrial changes, especially periglandular fibrosis, greatly inhibit the ability of the endometrium to supply nutrition to

the fetus. Mares with moderate to severe periglandular fibrosis (group III) probably have less than a 10 percent chance of carrying a fetus to term. The conception rate is also lowered. Although 6 of the eighteen mares bred in this group conceived, none were able to maintain the pregnancy.

Mares with diffuse severe periglandular fibrosis or too few endometrial glands (group IV) were believed to have a very poor chance of conception. Statistically there was no difference in the conception or foaling rates of prognosis groups III and IV. Because of the poor prognosis given to mares in prognosis groups III and IV, the mares were frequently retired from breeding.

Older mares (near 20 years of age) were consistently in the poorer prognostic groups. The conception and foaling rate of mares 15 years or greater was lower than that of younger mares. Several factors could be involved in decreasing the reproductive ability of older mares. For example, older mares have had more time to be exposed to agents that could damage the reproductive tract. Endocrine changes may also occur with age, which might decrease in the reproductive performance of these mares.

There was no relationship between species of bacterium and prognosis group or perineal conformation. Mares with poor perineal conformation were commonly in the poorer prognosis groups. Several mares which had received corrective surgery, however, were in the better prognosis groups.

Examination of the endometrial biopsy can be effectively used as a part of the routine reproductive exam. It is the most accurate

measure of the ability of the uterus to carry a fetus to term when it is taken during estrus and correlated with physical, clinical, cultural and behavioral findings. The histologic findings are sometimes indicative of problems indirectly affecting the uterus. The endometrial biopsy examination therefore, is a useful, economic method for obtaining direct, definitive information or the ability of the uterus to support fetal life as well as on other factors, affecting the uterus indirectly.

SELECTED BIBLIOGRAPHY

Aitken, W. A. 1927. Some observations on the oestrous cycle and related phenomena of the mare. Am. J. Vet. Med. Assn. 70: 481-491.

Aitken, W. A. 1926. The oestrous cycle of the mare. Vet Practitioner's Bull. 8: 178-187.

Andrews, F. N. and F. F. McKenzie. 1941. Estrus, ovulation and related phenomena in the mare. Res. Bull. Mo. Agric. Exp. Stn. 329: 5-117.

Asdell, S. A. 1964. Patterns of Mammalian Reproduction, 2nd Ed. Ithaca, N.Y.: University Press. 426.

Bergman, R. V. and R. M. Kenny. 1975. Representativeness of a uterine biopsy in the mare. Proc. Am. Assn. Equine Practnr. 355-361.

Brandt, G. W. 1970. The significance and interpretation of uterine biopsy in the mare. Proc. 16th An. Conv. Am. Assn. Equine Practnr. 279-294.

Brandt, G. W. and J. P. Manning. 1969. Improved uterine biopsy techniques for diagnosing infertility in the mare. Vet. Med./S.A.C. 64: 977-983.

Buckhardt, J. 1946. Anestrus in the mare and its treatment with estrogens. Vet. Rec. 59: 341-342.

Caslick, E. A. 1937a. The vulvo-vaginal orifice and its relation to genital health of the Thoroughbred mare. Cor. Vet. 27: 178-187.

Caslick, E. A. 1937b. The sexual cycle and its relation to ovulation with breeding records of the Thoroughbred mare. Cor. Vet. 27: 187-206.

Cole, H. H., C. E. Howell, and G. H. Hart. 1931. The changes occurring in the ovary of the mare during pregnancy. Anat. Rec. 49: 199-209.

Crowhurst, R. C. and W. Caslick. 1946. Some observations on equine practice and its relation to the breeding of Thoroughbred mares. N. Am. Vet. 27: 761-767.

Davis, L. E. and B. Abbitt. 1977. Clinical pharmacology of antibacterial drugs in the uterus of the mare. J. Am. Vet. Med. Assn. 170: 204-207.

Day, F. T. 1939. Sterility in the mare associated with irregularities of the estrous cycle. Vet. Rec. 51: 1113-1119.

Dimmock, W. W. and P. R. Edwards. 1928. The pathology and bacteriology of the reproductive organs of mares in relation to sterility. Res. Bull. Ky. Agric. Exp. Stn. 286: 157-237.

Dutt, R. H. and L. F. Bush. 1955. The effect of low environmental temperatures on the initiation of the breeding season and fertility in sheep. J. An. Sci. 14: 885.

Ewart, J. C. 1915. Studies of the development of the horse. Trans. Roy. Soc. Edin. 51: 287-329.

Gadd, J. D. 1975. The relationship of bacterial cultures, microscopic smear examination and medical treatment to surgical correction of barren mares. Proc. Am. Assn. Equine Practnr. 362.

Godley, W. C., R. L. Wilson, and V. Hurst. 1965. Effect of controlled environment on the reproductive performance of ewes. J. An. Sci. 25: 212.

Hammond, J. and K. Wodzicki. 1941. Anatomical and histological changes during the oestrous cycle in the mare. Proc. Roy. Soc. B. 130: 1-23.

Harris, A. S. 1956. Persistant hymen in a mare. Abstr. J. Am. Vet. Med. Assn. 129: 508.

Heape, W. 1900. The sexual season of mammals. Quar. J. Micro. Sci. 40. As referenced by F. N. Andrews and F. F. McKenzie. 1941. Estrus, ovulation and related phenomena in the mare. Res. Bull. Mo. Agric. Exp. Stn. 329: 5-117.

Hughes, J. P. 1973. Hormone therapy in equine reproduction. An. Am. Vet. Soc. for the Study of Breeding Soundness Conference. Symposium on Reproduction. 8-14.

Hurtgen, J. P. and H. L. Whitmore. 1978. Effects of endometrial biopsy, uterine culture, and cervical dilation on the equine estrous cycle. J. Am. Vet. Med. Assn. 173: 97-100.

Jennings, W. E. 1941. Some common problems in horse breeding. Cor. Vet. 31: 197-216.

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

Kenny, R. M. 1975. Prognostic value of endometrial biopsy of the mare. J. Reprod. Fert. Suppl. 23: 347-348.

Kenny, R. M., V. K. Ganjam, and R. V. Bergman. 1975. Non-infectious breeding problems in mares. Veterinary Scope. 19: 16-24.

Knudsen, O. 1964. Endometrial cytology as a diagnostic aid in mares. Cor. Vet. 54: 415-422.

KUpfer, M. 1928. The sexual cycle of female domesticated mammals. The ovarian changes and the periodicity of oestrum of cattle, sheep, goats, pigs, donkeys, and horses. 13th & 14th Reports of the Director of Veterinary Education. Dept. Agr., Union of So. Africa, Part II. 1209-1270 and supple.

Lieux, P. 1963. Infertility in the mare. Reproduction. In Equine Medicine and Surgery. J. F. Bone, E. J. Catcott, A. A. Gabel, L. E. Johnson, and W. F. Riley (Ed.). Santa Barbara, Calif.: American Veterinary Publications, Inc. 616-617.

Lieux, P. 1970. Relationship between the appearance of the cervix and the heat cycle in the mare. Vet. Med./S.A.C. 879-885.

Loy, R. G. 1968. Effects of artificial lighting regimes on reproductive patterns in mares. Proc. 14th An. Conv. Am. Assn. Equine Practnr. 159-165.

Loy, R. G. 1970. The reproductive cycle of the mare. Lectures of Stud Managers' Course. Lexington, Ky. 20-30.

Loy, R. G. and J. P. Hughes. 1966. The effects of human chronic gonadotropin on ovulation, length of estrus and fertility in the mare. Cor. Vet. 56: 41-50.

Lu, K. H., H. H. Huang, H. T. Chen, M. Kurcz, R. Mioduszewski, and J. Meites. 1977. Positive feedback by estrogen and progesterone on LH release in old and young rats. Proc. Soc. Exper. Biol. Med. 154: 82-85.

McDonald, L. E. 1969. Veterinary Endocrinology and Reproduction. Pennsylvania: Lea and Febiger. 386-388.

McEntee, K. 1970. The Female Genital System in Pathology of Domestic Animals, 2nd Ed. K. V. F. Jubb and P. C. Kennedy (Eds.). New York: Academic Press. 502.

McKenzie, F. F. and F. N. Andrews. 1937. Estrus and ovulation in the mare. Proc. Am. Soc. Anim. Prod. 64-70.

McKenzie, F. F. and C. E. Terrill. 1937. Estrus, ovulation and related phenomena in the ewe. Res. Bull. Mo. Agric. Exp. Stn. 264: 5-88.

McKenzie, F. F. 1926. The normal oestrous cycle in the sow. Res. Bull. Mo. Agric. Exp. Stn. 86: 5-41.

Osborne, V. E. 1966. Analysis of the pattern of ovulation as it occurs in the annual reproductive cycle of the mare in Australia. Austral. Vet. J. 42: 149-154.

Osborne, V. E. 1968. An appraisal of the efficiency of the official months of the Thoroughbred and Standardbred season in Australia. VIth. International Cong. Anim. Reprod. and Art. Insem. 2: 1593-1595.

Parkening, T. A., I.-F. Lau, S. K. Saksena, and M. C. Chang. 1978. Circulating plasma levels of pregnenalone, progesterone, estrogen, luteinizing hormone, and follicle stimulating hormone in young and aged C57BL/6 mice during various stages of pregnancy. J. Gerontol. 33: 191-196.

Porchester, L. and Veterinary Committee. 1965. Uterine infections in mares. Vet. Rec. 77: 110-111.

Rasenbeck, N. O. 1965. Effect of equine genital infections on reproduction with special reference to Thoroughbred and trotting mares. Nord. Vet. Med. 17: 305-317.

Ricketts, S. W. 1975. Endometrial biopsy as a guide to diagnosis of endometrial pathology in the mare. J. Reprod. Fert. Supp. 23: 341-345.

Roberts, S. R. 1971. Infertility in the Mare in Veterinary Obstetrics and Genital Diseases, 2nd Ed. Ithaca, N.Y.: Published by the Author. 26, 512-550.

Sano, E. M. 1949. Trichrome stain for tissue section, culture, or smear. Am. J. of Clin. Path. 19: 898.

Satoh, S. and S. Hoshi. 1932. A study in reproduction in mares. J. Jap. Soc. Vet. Sci. 11: 257-268. In An. Br. Abst. 1: 230-231.

Satoh, S. and S. Hoshi. 1933. A study of reproduction in the mare. II. A study on the oestrous. J. Jap. Soc. Vet. Sci. 12: 200-223. In An. Br. Abst. 1: 231.

Satoh, S. and S. Hoshi. 1934. Studies on the reproduction in the mare. III. The oestrous duration and oestrous cycle. J. Jap. Soc. Vet. Sci. 13: 237-250. In An. Br. Abst. 3: 358-359.

Scott, P. P. and M. A. Lloyd-Jacobs. 1955. Some interesting features in the reproductive cycle of the cat. In Studies in Fertility I. R. G. Harrison (Ed.). Proc. Soc. for Study of Fertility. Oxford, England: Blackwell Scient. Public. 123.

Seaborn, E. 1925. The oestrous cycle in the mare and some related phenomena. Anat. Rec. 30: 277-287.

Solomon, W. J., R. H. Schultz, and M. L. Fahning. 1972. A study of chronic infertility in the mare utilizing uterine biopsy, cytology and cultural methods. Proc. 18th An. Conv. Am. Assn. Equine Practnr. 55-86.

Stabenfelt, G. H., J. P. Hughes, and J. W. Evans. 1972. Ovarian activity during the estrous cycle of the mare. Endocrinology. 90: 1379-1383.

Stockard, C. R. and G. N. Papanicolaou. 1917. The existence of a typical oestrous cycle in the guinea pig--with a study of its histological and physiological changes. Am. J. Anat. 22: 225-285.

Sullivan, J. J., W. G. Parker, and L. L. Larsen. 1973. Duration of estrus and ovulation time in non-lactating mares given human chorionic gonadotropin during three successive estrus periods. J. Am. Vet. Med. Assn. 162: 895-898.

Swerczek, T. W. 1978. The first occurrence of contagious equine metritis in the United States. J. Am. Vet. Med. Assn. 173: 405-407.

Tobler, E. E. 1966. Collection of uterine fluid and uterine biopsy. Vet. Med./S.A.C. 61: 779-788.

Twisselmann, K. L. 1971. As referenced by J. T. Vaughan. The Genital System in Textbook of Large Animal Surgery. 1976. E. W. Oeheme and J. E. Prier (Eds.). Baltimore: The Williams and Wilkins Co. 496.

Vaughan, J. T. 1976. The Genital System. In Textbook of Large Animal Medicine and Surgery. F. W. Oehme and J. E. Prier (Eds.). Baltimore: The Williams and Wilkins Co. 496.

VITA - 2

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