

HISTOPATHOLOGICAL STUDY OF THE  
BOVINE UTERUS

by

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## INTRODUCTION

During the past two decades, much work has been done in a variety of disciplines by a large number of investigators, dealing in general with problems relating to reproduction and the female genital tract. Numerous fields of specialization within the biological sciences have contributed information to the rapidly advancing area of knowledge concerned with reproduction. The early '20s found anatomists, embryologists, physiologists, and zoologists investigating what was to be a most highly specialized area of veterinary and human medicine. Today many branches of science are joined in investigations concerned with the reproductive mechanism. Although much has been done and said about the uterus, it was Lord John Wilmot, who aptly summarized the importance of this organ, when he said, "On this soft anvil all mankind is made."

In recent years a great number of studies have been directed towards one of the most important problems of today, prenatal losses which involves a great number of repeat breeders in domestic animals. Repeat breeding may be due to (1) lack of fertilization, (2) loss of the embryo prior to placentation, (3) resorption and (4) abortion or stillbirth. Recent investigations of prenatal loss reveal that the majority of deaths result in early resorption of the embryo, predominantly during the period of placentation. It has also been resolved that the cause of these losses cannot be attributed to any single factor. The contributing factors may be anatomical abnormalities, bacterial infection, genetic inadequacies, hormonal imbalance, poor nutrition, intoxication, untoward

immunological reaction, or poor management. Sometimes apparently healthy and normal animals turn out to be a repeat breeder.

Embryo loss and repeat breeding in cattle have resulted in a tremendous loss to animal husbandmen of the United States and the World. According to United States Department of Agriculture, cattle breeders alone loose 250 million dollars annually by boarding infertile cows. This gives an indication of the huge loss in the world due to embryonic losses when such problems exist in every corner of the earth.

In recent years bacteriological studies on repeat breeder cows and on normal animals have given conflicting thoughts: (1) there are few, if any, organisms present in the normal uterus; (2) there are large numbers of organisms regularly present in the uterus. Histopathological studies on such animal uteri indicated endometritis, degeneration and pyogenic changes.

This study was motivated by incidental observations of infiltrations of large numbers and increased types of cells in the endometrium of apparently normal but repeat breeder cows and diseased uterine cases. The reason for such cellular infiltration, and variation in types of cells has been a matter of controversy for some time. This study was undertaken to determine the relationship of these cellular changes in the uterus to failure of conception.

#### REVIEW OF THE LITERATURE

Hallman (1919) described abnormalities of the reproductive organs of four cows slaughtered because of sterility and concluded

that deep-seated lesions of the uterine mucosa represented foci of infection with microorganisms of low virulence responsible for the reproductive disturbances of these animals. His observations have been supported by recent investigators like Miller (1950), Simon (1951), and Brus (1954), who, using biopsy technic, observed similar lesions in abnormal reproductive cases.

A good account of the earlier work on the histopathology of the bovine uterus came from Frei (1925) who described a large number of types of which only acute and chronic purulent forms, including pyometra, cystic, and necrotic types have been recognized by later workers. Frei (1925) reported the degree of catarrhal-purulent endometritis varied in different parts of the uterus. He described in the early stages of the chronic purulent form foci of polymorphs concentrated below the surface epithelium and accumulated around the glands. Lymphocytes were sometimes observed between the glands. As the condition progressed, the glands sometimes filled with polymorphs, and sometimes disappeared. In cases of pyometra the endometrium became ulcerated, and lost its surface epithelium. Occasionally several layers of regenerating epithelium were noted. In some cases pea-like abscesses were reported in the endometrium.

Frei (1925) considered necrotic endometria to be characteristic of acute septic puerperal disease: a dyptheretic membrane of necrotic tissue might form, become gangrenous and then desquamate and block the cervical canal. He did not connect cystic endometritis with cystic ovarian diseases. The characteristic change observed was the clustering of leukocytes both in and

around the gland cavities, with general fibrosis in the stroma and the disappearance of the gland epithelium. He attributed the cystic appearance of the gland to the blockage of the duct by desquamated epithelium.

The observations made by Frei (1925) were later supplemented and confirmed by other workers. Lasslo (1935) studied histologically 34 (11%) abnormal uteri from 300 random slaughter cattle and described permanent lymph follicles in the chronic purulent forms, infiltration by polymorphs and subsequent fibrotic replacement of the superficial glands. Erismann and Saxer (1933) confirmed Lasslo's finding that the deeper portions of the glands may remain intact and observed massive infiltrations by polymorph cells. Cembrowicz (1946), however, found and reported as did Frie (1925) that both the general and glandular infiltration was of mixed type and that many round cells participated.

Moss et al. (1956) and Simon and McNutt (1957b) observed the presence of lymph nodules in the endometrium. Neither of these workers reported the presence of polymorphs, but Schnider (1958), Cembrowicz (1946), and Erismann (1933) reported a mixed infiltration of lymphocytes and polymorphs. An increased number of plasma cells were observed by Simon and McNutt (1957b), Baur (1951) and Schnider (1958) accompanying bacterial infections while de Bois and van den Akkar (1957) found that the number of plasma cells rose with the age of the animal.

Weber et al. (1950) studied the presence of tissue mast cells in virgin heifers and reported that these cells accumulated in large numbers in the central pitted areas of the caruncles at the



onset of the massive metrorrhagia, but cyclic variations in the number of mast cells were not demonstrable in other areas of the endometrium, myometrium, or perimetrium.

Shelly and Juhlin (1961) reported that mast cells and basophils liberated histamine in the tissues or the blood vessels by dissolution and liberation of granules from the cytoplasm. The liberated histamine was shown to be responsible for the anaphylactin reaction of patients sensitized by treatment with an antigenic stimulus (penicillin or cold). At the same time normal, non-sensitized individuals showed no change in the granulation of the cytoplasm of basophils or mast cells.

Tagliavini (1935) described the presence of plasma cells in pyometra. He claimed in opposition to Frei (1925) that the surface epithelium often survived in a vacuolated form. In other respects he agreed with Frei (1925), Cembrowicz (1946), and Quinlan (1929).

Tagliavini (1935) encountered cystic endometritis in few uteri of cows. Some of the cases associated with ovarian cysts were considered coincidental and of no significance. He emphasized hypersecretion and blockage of the ducts by plasma cells to be the cause. Erismann and Saxer (1933), Quinlan (1929), and Frei (1925) held the view that the uterine cysts were due to duct blockage; however, some of Quinlan's animals had ovarian cystic diseases. Leszlo (1935) also observed ovarian cysts in some cases of cystic endometritis. He stressed the virtual absence of polymorphs; the infiltration consisting mainly of round and plasma cells. Quinlan (1929) agreed and also observed eosinophils and macrophages in

some numbers. However, Quissar (1941) found cystic endometritis characterized by increased polymorph infiltration as well as by glandular dilatation and stromal edema in 13 of 14 cows with cystic ovarian diseases.

Dawson (1958) observed cystic gland dilatation in over 40% of 48 cows with cystic ovaries while most of the remaining 60% showed mixed infiltration and fibrosis severe enough to indicate previous cystic glandular dilatation. A few cases of cystic dilatation were observed by Simon and McNutt (1957) among 109 infertile cows and by Moss et al. (1956) in 40 infertile cows.

Lasslo (1935) reported 34 uteri as histologically abnormal out of 300 random slaughter cattle. Aziz-ud-Din (1948) in the same way, reported 35% in the series of 230 cows, while Gembrowiz (1946) noted less than 6% in 1,030 cattle. Bhattacharya et al. (1954) reported abnormalities in 27% of randomly selected uteri from 1,020 buffaloes. All the uteri used in the above studies were apparently abnormal grossly. Dawson (1949) observed that many uteri (6 to 35% as a minimum) may have abnormal microscopic changes but appear normal grossly.

In a histological study of 252 cows discarded for failure of breeding, Dawson (1958) described oviducal lesions in 52% and found evidence that the chief cause was uterine infection. Of the cows with salpingitis the endometrium was histologically normal in 32%. At least as great a percentage of infertile cows with normal oviducts had lesions of the endometrium. This emphasizes the recuperative ability of the endometrium during the final lactation period.



Eriemann and Saxer (1933) observed changes of the endometrium in 65 out of 68 discarded cows. The changes described were infiltration of cells (mostly polymorphs), clustering of lymphocytes, and fibrotic replacement of superficial glands, while no changes were observed in normal controls. Roark (1953) noted severe erosions of endometrial tissue in 25% of 52 discarded cows and obstruction of the genital tract in an additional 29%. He could not observe these changes in the 33 animals with normal history. Fugimoto (1956) found simple endometritis in 45% of 57 discarded cows and glandular hyperplasia in a further 7%. He also reported infiltration of epithelium and subepithelium with polymorphs, lymphocytes, and plasma cells and sometimes with mast cells. The changes were conspicuous in periglandular and subepithelial tissues at times with cellular foci in the connective tissue. He also reported subacute purulent endometritis (1.1%), pyometra (1.1%), endometritis catarrhal eosinophilia (1%), and perimetritis (6.9%).

Koike et al. (1959) reported the isolation of Staphylococcus, Streptococcus, Corynebacterium, and some other bacteria as well as chronic catarrhal endometritis (27.7%) in uteri in infertile cows. Desquamated epithelium, edema, and cellular infiltration with lymphocytes and plasma cells were the lesions noted. Of 47 cattle 6.4% had cystic glands. Maekawa et al. (1959) observed chronic catarrhal changes in the uterine mucosa, relatively slight in four out of seven cases. They emphasized this as the cause of infertility in the repeat breeding cattle. They also observed cellular infiltrations and lymphocytic nodules in the endometrium,

and noted cases of meso-ovaritis, meso-salpingitis and perimetritis related to oviducal adhesion, and thought them to be concerned with sterility. Sugawa et al. (1959) observed comparatively severe eosinophilic infiltration in the endometrium of myxometra cases of cows.

Dawson (1961) was of the opinion that the majority of cows culled for infertility had persistent lesions in either the endometrium or the oviducts, while in others an endometritis had probably healed by the time of slaughter. He reported it a probability, however, that many cows are rendered temporarily infertile by an endometritis which later resolves partially or completely. Moss et al. (1956) threw some light on these two aspects by studying uteri from 25 normal cows and 30 with a history of infertility. With regard to the first aspect, the correlations of the lesions with breeding history--he found only four out of 25 normal cows with endometritis while none had lymph nodules in the endometrium. At the same time he observed 21 out of 30 infertile cows had abnormal tissue changes and eight had lymphatic nodules as well. With regard to the second aspect--the amount of damage allowing normal reproductive function--Moss et al. (1956) observed that seven out of eight uteri with lymph nodules contained normal embryos, conception having followed a period of infertility. They also noted that a higher incidence of abnormality existed in the body than in the horns. Statistically Moss et al. (1956) reported the most significant lesion in infertility to be the encapsulation of the endometrial glands. A second series of 12 normal and ten infertile animals from another locality yielded typical tissue

lesions in only two of each group. However, Simon and McNutt (1957b) found pronounced endometritis in only one case and minor abnormalities in 49 of 109 cows that were not settled by four services.

While studying biopsy samples of 100 cows returned to service twice, Brus (1952) analyzed the tissue changes histopathologically and correlated them with the subsequent service history. A simple grading procedure for the tissue changes was established: (a) those having only a few inflammatory cells near the surface of the endometrium; (b) those with many cells uniformly distributed throughout the tissue; and, (c) those having numerous leucocytic cells forming foci around the glands or blood vessels. Only 29 of the cows showed no inflammatory change. He observed in a second group of cows, one with the samples from a *Brucella* infected herd or trauma, that 20% were normal, 50% belonged to group (a) and (b) and 30% to group (c). In the normal group, he designated 36% normal, 53% in group (a), and 9% in group (b), and only 2% in group (c). Of 100 cows that conceived within six weeks after biopsy, 20 had yielded normal biopsy, 33 of type (a), and only six of types (b) and (c). Nine cows conceived three months or more after biopsy study. Samples from four of these were classified as (b) and (c). Of the 19 discarded as permanently infertile, two had shown normal biopsy and 10 were b and c types. Brus (1952) thus emphasized that endometritis as represented by b and c samples was frequently associated with trauma and in those cases recovery was poor. Only ten of 30 such cases were known to breed again and ten were culled as barren.

De Bois and van den Akkar (1957) took biopsy samples from both uterine horns of 137 cows about 13.5 weeks post-partum. They diagnosed endometritis only when the sample contained foci of inflammatory cells. Positive samples were graded according to the number of foci per section; one to two foci per section was designated as grade I, two to five as II, five or over as grade III. Of the 57 cows that conceived at the first service 28 were classified as normal, 18 in group I, nine in group II, and two in group III, suggesting that a moderate degree of tissue change may not always delay conception.

Dawson (1961) concluded that some signs of endometritis occur in a small majority of cows at 13.5 weeks post-partum, and that a significant depression of the conception rate is reached only with five or over of the inflammatory foci in the section or with grade III by the de Bois method.

De Bois and van den Akkar (1957) along with Brus (1954) were of the view that prognostic value of the biopsy is much greater by the time infertility is recognized, presumably because the tissue lesions are then of a more refractory type than those detected before service. Brus (1954) considered the prognosis worse when lymphocytes predominated in the infiltration. However, in the earlier stages de Bois and van den Akkar (1957) noted mainly lymphocytes and some eosinophils which did not seem to affect conception rate.

Research work in the past has been directed toward bacterial infections with tuberculous endometritis being dominant. Quinlon (1921) observed two distinct groups of uterine lesions: true

tubercles lying deep in the uterine walls and pinhead size miliary lesions apparently on the surface of the mucous membrane. Klimmer, Haupt and Roots (1929) found nine of 12 tuberculous uteri to be of miliary types. Stamp (1944) who made an intensive study of the infection throughout the body explained that the miliary involvement resulted from infection descending directly from the peritonium through the oviducts while the deeper tubercles were due to blood born infection. Cembrowiez (1946) found both types of lesions and observed that the miliary lesions often involved the glands. He thought that direct infection of the oviduct was possible from tubercles originating in the deeper layers. Baur (1951) observed only the miliary types of lesions in eight out of 30 infertile cows. Typically the foci were rich in lymphocytes, giant cells, and epithelial cells. Schnider (1956) referred specifically to the diagnosis of uterine tuberculosis by biopsy. He described the sample in his cases as "highly characteristic", but gave no further details beyond mentioning that fibrosis was conspicuous whereas it was not in other types of endometritis. Epithelioid giant cells were conspicuous also.

Two detailed studies have been reported on the changes in uteri due to Vibriosis infection. Simon and McNutt (1957a) found an endosalpingitis but no pronounced endometrial changes in three heifers killed 11 days after the injection of the Vibrio foetus into the uterus during estrus. Heifers infected in the fifth month of pregnancy showed a variety of changes ranging from erosion of epithelium, necrosis, edema, hemorrhage and infiltration with



polymorphs, lymphocytes, and plasma cells. Huysman (1957) reported a normal picture upto three weeks after an infective service, then between the third and the sixth weeks there occurred a filling of the gland lumina with necrotic nuclei and infiltration of lymphocytes and a few eosinophils. These changes, which regressed after the ninth week, were not observed in animals treated with streptomycin.

Dawson (1949) described the changes in 19 cows which had yielded uterine pus with a high concentration of Corynebacterium pyogenes. At post-mortum examination he found pus in five uteri (two of them from large endometrial abscesses), and a further 12 showed ascent of infection to the oviducts. He also observed considerable microscopic changes in all of the uteri. A typical finding was the replacement of large areas of endometrium by dense fibrosis. The sections rarely showed superficial glands present. A few surviving deep glands were surrounded by a fibrous ring, had necrotic epithelium and contained many polymorphs and lymphocytes. Cells of these types were also commonly seen in the surrounding stroma.

Baur (1951) confirmed these findings and reported in four animals with Corynebacterium pyogenes, endometritis, and accumulation of plasma cells, fibroblasts and polymorphs in necrotic foci. He claimed that histopathological observations of uteri of each group of animals infected with M. tuberculosis, C. pyogenes, Trichomonas foetus, and Streptococci pyogenes, infection were characteristic. He believed that mixed infiltration of lymphocytes and polymorphs were rare and eosinophils characterized a non-purulent hyperemic



reaction. Huysman's (1957) description of the changes in Vibrinosis coincided with the above. De Bois and van den Akkar (1957), correlated pathological findings with bacterial infections. Beta-haemolytic streptococci were recovered predominantly, and were associated with a high density of the inflammatory foci.

Conklin et al. (1931) reported 88.7% of 80 pregnant uteri contained bacterial flora comprising Micrococcus, Bacillus, Escherichia, Streptococcus, Staphylococcus, Eberthella, Aerobacter, Sarcina, Salmonella, Actinobacillosis, Rhodococcus, Proteus, Corynebacterium, Mycobacterium, etc. They also reported the presence of gross pathological lesions in 19 uteri.

Descriptions of changes in the uterus during or after brucellosis are rare. Wagner and Bisping (1959) described the changes briefly as edema, infiltration, and necrosis. Payne (1959) described the changes in the pregnant uteri as essentially the destruction of uterine glands, invasion by large number of lymphocytes, plasma cells and polymorphs and the appearance of edema in the stroma. Payne (1960) found no brucella in the empty uteri of heifers infected intraocularly, although Munro (1957) indicated intra-uterine infection may cause infertility rather than abortion.

Bacterial isolations from one group of bovine uteri followed through slaughter was made by Poggio (1962), Department of Bacteriology, Kansas State University. The isolation results of the bacteria are summarized here to give a picture of the contamination of the uterus in different group of animals.

The pregnant control group had only four uteri out of 14 contaminated with bacteria. In one case the bacterium isolated was a Gram negative rod; one suffering from mastitis had Corynebacterium; another having a yellow fluid in one horn gave a Bacillus; and Staphylococci and Corynebacterium were isolated from another in which the uterus had been punctured previously by some foreign object.

In the group of repeat breeder cows, a much more frequent contamination of uteri was observed. Of 32 uteri in this group 21 were positive for bacterial isolations. Streptococci were isolated nine times, Corynebacterium eight times, Micrococcus, Pseudomonas and Staphylococci, each four times, along with bacilli twice, and yeast once. In certain cases a combination of three or four microorganisms was identified, but most of the cases gave one to two types of organism.

Of the uteri of post-partum cows, only 50% were found to be contaminated. Streptococcus occurred three times, and Pseudomonas, Corynebacterium, E. coli, and Micrococcus each once. All three diseased uteri were contaminated, two having Corynebacterium, associated with Micrococcus in one, and one having Streptococci, Bacilli, and a gram negative rod. Two of the four of the "Unclassified cows" tested for bacteria were found positive, one having Corynebacterium and other Streptococcus.

Considering all isolations Streptococcus was recovered 15 times, Corynebacterium 14 times, Staphylococcus and Micrococcus six times each, Pseudomonas five times, Bacillus four, gram negative rods twice, and yeast, Sarcina and E. coli each once.

Lloyd (1961), dealing with a group of post-partum cows, observed that there was a distinct correlation between length of the post-partum period and the presence of microorganisms. From 0 to 15 days post-partum approximately 85% of the uteri examined were found to be contaminated with microorganisms. Frequency of positive cultures decreased until 60 days post-partum, when only 10% of the uteri showed the presence of microorganisms. The bacteria isolated in this group of the post-partum cows were: Proteus, Staphylococcus, Micrococcus, Streptococcus, Escherichia, Aerobacter, Pseudomonas, Corynebacterium, Microbacterium, Actinomyces, Alcaligenes, Arthrobacter, Clostridium, a non-spore forming anaerobe, Vibrio, and also the mold, Aspergillus. He reported the bovine uterus to be essentially free from bacteria by 60 days post-partum.

Lukert (1961) cultured 47 bovine uterine samples, mostly from repeat breeder cows, for the presence of viruses, using embryonated egg and bovine kidney cultures. The yolk sac, allantoic cavity and the corioallantoic membrane of the embryonated egg were inoculated and three to seven blind serial passages were made. One to four passages in bovine kidney cell cultures were made and examined for cytopathogenic effects. Evidence of viral infection was not detected by these methods.

Szeky and Dozza (1955) and others described histologically the uteri of animal with true retention of corpus luteum. They agreed that the glands showed proliferation and that increased vascularity occurred. Also numerous deposits of hemosidrin were observed in the stroma. They reported round cells and polymorphs

in the stroma of some cases. Only two of Ajello's (1954) 16 random cases had clinically purulent endometritis, and only in these were secondary inflammatory changes evident. Ajello (1954) considered a persistent corpus luteum to be usually actively functional. According to Dawson (1961) the persistence is often associated with pyometra as are small empty ovaries and ovaries with cystic diseases.

## MATERIALS AND METHODS

### Tissue Collection and Preparation

Material for the present study was collected from the viscera table following slaughter. The 117 cows with Kansas State Dairy herd number (Tables I to III), had known histories for breeding, reproduction and clinical treatments. These cows, in various stages of reproduction, were followed through slaughter at the abattoir at Salina, Kansas. Embryos, if present, were compared with accumulated records by size and weight for normality of development. All macroscopic abnormalities were recorded.

Another group of 90 cows in various stages post-partum were followed through slaughter at the Armour and Co., Packing plant at Kansas City, Kansas. Uteri of these cows were aged (Marion and Gier, 1959) on the basis of uterine size, condition of cruncles, the histological condition of the uterine epithelium. Bacterial samples from these cows were collected from the intact horns and body, immediately after the removal of the uterus.

Tissues from at least the recent pregnant horn of the uterus were excised and fixed immediately after removal in Bouin's, Flemming's or buffered 10% formalin, dehydrated in isopropyl

alcohol and embedded in paraffin. Enough sections were cut at 6  $\mu$  to fill at least three standard microscope slides for 24 by 50 mm cover glasses.

### Staining

Sections were stained with Haematoxylin-acid fuschin orange G, Periodic acid Schiff (PAS), and Giemsa. The PAS stain was made according to Gomori (1952). The Giemsa stain adopted was a modification of the Wolbach (1931) method for staining bacteria in the tissues:

- (1) The stain was made in the proportion of 10 ml of stock Giemsa solution; 12.5 ml of methanol; 400 ml of distilled water.
- (2) The tissues were transferred from water to this solution for 30 minutes.
- (3) Rinsed in running water.
- (4) Placed in a fresh Giemsa solution, made as above, for 30 minutes to one hour.
- (5) Differentiation was accomplished in 95% alcohol, to which a few drops of eosin-y solution were added for better contrast.

### Method of Study

General histological structure of the uterine tissues were studied on haematoxylin and PAS preparations. Cellular differentiation, and the differential counts were made on the Giemsa preparation. Random fields throughout the length of the selected



section were studied for:

- (a) The nature of the epithelium, its size and height compared in terms of nuclear thickness.
- (b) The nature of the epithelium of the uterine glands and development of the fibrous connective tissue around the glands.
- (c) Any abnormalities in the structure of the endometrium.

In addition to these general observations, ten random microscopic fields were examined for lymphocytes in the epithelium, lymphocytes in the stratum compactum; lymphocytic nodules in the compactum; and eosinophils, mast cells, plasma cells, neutrophils, and histiocytes in the compactum and in the stratum spongiosum. Infiltration of lymphocytes into the glandular epithelium was also recorded. The average occurrence of each cell type was recorded as the simple average of the ten fields counted.

The observed animals were classified into groups: normal pregnant; repeat breeders; post-partum; diseased uteri; and unclassified. The "normal pregnant" were animals with known normal histories with never more than three services required; the "repeat breeders" were animals which had been bred three or more times whether settled or not. The "post-partum" group contained animals up to 60 days after parturition. The animals with known diseased uteri were grouped separately. Other animals with complicating histories that did not fit into the above groups were listed as "Unclassified".



### Identification of Cells

The various types of cells that infiltrated the endometrium were differentiated on the following characteristic features from Giemsa stain preparations.

Neutrophils (Plate I, Fig. I). These are spherical to irregular cells, seven to nine microns in diameter with a lobed or polymorphonuclear nucleus. The cytoplasm in an ideal stain is light pink and the small, numerous evenly distributed fine granules have a light pink color; while the nucleus is deeply basophilic. (Diggs *et al.*, 1954).

Mast cells (Plate I, Fig. II). Tissue basophils vary from 15 to 40 microns in diameter and are oval, round, or irregular in shape. The nucleus is relatively small, round or oval, and has an intermediate or mature nuclear chromatin structure. The cytoplasm is filled with intensely stained violet-blue (metachromatic) granules. The granules are uniformly round and are approximately the same size (about 0.1 to 0.3 microns). Sometimes the bluish cytoplasm is visible, but as a rule the cell is so packed with granules that the cytoplasm is not seen. The granules frequently overlie the margins of the relatively pale nucleus or may partially or completely obscure the nucleus. The cytoplasmic granules in certain cases appear to be washed out or dissolved, leaving the cells with light bluish cytoplasm and few metachromatic granules (Diggs *et al.*, 1954 and Smith, D. N. *et al.*, 1955).

Eosinophils (Plate I, Fig. III). These are oval to irregular cells with elongated and tapering cytoplasmic extension, containing relatively large spherical eosinophilic cytoplasmic granules.

PLATE I

Explanation:

- Fig 1: Endometrial glands packed with neutrophils in the endometrium of repeat breeder cow No. 459. This cow was found to be infected with Streptococcus and Pseudomonas. Neutrophils can be seen in the stroma as well as the lumen of the glands. Giemsa stain. 500X.
- Fig 2: Mast cells in the endometrium of the non-pregnant cow No. 369. These are large, oval to round cells, with relatively small, round to nucleus with intermediate to mature chromatin structure. The cytoplasm is completely filled with intensely stained uniformly round granules. Giemsa stain. 1250X.
- Fig 3: Eosinophils in the endometrium of cow No.225, a nymphomaniac cow.. These are oval to irregular cells containing relatively large eosinophilic cytoplasmic granules, with relatively small, spherical to oval or lobulated nuclei. Giemsa stain.. 1250X.
- Fig 4: Lymphocytes in endometrium of cow No. 376 with pyometra. These are relatively large spherical cells, with round nuclei which comprise most of the cellular structure.. Giemsa stain. 1250X.

## PLATE - 1

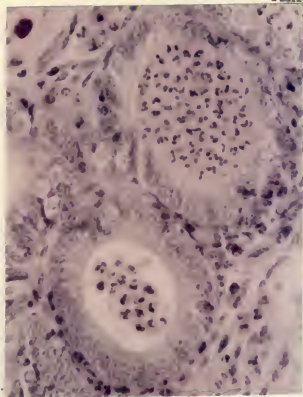


Fig 1

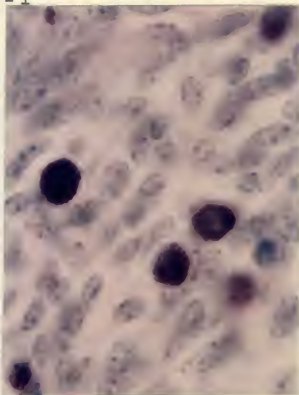


Fig 2

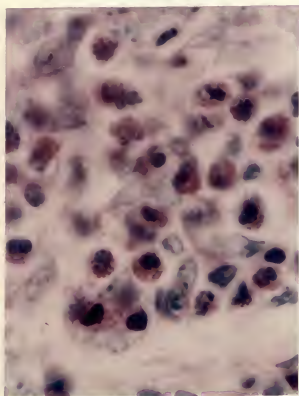


Fig 3

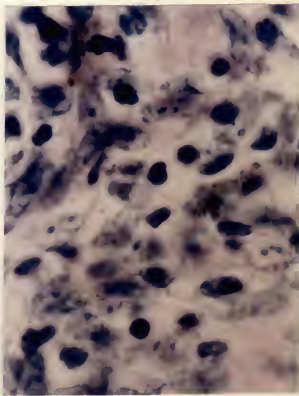


Fig 4

The nuclei of these cells are relatively small, spherical to oval or lobulated, having a well defined reticular chromatin and often nucleoli. The cells are amoeboid in nature (Diggs et al., 1954).

Lymphocytes (Plate I, Fig. 4). These cells are spherical, usually 9 to 12 microns in diameter, but occasionally as much as 30 microns in diameter. The cytoplasm of these cells stains light sky-blue to dark blue. Tiny colorless globules are observed in good preparations, giving the cytoplasm an appearance of uneven staining.

The cells, like the nuclei, are usually round. The nucleus is relatively large and comprises most of the cellular structure, leaving a narrow cytoplasmic rim around the nucleus. The chromatin is clumped and there is a tendency towards peripheral condensation. Light coming up from below and striking the darkly stained nucleus is reflected and high-lights the surrounding non-granular cytoplasm, giving a perinuclear halo or "silver lining" effects (Diggs et al., 1954).

Plasmocytes (Plate II, Fig. 1). These cells vary greatly in size. They are round, oval, or pear shaped with relatively smooth cytoplasmic margins, but may be irregular, with pointed or filamentous cytoplasmic projections.

They have a round, relatively eccentric nucleus with coarse chromatin characteristically arranged radially like the spokes of a wheel, or clock-face appearance. The cytoplasm adjacent to the nucleus is lightly stained in contrast to the periphery of the cells, which has a higher degree of saturation of red and blue dyes. In some cells the peripheral cytoplasm has a greenish or

PLATE II

Explanations:

- Fig 1: Plasmocytes in the endometrium of cow No. 38, with abnormal adhesion of anterior part of uterus to the body wall. These are round to pear shaped cells, with a round, relatively small, eccentric nucleus and a light bluish, foamy cytoplasm. Giemsa stain. 1250X.
- Fig 2: Monocytes in the endometrium of cow No. 468, which yielded cultures of *Streptococcus* and *Corynebacterium* from the uterus. Monocytes are larger cells, with even distribution of granules in the relatively voluminous cytoplasm, and somewhat kidney shaped nucleus with linear pattern of chromatin. They occur in some cases with acute bacterial infections. Giemsa stain. 1250X.
- Fig 3: Histiocytes in the endometrium of cow No. 384, 15 days post-partum. These are large cells with blunt, non-granular pseudopodia, shaggy margins, and cytoplasm filled with brown pigments. Found primarily in post-partum cases. Giemsa stain. 500X.
- Fig 4: Lower magnification of endometrium of cow No. 384, showing the brown pigmented cells (histiocytes) distributed through the endometrium. The uterine epithelium is the darkly staining band at the right of the picture. Giemsa stain. 125X.



PLATE - 2

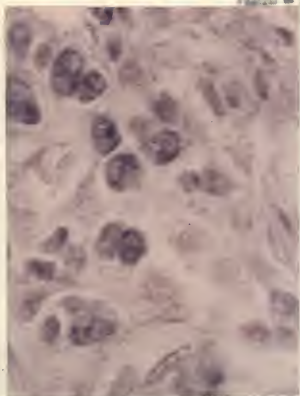


Fig 1

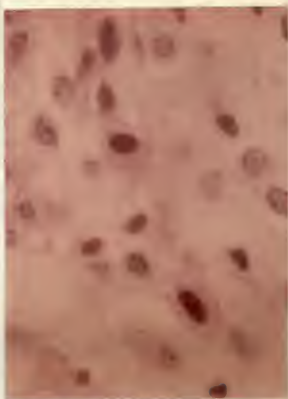


Fig 2

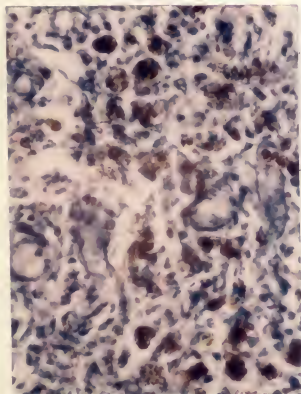


Fig 3

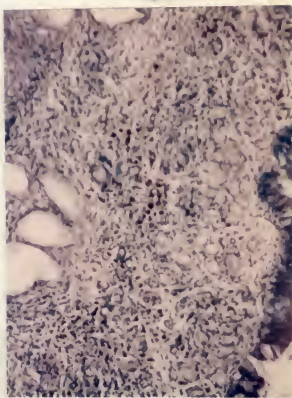


Fig 4



larkspur-blue color. The cytoplasm contains small and relatively unstained globules, giving the characteristic mottled and foamy appearance and brilliant translucency (Diggs et al., 1954).

Monocytes (Plate II, Fig. 2). These cells are larger, varying from 15 to 25 microns, and are often seen with one to two pseudopods.

There is a relatively large amount of cytoplasm in relation to the nucleus. The cytoplasm stains dull gray-blue. The granules are very fine and evenly distributed, giving the cells a dull, opaque or ground glass appearance. There may be a few larger unevenly distributed azurophilic granules. The cytoplasm often shows phagocytized bacteria and particles present within it.

The nuclei of these cells are usually round or kidney-shaped with superimposed lobes, giving the nucleus the appearance of brain-like convulsions. The chromatin tends to be loose with light spaces between the chromatin strands, giving a coarse linear pattern in contrast to the lymphocytes (Diggs et al., 1954).

Histiocytes (Plate II, Fig. 2). These are large cells which have digestive vacuoles, and/or phagocytized substances within their cytoplasm. Some of them show blunt, non-granular pseudopods, a manifestation of their amoeboid activity, while others tend to be round, or some have shaggy cell margins.

These cells have a relatively small, vesicular nucleus and a large amount of cytoplasm. The nucleus is usually round or oval and has a coarse linear chromatin pattern. The cytoplasm is light blue to bluish gray or light greenish in color. Many of the cells appear foamy. Several cells are quite commonly seen clumped

together. The phagocytized materials include whole cells, fragments, granules, pigment particles, fat globules, and various etiological agents (Diggs et al., 1954).

## RESULTS

### Histopathological Changes

The following differential analyses was made on Giemsa-stained materials:

Lymphocytic nodules (Plate III, Fig. 1). These consisted of masses of small round cells with dense nuclei (lymphocyte cells) aggregated to form spherical or ovoid nodules, distributed at random within the otherwise normal endometrial stroma. Frequently a fibrous connective tissue capsule surrounded the nodule. A majority of times they were provided with small blood vessels. (Plate III, Fig. 2). They did not contain PAS positive material. Although these nodules were observed in all layers of the endometrium, they were predominantly in the stratum compactum.

Of the 77 repeat breeder cows not settled, 16 (21%) were positive for these nodules. They varied from few to many in number. Of the 26 repeat breeders pregnant less than 100 days, seven (27%) were positive; of the 10 pregnant over 100 days, two (20%) had lymphocytic nodules (Plate V).

Only two cows out of 28 (7%) normal animals placed in the group and pregnant less than 100 days were observed to have lymphocytic nodules in their tissue, while two of 24 (8%) of the normal cows pregnant more than 100 days showed the presence of the nodules (Plate V).

PLATE III

Explanation:

- Fig 1: Lymphocytic nodules, randomly distributed in the endometrium of cow No. 76, 40 days post-partum. The nodules are seen as circular areas of different sizes, filled with small lymphoid cells, between the endometrial glands. PAS stain. 50X.
- Fig 2: Details of lymphocytic nodules in endometrium of cow No. 464, 61 days post-partum. Blood vessels penetrate the nodules and come to lie between the lymphoid cells. Giemsa stain. 500X.
- Fig 3: Lymphocytic cells beneath the epithelium in endometrium of cow No. 376, with pyometra. In many cases involved with bacterial infection there are greatly increased number of these cells in the stratum compactum, spongiosum and uterine epithelium. Giemsa stain. 500X.
- Fig 4: Mast cells in the endometrium of repeat breeder cow No. 369, having a bacterial infection. These cells when present were usually in the stratum compactum, as shown here, but were at times found also in the spongiosum. Giemsa stain. 500X.

PLATE - 3

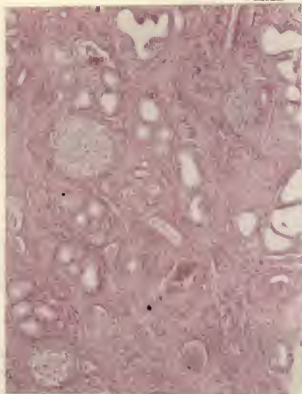


Fig 1

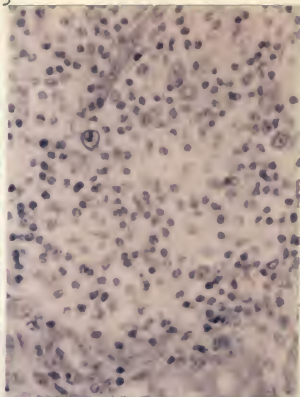


Fig 2

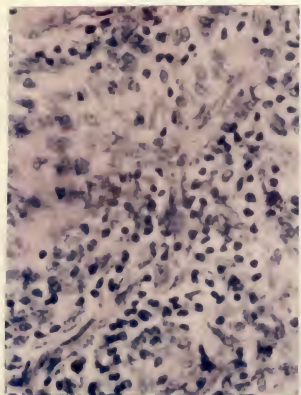


Fig 3

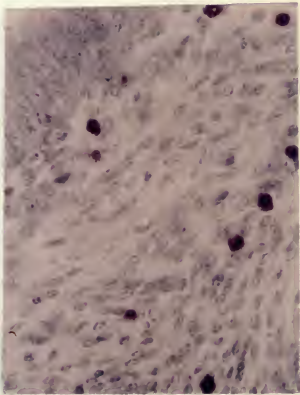


Fig 4

PLATE IV

Explanation:

- Fig 1: Normal endometrial glands in the endometrium of cow No. 388, a normal pregnant case. Large columnar epithelial cells of the gland indicate an active secretory stage. Giemsa stain. 500X.
- Fig 2: Peri-glandular encapsulation in endometrium of cow No. 388. Early development of spindle shaped connective tissue cells are becoming oriented around the glands. Giemsa stain. 500X.
- Fig 3: Encapsulated gland in the endometrium of post-partum repeat breeder cow No. 384, infected with *E. coli*. There is extreme development of peri-glandular connective tissue, the glandular epithelium has degenerated and the lumen is filled with a degenerating mass. Giemsa stain. 125X.
- Fig 4: Cystic endometrial glands in the endometrium of cow No. 424, a chronic repeat breeder. Dilated lumens, low epithelium and peri-glandular connective tissue characterize this condition. PAS stain. 125X.



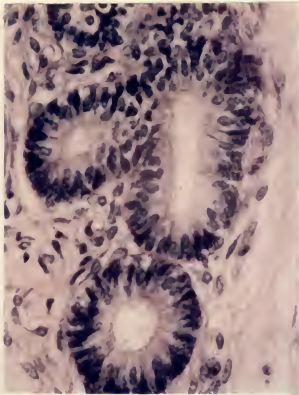


Fig 1

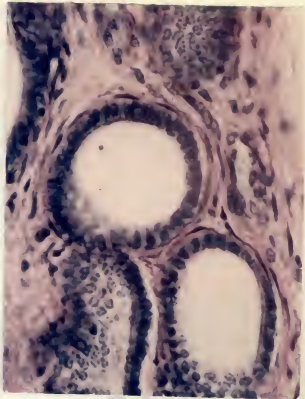


Fig 2

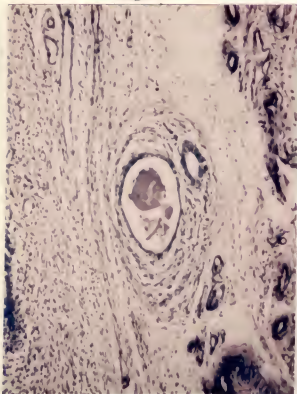


Fig 3

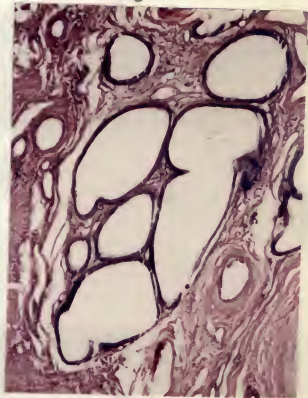


Fig 4



The most interesting of all was probably the incidence of lymphocytic nodules in the uteri post-partum cows. Of 19 cows five to 50 days post-partum, 13 (68.5%) showed the presence of these nodules. Out of 90 slaughter house cows five to 60 days post-partum, 47 (52%) were found to have lymphatic nodules. The nodules in the post-partum cows were comparatively larger and more numerous than in other uteri. There was a tendency for these nodules to form 12 to 15 days post-partum, become static up to 40 days post-partum, then decline (Plate VI).

Bacterial isolations from the above group of cows with unknown histories, indicated that of the 47 animals showing lymphatic nodules, 32 were from uteri from which some microorganisms were cultured (Plate VI). The 15 uteri from which no isolations were made were mostly beyond 35 days post-partum.

These nodules were also predominant in known diseased uteri. Out of 31 such cases studied, 17 (55%) showed a presence of these nodules in their tissues. In the unclassified group of animals, three out of eight were positive for these nodules. (Plate V).

Lymphocytes (Plate III, Fig. 3). Lymphocytes were observed to be present in nearly all the cases studied, but their number and frequency varied in the different conditions. The number of lymphocytic cells per field was invariably higher in repeat breeder cows, in those contaminated with bacteria, and in a few instances in the cows in later periods of normal pregnancy (Table I and III).

There was a tendency for a greater occurrence of lymphocytes in the presence of microorganisms directly proportional to the

pathogenicity of the microorganism. In repeat breeder cows not settled even after 11 services the uteri were not infiltrated with increased numbers of lymphocytes and showed only a few cells per field (Table III).

Tissues from all of the diseased uterine cases were infiltrated with lymphocytes in varying numbers and distribution (Table II).

Monocytes (Plate II, Fig. 2). The occurrence of monocytes was much less than was that of lymphocytes (Table I and III). The number in most cases was moderately low with only few cells per field. The presence of monocytes in the tissues tended to be increased when the uterus was infected with microorganisms, particularly in repeat breeders, post-partum, and diseased uteri. Monocytes were probably involved in phagocytic destruction and removal of foreign particles as evidenced in some cells which were loaded with phagocytized particles.

Neutrophils (Plate I, Fig. 1). Neutrophils were found in all those tissues which macroscopically showed the presence of pus in the uteri. In a study of 55 repeat breeder cattle, neutrophils were found in the uterine tissues of only nine cows (Table III), whereas five out of ten post-partum cows showed these cells (Table II). Out of 15 diseased uteri under study only six were found infiltrated with these cells (Table II). None of the normal pregnant animals were observed with neutrophils in the tissues (Table I).

A significant increase in the number of these cells was associated with infection of Streptococcus and Corynebacterium which were isolated six and three times respectively out of the 13 uteri found to have an infiltration of neutrophils. Other

organisms were also isolated in association with neutrophilic infiltration six times. They were Micrococcus three times and once each Staphylococcus, Pseudomonas, E. coli, Sarcina, Gram-negative rod, and Bacillus (Tables I, II, and III).

Ten out of 13 uteri with neutrophilic infiltration in the tissues were associated with the presence of certain pyogenic microorganisms.

Plasma cells (Plate II, Fig. 1). Plasma cells were found in only three cases all in 15 cows with diseased uteri. The history of these cattle and the condition of the organ at the time of slaughter indicated that they all had a chronic diseased condition (Table II).

Eosinophils (Plate I, Fig. 3). Eosinophils were observed in only 12 uteri of the 117 cases examined. In the normal pregnant group, two out of 25 were found with eosinophils, whereas in repeat breeders and diseased uteri there were six out of 55 and two out of 15 respectively. They were also present in two of the three heifers which had never been bred. When present they were in small numbers. Their presence could not be associated with the estrus cycle. (Plate VII).

Mast cells (Plate III, Fig. 4). The morphological picture of the mast cells differed in various cases. In certain cases the cytoplasm of the normal mast cell was completely filled with metachromatic granules, whereas in others the granules were dissolved out leaving only a few faintly staining shadows. In some cases granules comparable to those of basophils were seen scattered outside the cells.

Mast cells (Table I, II and III) were found in 40 cases of the 117 cattle studied (Table I, II and III), and were observed in all the groups in somewhat the same patterns but variable in numbers. No correlation with the estrus cycle could be demonstrated; however, in 16 of the 24 cases in which mast cells were found the uterus was infected with microorganisms (Plate VIII). These usually were Streptococcus and Corynebacterium.

The occurrence of these cells was much higher in repeat breeders (21 out of 55) in contrast to five out of 25 cases of the animals with normal pregnancies. The diseased uteri were positive for mast cells in four out of 15 as were five of ten post-partum cases of known history. Of the specimens obtained from the slaughter house with no histories available, 58 out of 106 cases were positive for mast cells.

Histiocytes (Plate II, Fig. 4). Histiocytes were observed in all the groups and in the majority of the cattle under study. In "normal pregnant cows" the number of cells was few and sometimes rare. The uteri of the repeat breeders were heavily infiltrated with histiocytes. A detail study of these cells was undertaken in post-partum cows as this condition presented maximum numbers and occurrence. These cells distinctly increased in the endometrium after 15 days post-partum, reached a maximum at 35 to 40 days, then declined to near complete disappearance by 60 days. Bacteria were cultured from 38 out of the 65 uteri found to contain histiocytes (Plate IX).

Histiocytes were observed in 11 of the 15 grossly diseased uteri studied. Their number varied from few per field to many,

depending upon the individual case and condition (Table II).

Endometrial glands. The uterine glands were normally distributed throughout the endometrium; the deeper glands having smaller lumina than the superficial ones. These glands were lined with epithelial cells varying from low cuboidal to columnar (Plate IV, Fig. 1).

In addition to the normal appearance of the glands, two types of abnormalities were observed. One type involved primarily a modification of periglandular connective tissue while the other was a change in the gland proper. In the first type of abnormality (Plate IV, Fig. 2), the gland cells themselves do not differ appreciably from those of normal glands; the lumen, however, is slightly extended. The connective tissue around the glands was hypertrophied and distinctly modified and formed a capsule of closely packed spindle-shaped cells parallel to each other. These glands were distinctly different from neighboring glands (Plate IV, Fig. 3).

The second type of glandular abnormality was typical cystic glands (Plate IV, Fig. 4) with wide branched lumina and low cuboidal or flattened epithelium. In most cases epithelial degeneration was evident. The periglandular connective tissue ordinarily was not altered. In a few cases there was a combination of disturbances in the gland cells proper as well as in the periglandular connective tissue.

Enlarged lumina of the glands were commonly seen in the normal pregnant animals. These glands had a normal columnar epithelium, with no evidence of periglandular encapsulation. The



epithelial cells in certain cases indicate an infiltration of different leucocytic cells, mostly lymphocytes and neutrophils but sometimes monocytes.

The different groups of cows under study provided a varying picture of these glands. In normal pregnant cattle, about 35% have slight periglandular encapsulation, while four out of seven (58%) associated with microorganism contamination had some indication of the disorder. In the repeat breeder group, 79% showed slight periglandular encapsulation to highly encapsulated and cystic glands. Epithelial degeneration with some plugging of the lumen occurred in four cases. This group included 19% cystic uteri of which 55% were associated with bacterial contamination of the uteri. The diseased uteri had 92% affected mostly of higher modification of the endometrial glands.

Table 1. Normal pregnant group<sup>a</sup> (with known history).

Book Number	Bact. Isolations	Streptococci	Staphylococci	Corynebacterium	Pseudomonas	Others	Lymph. nodules	Uterin Glands	Monocytes	Lymphocytes	Neutrophils	Acidophils	Mast cells	Histiocytes
243	-									+				
245	-									+				
248	-									+				
306	-							+	+	+			+	
313	-								+	+				+
315	-								+	+				+
327	-								+	+				+
331	-							+	+	+				
370	+									+			+	
386	+						+		+	+				
387	+									+				
388	+									+				
389	+							+		+				
392	+									+				+
395	+							+	+	+			+	
396	+						+	+	+	+			+	+
399	-								+	+				
400	-							+	+	+				+
401	+								+	+			+	+
413	+					+				+		+		+
444	+		+				+			+				+
447	+					+		+	+	+		+		+
465	+	+	+					+		+			+	
492	-									+				+
493	-								+	+				

<sup>a</sup>This group consisted of animals with known histories of normal pregnancy settled within three services.

The bacterial isolations shown were obtained from Joe Poerio's (1962) work, Department of Bacteriology, Kansas State University, Manhattan.

<sup>b</sup>The plus sign in this column indicates that a bacterial isolation was undertaken and the bacterial species isolated are indicated following appropriate columns. A minus sign indicates that no bacterial isolations were attempted.

Table 2. Diseased uteri (with known history).

Book Number	Bact. Isolations	Streptococci	Staphylococci	Corynebacterium	Pseudomonas	Others	Lymph. nodules	Uterine glands	Monocytes	Lymphocytes	Neutrophils	Acidophils	Mast cells	Histiocytes
19	-						++	++	+				+	+
28	-							++			+++			+++
29	-							+++	+	++				+
31	-							++		+				+
38	-							+	++	++	+			+
43	-							+	+	+			+	
45	-							+		P+++				+
48	-									+			+	+++
220	-						+	+++	+	P+++				
225	-						++	+	+	P++		+++		
226	-						++	+	++	+	+			
293	-							++	+	+				
513	+			+		+	++	++	+	+++	+	+++		+
516	+			+				++	+	++	++			+++
517	+	+				+	++	+++		++	+++		+	+

Table 2. Post-partum and unclassified group (with known history).

Book Number	Bact. Isolations	Streptococci	Staphylococci	Corynebacterium	Pseudomonas	Others	Lymph. nodules	Uterine glands	Monocyte <sup>s</sup>	Lymphocytes	Neutrophils	Acidophils	Mast cells	Histiocytes
71	PP	-	-							++	+		+	+
72	"	-					+	+		+				+
384	"	+				+	+	++	+	+++	++			++
406	"	+							++	++				++
421	"	+	++		+			++		++++				++
422	"	+	++	+		++		+	++	+	++++		+	+
423	"	+						++	+	+				++
464	"	+					++	++	+	+			++	++
466	"	+	++	+				++	+	+++	+		+++	+++
467	"	+						++	+	+	+		++	+
403	Un	+							++	++				+
408	"	+						++	+	+				
409	"	+		+			++	+	++	+				++
416	"	+	+			+		+	++	+	+			
454	"	-							+	+			+	+
455	"	-							+	+			+	+

Table 3. Repeat breeding group. (With known History)

Book Number	Bact. Isolations	Streptococci	Staphylococci	Corynebacterium	Pseudomonas	Others	Lymph. nodules	Uterine glands	Monocytes	Lymphocytes	Neutrophils	Acidophils	Mast cells	Histiocytes
251								+						
252							++	++	+	++				+
258							+		+		+			+
259							++	++		++			++	+
260							+	+		++		+	+	
274							++	++		++				+
275							++	++		++				+
277							++	++		++			+	+
278							++	++		++				+
284							+	++		++				+
292							++	++		++				++
296							++	+		++	+	+		++
303							++	+		++			+	++
307							++	+		++	+		+	++
311							++	+		++				++
312							++	++		++				++
314							++	+		++				++
317							++	+++		+++			++	++
369	+		+				++	++		+++			+++	++
376	++						++	+		+++			++	++
382	++							+		++	+		++	++
383	++							+		+++			++	++
391	++			+			++	++		+++		+	++	++
398	++							+		+++			++	++
402	++		+		+		+			+++			++	++
405	++							++		+++			++	++
407	++							+++		++			++	++
411	++		+		+	+	++	+		++			++	++
412	++		+		+		++	++		++			++	++
424	++		+	+	+		++	+++	+	++			++	++



Table 3. (continued)

Book Number	Bact. Isolations	Streptococci	Staphylococci	Corynebacterium	Pseudomonas	Others	Lymph. nodules	Uterine glands	Monocytes	Lymphocytes	Neutrophils	Acidophils	Mast cells	Histiocytes
443	+			+				++	+	+		+		+
445	+	+						++	+	+			+	+
446	+	+	+					++	+	+	++			
448	+			+				+		+				
453								++		+		++	+	+
456								++	+	+			++	++
457								++	+	+			+	+
459	+	+			+			++		+	+++		++	+
460	+	++						++		++		+++	++	
461	+	++						++	+	+	++		++	+
462	+	++						++	+	+			++	+
463	+	+++				+		++	+	+		+++	++	+
468	+	++		++				+	+	+		+	+	
469	+							++		+				
470	+					++		++		+			++	+++
471	+					+		++	+	+			++	+++
481	+							++	+	+				++
482	+			+				++		+			+	+
483	+							++	+	+			+	+
485	+							++	+	+			+	+
491								+++	+	+++				+
508								++	+	++				+
514	+			+		+		++	+	+		++	++	+
521	+	+				++		++	+	+		++	++	+

## DISCUSSION

It is an established fact that many conditions or factors may play a part in the causation of sterility in cattle. Endometritis probably is one of the important conditions responsible for many repeat breeders. Endometritis characterized by a heavy leukocytic infiltration and other abnormal changes of the endometrium have been marked criteria of pathological conditions associated with infertility.

It is interesting to note an increased lymphocytic infiltration in nearly all repeat breeders and even in normal pregnant animals. Such infiltration of lymphocytes is probably a response to an antigenic stimuli which is a microorganism in the majority of the repeat breeding cows. The presence of the large numbers of these cells in normal pregnant animals is possibly also an antigenic stimuli response towards the foetus of the animal. This view is supported by recent work (Poerio 1962) in rabbits, in which antibody reaction was developed in does against embryo extract. The number of these cells had a tendency to increase with the progression of the pregnancy. It has been established that plasma cells and lymphocytes are responsible for antibody production (Carpenter, 1956) and play a role in the body's defense. An increase of these cells in proportion to age as described by de Bois and van den Akkar (1957) could not be detected.

Lymphocytic nodules have been suggested as one of the criteria of infertility by Moss *et al.* (1956) when they reported the presence of such nodules in eight out of 30 such animals. This change was

also reported by Simon and McNutt (1957b) and had been observed by previous workers (Fugimoto, 1956 etc.). The author also finds it one of the significant changes in 25 out of the 113 repeat breeders, in comparison to four occurrences in 52 normal pregnant cows. The high incidence (55%) of these nodules in known diseased uteri makes it more appropriate to regard them as a part of endometritis and a pathological change (Plate V). The presence of these nodules in post-partum cows (68.5% occurrence) along with increased numbers in the tissues and their association in 32 cases out of 47 with some microorganisms indicates that microbes may be one of the causes of their formation (Plate VI). The 12 cases of the lymphocytic nodules not associated with bacteria were in cows over 30 days post-partum, so possibly the contamination acquired at the time of parturition had cleared by the time of slaughter while the characteristic changes persisted. The heifers that were never bred showed no such nodule formation in the endometrium.

The presence of monocytes in the tissue is not of much significance apart from the basic fact that being the capable cell of phagocytosis they have been drawn towards the site of action which has been infected with microorganisms or certain other foreign particles.

It could not be determined what role the acidophile cells play or what may be the cause for their presence in the tissue. The percentage of such cells was low and the cases affected were few. Extensive eosinophilic infiltration of the endometrium, as in myxomatous cases reported by Sugawa and Homma (1957) was not observed.

It was not always possible to ascertain the part played by mast cells in these tissues. There was no correlation between these cells and any phase of the estrus cycle (Plate VIII). In spite of the fact that mast cells produce heparin, an anticoagulant, and histamine (Wintrobe, 1961), it was not possible to correlate the function of these materials in the tissues. It is a probability which cannot be ruled out that the mast cells have a role in liberating histamine and producing the anaphylactic reaction due to foreign proteins of any source. The degranulated mast cells described by Shelley and Juhlin (1961) lend good support to this probability.

In the present study, neutrophil cells were found to be associated with pyogenic microorganisms (Streptococcus, Corynebacterium, and Pseudomonas) with macroscopic pus formation in some cases. Pyogenic forms of endometritis have been described by several previous workers (Frei, 1925) who have also reported the presence of polymorphs, probably neutrophils, below the epithelium. These findings were supplemented and supported by a large number of workers like Laszlo (1935), Erisman and Saxer (1933), and Cembrowicz (1946). It was well established by these workers and others (Dawson, 1949; Baur, 1951) that pyogenic infection is always associated with neutrophils.

Histiocytes, as a separate cell type in the uterus have never been adequately reported by previous workers. In this study these cells were observed in most of the cases where phagocytosis and cleaning of the organ were required, as in bacterial or other contamination. Their incidence in repeat breeder cows was high in

comparison to normal pregnant animals. Their actual role can well be understood by the study done in post-partum cows, which showed the highest incidence of these cells. They were mostly associated with bacterial infection up to 30 days, the association then becoming less and less with the reduction of microorganisms. It is interesting to note that histiocytes are first observed to increase at about 10 day post-partum, increasing to a maximum at about 35 to 40 days, and then showing a reduction in numbers to almost complete elimination by 60 days post-partum (Plate IX). This indicates that these cells function to remove the infection acquired at the time of parturition or to phagocytise and remove the debris left in the uterus after parturition. Both these functions are completed normally by 50 to 60 days post-partum, and then the histiocytes disappear.

Endometrial glandular changes have frequently received notice by workers who reported them encapsulated or cystic. Tagliavini (1935) reported them and considered hypersecretion to be the cause, whereas Frei (1925) followed by Erisman and Saxer (1933) and Quinlan (1929) attributed them to duct blockage. Queisser (1941) correlated cystic endometritis with cystic ovarian diseases. This idea was later supported by Dawson (1958), who observed cystic glandular dilation in about 40% of the cases of cystic ovaries. This contrast according to Dawson (1961) suggests that two types of cystic endometritis may be present, one associated with ovarian cystic diseases and other due to duct blockage. In the present study, a higher incidence of periglandular encapsulation was observed,



sometimes with necrosis and degeneration of epithelial cells in repeat breeder and diseased uteri as compared to normal pregnant cows, although the latter in certain cases indicate a slight development of connective tissue around the glands. The higher incidence associated with microorganism infections indicates the possibility that encapsulation may be a barrier against further spread of infection to the healthy tissue. This interpretation may also hold true for the pregnant cases involving such changes, possibly indicating previous infections which have been cleared up in the later stages. As a matter of fact this lesion can be considered only indirectly an indication for repeat breeding, It is an indication of previous infection with a direct relationship between degree of infection and extent of lesions. Cystic glands may be due to duct blockage by degenerating epithelium and cellular infiltration as previously reported by Frei (1925), Dawson (1961) and Moss et al. (1956). A high frequency of the encapsulation of the endometrial glands was also observed in the sterile cows, making this the most significant lesion of infertility, as described by Moss et al. (1956).

It was surprising to find such a low incidence of lesions as reported by Simon and McNutt (1957b) who in the series of 109 cows that were bred four times could find a pronounced endometritis in only one cow and minor abnormalities in 49. This author is in full agreement with Brus (1952), Moss et al. (1956), and Dawson (1961) that endometritis and these changes observed in the endometrium constitute the characteristic changes in the uterus of a

majority of the repeat breeder cattle. Many cows culled for infertility have a persistent lesion in either the endometrium or in other parts of the reproductive system, while in others an endometritis has probably healed by the time of slaughter. It seems probable, however, that many cows are rendered temporarily infertile by infections and endometritis. These changes may resolve partially or completely later on with restoration of tissues to near normal.

#### SUMMARY

Uterine tissues were obtained for this study from a group of 117 cows with known reproductive histories and 90 post-partum cows of unknown histories.

In repeat breeder cows there was a distinct endometritis characterized by a mixed cellular infiltration of lymphocytes and polymorphs, along with other changes found when compared with normal pregnant uteri. A significant increase in size and number of lymphocytic nodules was observed, usually associated with bacterial infections in post-partum cows, and reached a maximum at 30 to 40 days post-partum. Persistence of these nodules was frequent in repeat breeders. Increased lymphocytic infiltration in both normal pregnant uteri and uteri from repeat breeders was considered to be an antigenic response to the embryo and to bacteria respectively. The uterine glands in repeat breeders showed a higher incidence of encapsulation and cystic glandular endometritis than did the normal pregnant cows.

Histiocytes were correlated with bacterial infections, making their appearance about 10 days post-partum, becoming maximum at 35 to 40 days, then declining and mostly disappearing by 60 days. This was the identical pattern which is followed by bacteria in the post-partum uterus.

In a majority of cases neutrophils were found to be associated with pyogenic microorganisms.

Acidophils were observed in a few cases. Their number was small, and showed no relationship to days of the estrus cycle. There was no relationship of mast cells with days of the estrus cycle. Their association with bacteria indicates a possibility that they play a part in anaphylactic reactions.

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## BIBLIOGRAPHY

- Ajello, P.  
Zootec. e. Vet. 2:47. 1954. F. L. M. Dawson. The Bovine Uterus, A Histopathology. Vet. Rev. and Ann. 7(1):29. 1961.
- Aziz-Ud-Din, I. M.  
The visual lesions in certain pathological conditions of the genital tract in cows. Vet. Rec. 60:345, 1948.
- Asdell, S. A.  
Cattle fertility and sterility. Bost. Little-Brown & Co. 1955.
- Asdell, S. A., J. de Alba and S. J. Roberts.  
Studies on the uterus cycle of the dairy cattle: cycle length, size of corpus luteum, and endometrial changes. Cornell Vet. 39:389-402. 1949.
- Baur, E.  
Inaug. Diss. Munich. 1951. F. L. M. Dawson. The bovine uterus, a histopathology. Vet. Rev. and Ann. 7(1):23. 1961.
- Bhattacharya, P., S. N. Luktuke, A. S. P. Rao, and S. K. De.  
Incidence of fertility under various casual groups in buffalo cows in INDIA. Curr. Sci., 23:335-36. 1954.
- Black, W. G., L. C. Ulberg, H. E. Kidder, J. Simon, S. H. McNutt and L. E. Casida.  
Inflammatory response of the bovine endometrium. Ame. Jour. Vet. Res. 14:179. 1953.
- Black, W.G., J.Simon, H.E. Kidder, J.N. Wiltbank.  
Bacterial activity in the uterus of rabbit and cow. Ame. Jour. Vet. Res. 15(55):247. 1954.
- de Bois, C.H.W. and vanden Akkar, S.  
Tijdschr. Diergenesck. 82:951. 1957. F.L.M. Dawson. The Bovine Uterus, Histopathology. Vet. Rev. & Anno. 7(1):29. 1961.
- Broom, A.W., G.E. Lamming and M. Woodbine.  
Studies on the relationship between ovarian hormones and uterine infections. Jour. Endo. 18:209-219, 220-228, and 229-235. 1956.
- Brus, D.H.J.  
Proc. IIInd. Int. Cong. on Anim. Reprod. 2:175. 1952. F.L.M. Dawson. The Bovine Uterus, Histopathology. Vet. Rev. and Anno. 7(1):29. 1961.



- Brus, D. H. J.  
Biopsia Uteri. Proefschrift. Rijksuniversiteit Utrecht.  
The Netherlands. 1954. S. Moss, J. F. Sykes and T. R.  
Wrenn. Some abnormalities of the bovine endometrium.  
Jour. Anim. Scie. 15:631. 1956a.
- Bunting, C. H.  
The granular leucocytes, in Cowdry's special cytology.  
2nd. Ed. N.Y. (2). 1932.
- Carpenter, P. L.  
Immunology and Serology. Philadelphia and London. W. B.  
Saunders Co. 1956 Ed.
- Cembrowicz, H. J.  
Diss. Cambridge. 1946. F. L. M. Dawson. The bovine uterus,  
histopathology. Vet. Rev. and Ann. 7(1):23. 1961.
- Compton, A. S.  
A cytochemical and cytological study of connective tissue  
mast cells. Ame. Jour. Anat. 91:301. 1952.
- Conklin, R. L., J. B. McCarthy, R. R. Thompson and L. I. Pugsley.  
Clinical, Bacterial and Physico-chemical studies of preg-  
nant bovine uterus. Cornell Vet. 21:177-186. 1931.
- Dawson, F. L. M.  
The microbial content and the morphological character of the  
normal bovine uterus and oviducts. Jour. Agri. Scie. 40:  
150-156. 1950.
- 
- Bovine cystic ovarian diseases. An analysis of 48 cases.  
Brit. Vet. Jour. 114:96. 1958.
- 
- Bovine endometritis. A Review. Brit. Vet. Jour. 116, 12:  
149. 1960.
- 
- The bovine uterus, histopathology. Vet. Rev. and Ann. 7(1):  
29. 1961.
- Diggs, L. W., Dorothy Sturn, and Ane Bell.  
Morphology of blood cells. Abbott Lab., Ill. 1954.
- Downey, H.  
The development of histiocytes and the macrophages from  
lymphocytes. Jour. Lab. and Clin. Med. 45:499. 1955.
- Elliott, Lloyd.  
The bacterial flora of the uterus of the post-partum cow. A  
Master's Thesis. Kansas State University, Manhattan, 1961.

- Erismann, H. and E. Saxer.  
Schweiz. Arch. Tierheilk. 75:534. 1933. F. L. M. Dawson.  
The bovine uterus, histopathology. Vet. Rev. and Ann. 7(1):  
23. 1961.
- Frei, W.  
In Joest's Handbuch derspez. path. Anatomie der Haustiere.  
4:106. 1925. F. L. M. Dawson. The bovine uterus, histology.  
Vet. Rev. and Ann. 7(1):23. 1961.
- Fugimoto, Y.  
Pathological studies on sterility in dairy cows. Jap. Jour.  
of Vet. Res. 4(4):130. 1956.
- Gomori, G.  
The periodic acid Schiff stain. Amer. Jour. Clin. Path. 22:  
277. 1952.
- Hallman, E. T.  
A preliminary report on the pathology of the reproductive  
organs in sterility. The Cor. Vet. 2:28. 1919.
- Huysman, A.  
Vlaams diergeeneesk. Tijdscher. 26:299. 1957. F.L.M.  
Dawson. The bovine uterus, histopathology. Vet. Rev. and  
Ann. 7(1):29. 1961.
- van der Kaay, F. C.  
Vlaams diergeeneesk. Tijdscher. 26:299. 1957. F. L. M.  
Dawson. The bovine uterus, histopathology. Vet. Rev. and  
Ann. 7(1):23. 1961.
- Klimmer, M., H. Haupt and E. Roots.  
Abt. Bakt. (Orig.) 110:62 & 111:207. 1929. F. L. M. Dawson.  
The bovine uterus, histopathology. Vet. Rev. and Ann. 7(2):  
23. 1961.
- Koike, Toshio and Keuchiro Kawata.  
Studies on tubal potency of cows. Jap. Jour. of Vet. Res.  
7(e):61-70. 1959.
- Kolouch, F. Jr.  
The lymphocytes in acute inflammation. Ame. Jour. Path. 15:  
413. 1939.
- Lagerlof, N. and H. Boyd.  
Ovarian hypoplasia and other abnormal conditions in the sexual  
organs of cattle of the Swedish highlands breed:Results of  
post-mortum examination of over 6000 cows. Cornell Vet. 43  
(1):64-79. 1953.
- Laszlo, F.  
Dtsch. tierarztl Wschr. 43:371. 1935. F.L.M. Dawson. The  
bovine uterus, histopathology. 7(1):23. 1961.

- Lukert, P.D.  
Virological Examination of Bovine Uteri. Vet. Med.  
56(11):458-460. 1961.
- Maekawa, Hikaru and Keiichi Kawata.  
Histo-pathological observations on the genital organs  
of the Repeat Breeding Heifer's. Jap. Jour. Vet. Res.  
7(4):171-178. 1959.
- Maximov, A.  
The lymphocytes and plasma Cells in Cowdrys special  
cytology. 2nd. Ed. N.Y. (2):601. 1932.
- Menkin, V.  
Factors concerned in the mobilisation of the leukocytes  
in inflammation, in symposium on leukocytic function.  
Ann. N.Y. Acad. of Sc. 59:950-985. 1955.
- Miller, J.G.  
A technique of endometrial biopsy in the Bovine Animals.  
Jour. Am. Vet. Med. Asso. 119:368. 1951.
- 
- A method of endometrial biopsy in the bovine and his study  
of biopsy specimens in cases of infertility. Thesis, M.S.  
Cornell University. 1950.
- Moss, F., J.F. Sykes and T.R. Wrenn.  
Some abnormalities of the Bovine Endometrium. Jour.  
Animal Scie. 15:631. 1956a.
- Munero, I.B.  
Infectious and non-infectious Herd infertility in East-  
Anglia. Vet. Rec. 69:125. 1957.
- Ogilvie, R.F.  
Pathological Histology. 2nd. Ed. Williams & Wilkins  
Co. Baltimore. 1951.
- Payne, J.M.  
The Pathogenesis of the Experimental Brucellosis in the  
pregnant Cow. Jour. Path. and Bact. 78:447. 1959.
- 
- The Pathogenesis of Experimental Brucellosis in Virgin  
Heifers with and without continuous Progesteron Treatment.  
Jour. Endocrinology. 20:345. 1960.
- Plant, A.  
Historical and Cultural aspect of the Uterus. Ann. N.Y.  
Acad. Sc. 75:389. 1959.

- Poerio, J.  
Bacteriological and Immunological Studies in relation to Embryo Loss. Ph.D. Dissertation. Kansas State University. Manhattan. 1962. (manuscript in preparation)
- Queisser, J.  
Inaug. Diss. Hanover. 1941. F.L.M. Dawson. The Bovine Uterus, Histopathology. Vet. Rev. and Ann. 7(1):29. 1961.
- Quinlan, J.  
15th. Ann. Repro. Director Vet. Serv. Onderstepoort. Pg. 339. 1929. F.L.M. Dawson. The Bovine Uterus, Histopathology. Vet. Rev. and Ann. 7(1):23. 1961.
- Rebuck, J.W. and Helen L. Woods.  
Electron microscopy of Blood Cells. Jour. Haemato. 3:175. 1948.
- Riley, J.F.  
Histamine in tissue mast cells. Science. 118:332. 1953a.
- Riley, J.F. and G.B. West.  
Histamine in tissue mast cells. Jour. Physio. 117:72. 1953.
- 
- The presence of histamine in tissue mast cells. Jour. Physio. 120:528. 1953.
- Roark, D.B.  
Studies on Breeding Failure in Dairy Cattle. Jour. Anim. Sc. 12:897. 1953.
- Rowson, L.E.A., G.E. Lamming and R.M. Fry.  
The relationship between ovarian hormones and uterine infections. Vet. Rec. 65(22):335-340. 1953.
- Runnels, R.A., W.S. Monlux and A.W. Monlux.  
Principals of Veterinary Pathology. Pg. 550. 1960. Pub. The Iowa State University Press. Ames, Iowa.
- Schnider, P.  
Tierarztl. Umsch. 13:149. 1958. F.L.M. Dawson. The Bovine Uterus, Histopathology. Vet. Rev. and Ann. 7(1):23. 1961.
- Shelley, W.B. and L. Juhlin.  
A new test for detecting anaphylactic sensitivity: The basophil reaction. Nature. 191. No. 4793:1056. 1961.

- Simon J. and S. H. McNutt.  
Histopathological alterations of the bovine uterus I, Studies with the *Vibrio foetus*. *Ame. Jour. Vet. Res.* 18:53. 1957a.
- 
- Histopathological alterations of the bovine uterus II, Uterine tissue from the cows of the low fertility. *Ame. Jour. Vet. Res.* 18:241. 1957b.
- Skjerven, O.  
Endometrial biopsy studies in reproductive normal cattle. *Acta. Endc. Copenhagen Suppl.* 26. 1956.
- Smith, H. A. and T. C. Jones.  
Veterinary Pathology. IInd. Ed. Pg. 923-925. 1961. Lea & Febiger, Phil.
- Smith, D. N. and Y. S. Lewis.  
Electron microscopy of mast cells after X-radiation. *Ame. Jour. of Phys.* 183:662. 1955.
- Stamp, J. T.  
A review of the pathogenesis and pathology of bovine tuberculosis with special reference to practical problems. *Vet Rec.* 56:443. 1944.
- Strikwerda, R.  
*Tijdschr. Diergeneesk.* 81:705. 1956. F. L. M. Dawson.  
The bovine uterus, histopathology. *Vet. Rev. and Ann.* 7(1): 23. 1961.
- Sugawa, A. and S. Homma  
*Jap. Jour. Animal Repro.* 3:35. 1957. Hikaru Maekawa and Keiichiro Kawata. Histopathological observations on the genital organs of the repeat breeding heifers. *Jap. Jour. Vet. Res.* 7(4):171-178. 1959.
- Szeky, A. and Dozsa, L.  
*Acta. Vet. Acad. Scie. hung.* 5:99. 1955. F. L. M. Dawson.  
The bovine uterus, histopathology. *Vet. Rev. and Ann.* 7(1): 23. 1961.
- 
- Acta. Vet. Acad. Scie. hung.* 6:259. 1956. F. L. M. Dawson.  
The bovine uterus, histopathology. *Vet. Rev. and Ann.* 7(1): 23. 1961.
- Tagliavini, A.  
*Azione vet.* 4:310. 1935. F. L. M. Dawson. The bovine uterus, histopathology. *Vet. Rev. and Ann.* 7(1):23. 1961.



- Terpstra, J. I.  
Proc. IIIrd. Int. Cong. on Anim. Repro. Plenary Papers. Pg. 34. 1956. F. L. M. Dawson. The bovine uterus, histopathology. Vet. Rev. and Ann. 7(1):23. 1961
- Weber, A. F., B. B. Morgan and S. H. McNutt.  
A histological study of the Metrorrhagia in the virgin heifers, Ame. Jour. Anat. 83:309-328. 1948.
- 
- Tissue mast cells in the virgin bovine uterus during the estrus cycle. Cornell Vet. Vol. XL:34-38. 1950.
- 
- Clinical and post-mortum observations on metrorrhagia in virgin heifer. N. Ame. Vet. 29:705. 1948b.
- Weeth, H. J. and H. A. Herman.  
A histological and histochemical study of the bovine oviducts, uterus and placenta. Mo. Agr. Exp. Sta. Res. Bul. 501. 1952.
- Wegener, K. and Bisping, B.  
Berl. Munch. tieraratl. wscrh. 72:255. 1959. F. L. M. Dawson. The bovine uterus, histopathology. 7(1):23. 1961.
- White, Abraham and T. F. Dougherty.  
The role of lymphocytes in normal and immune globulin production and the mode of release of globulin from lymphocytes. Ann. of N. Y. Aca. Sci. 56(Art. 8):859. 1946.
- Wintrobe, M. M.  
Clinical Hematology. 5th. Ed. Philadelphia. Lea & Febiger. 1961.
- Year Book of Agriculture.  
Animal Diseases. Year Book of Agriculture. 1956. Published by the United States Department of Agriculture. Washington, D. C.

## APPENDIX

PLATE V

DISTRIBUTION OF LYMPHOCYTIC NODULES IN  
ANIMAL GROUP OF THE KNOWN HISTORIES ,

Explanation:

In contrast to counts for numbers of cells of various types all section on atleast one slide ( 25 to 100 sections) were scanned for the presence of lymphocytic nodules. Presence of nodules in the different groups of cows, by percent, are plotted on this chart.

DISTRIBUTION OF LYMPHOCTIC NODULES IN ANIMAL GROUPS OF THE KNOWN HISTORIES.

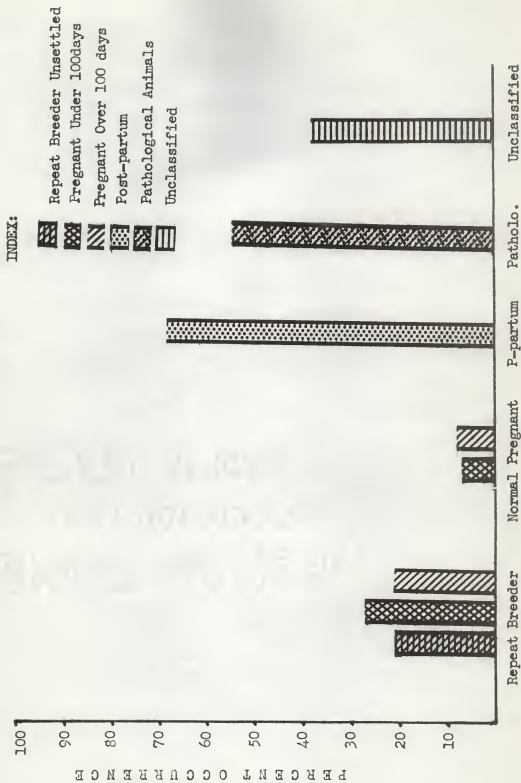


PLATE VI

DISTRIBUTION OF LYMPHOCYTIC NODULES IN  
POST-PARTUM CASES (K.C.S.H.)

Explanation:

Examination of many sections of endometrial preparations resulted in grouping lymphocytic nodules as:

None: no nodules were found in the entire section's series.

Rare: Only one or two nodules found in atleast 40 sections.

Common: Nodules present in several sections, but only a few in any one sections.

Many: Nodules in every sections, frequently several nodules in a sections.

The cows used for this study were the same ones as were studied bacteriologically by Elliott (1961). Solid dots indicates cases in which bacteria were present. Open circles indicates no bacteria were cultured. No nodules were found in the number of uteri which definitely contained bacteria and many nodules were present in some uteri from which cultured failed.



DISTRIBUTION OF THE LYMPHOCTIC NODULES  
IN POST-PARTUM COWS(K.C.S.H.)

INDEX:  
 • Nodules Associated with Bact.  
 ○ Nodules Without Bacteria.



PLATE VII

DISTRIBUTION OF ACIDOPHILS

Explanation:

The "number occurrence" is the average number of acidophils counted in ten fields in a well stained typical section. Counts were made on 117 preparations. Occurrence of acidophils at any stage is closely proportional to the number of animals examined at that stage, so no correlation was found between presence of acidophils and stage of cycle or days of pregnancy.

## DISTRIBUTION OF ACIDOPHILS

INDEX :

- Not Settled Cases
- Pregnant Animals

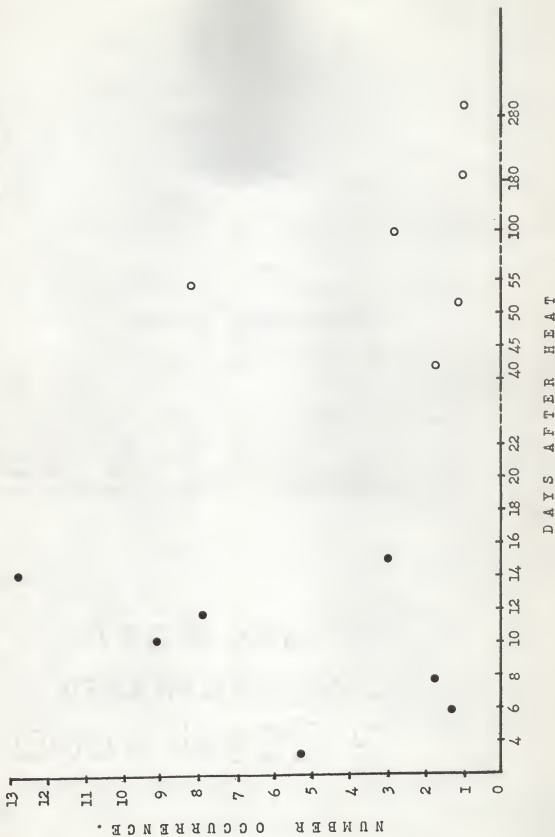


PLATE VIII

DISTRIBUTION OF MAST CELLS IN RELATION  
TO ESTRUS CYCLE

Explanation:

Mast cells were found in 23 of 77 cycling cows examined for them. The "number occurrence"(ordinate) is the average number of mast cells found in ten microscopic fields in a well-stained typical section of the preparation. Occurrence of positive cases within the estrus cycle is closely proportional to the distribution of the total sample.

DISTRIBUTION OF MAST CELL IN RELATION  
TO ESTRUS CYCLE

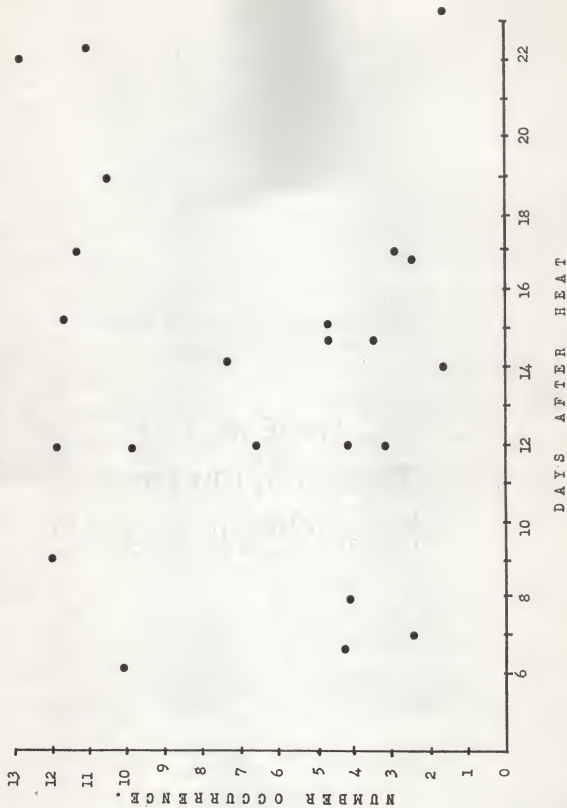




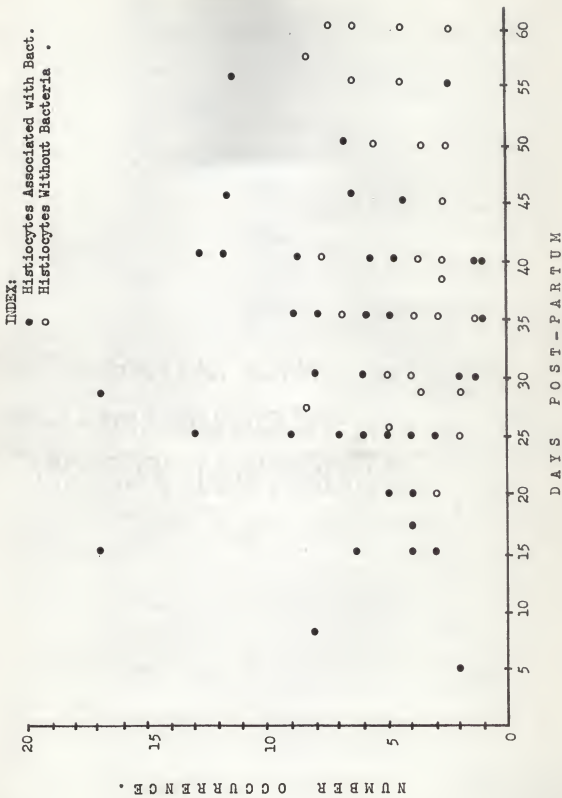
PLATE IX

DISTRIBUTION OF HISTIOCYTES IN  
POST-PARTUM CASES

Explanation:

The number of histiocytes per occurrence ( the ordinate) was taken as the average of ten fields in a well stained typical section of the endometrium. The material studied for this group was the same as that used by Elliot(1961) for the bacterial study of post-partum cows, and results are separated into "with bacteria" designated by solid dots and "without bacteria" designated by open circles. Histiocytes occurrence is more closely correlated with time after parturition than with the presence of bacteria.

DISTRIBUTION OF HISTIOCYTES IN  
POST-PARTUM CASES



HISTOPATHOLOGICAL STUDY OF THE  
BOVINE UTERUS

by

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B. Sc., University of Lucknow, India, 1955  
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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the  
requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1962

## ABSTRACT

The repeat breeder cow has been the focus of many investigation throughout the past years due to the economic importance of these animals. The great economic loss to the cattle industry and the nation resultant from reproductive failures, stimulated this study of the histopathological picture of uteri from repeat breeder cows. The immediate goal was to determine the abnormalities and the pathological conditions which might be responsible for failure of fertility and to determine the cause of such pathological changes.

Uterine tissues were collected from 117 cows with known histories of reproduction and 90 post-partum cows without known histories. These tissues were obtained at time of slaughter in the abattoirs located at Salina, Kansas and Kansas City, Missouri. Gross pathological examinations were conducted on all reproductive organs at time of slaughter.

Tissues were sectioned in paraffin at six microns, and one slide stained with each of the following stains: Haematoxylin, Periodic-acid-Schiff (Gomori, 1952), and modified Giemsa with eosin. The first two were used to study the general structure of the tissue, while the Giemsa-stained sections were used for the differential cell studies.

A greater incidence of lymphocytic nodules was observed in the uteri from repeat breeders than were found in normal uteri. However, a maximum number of nodules were found in uteri from post-partum and pathological cases. In post-partum uteri a correlation of bacterial infections and lymphatic nodules was observed,

which decreased with the decline of bacterial infection. Increased infiltration of lymphocytes in both the normal pregnant uteri and uteri from repeat breeders was considered to result from antigen stimulus produced by the embryo and the bacterial proteins respectively.

Neutrophils were observed to be associated with pyogenic infections. Acidophils were seen in only a few cases, and in small numbers. No relationship was found between their occurrence and the estrus cycle, pregnancy or bacterial infections. Mast cells were observed in 34.2% of the cases, but exactly what is their role in allergic and anaphylactic reactions. There was no correlation with their infiltration and the estrus cycle.

Monocytes and histiocytes were directly associated with phagocytic action and clearing up of infection from the uterus. Histiocytes were observed in maximum numbers in the post-partum cows, showing at about 10 days post-partum and increasing up to 35 to 40 days, hence they show a tendency to decline and mostly becoming free by or after 60 days. Their cycle runs parallel to the microorganism occurrence and disappearance, indicating that they play an important role in clearance and removal of foreign matter of the uterus after parturition. Plasmocytes were seen only in a few pathological cases.

An increased incidence of endometrial gland modifications were observed in the repeat breeder cows, which was either in the form of periglandular encapsulation or dilated lumen. Mostly these two forms were seen separately, but a few times were observed to exist



simultaneously as encapsulated and cystic glandular endometritis. A high incidence of glandular encapsulation with bacterial contamination was observed.

This study suggests endometritis, characterized by the above changes, as one of the probable causes of repeat breeding. The lesions described above along with endometritis are the persistent lesions in most of the infertile cows, but others may show a repaired condition by the time they go to slaughter. It is indicated that these changes are the result of chronic bacterial infections.