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The Oestrous Cycle of the Ewe; Histology of the Genital Tract

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ABSTRACT

Nine ewes were killed at known stages of the oestrous cycle. Their genital tracts were removed and sections fixed for the study of the cyclic changes of the mucosa of different regions of the tract. The histology of the vagina, uterus, and Fallopian tubes with reference to the oestrous cycle has been considered in detail. Some consideration also has been given to observations on the cervix and the fimbriated end of the Fallopian tubes.

Active proliferation of the vaginal epithelium occurred during metoestrum and dioestrum. Leucocytes were never entirely absent but were fewest in late oestrum and most abundant from late metoestrum throughout dioestrum. Some cornified layers were present at all stages; greatest amount of cornification and also desquamation occurred in late oestrum and the greater part of metoestrum.

Active proliferation of the uterine epithelium occurred during metoestrum. An intense leucocytic invasion of the epithelium occurred during metoestrum and early dioestrum. Vacuolization of the proximal ends and crowding of nuclei to distal ends of cells of the supra-cotyledonary epithelium was observed in early metoestrum. Secretory activity in the cervical glands, which was observed throughout the greater part of dioestrum, became especially marked in prooestrum.

Greatest height of epithelium of the Fallopian tubes was shown in late oestrum and throughout metoestrum. The cells of the connective tissue stroma of the folds were swollen and vacuolated during much of metoestrum. Cytoplasmic projections from the epithelial cells of the tubes were present in dioestrum but became taller and more numerous in prooestrum and early oestrum. Similar projections were present throughout the cycle in the fimbriated ends of the tubes. They were tallest and most numerous in that region during metoestrum.

In the ovaries, corpora lutea were largest in dioestrum and the mean diameter of the follicles increased during late oestrum and metoestrum. A tabular synopsis is given.

The Oestrous Cycle of the Ewe; Histology of the Genital Tract

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INTRODUCTION

Much attention has been paid to the phenomena connected with the oestrous cycle during the past decade and a half. As a result of the large amount of work which has been done in the field, a clearer understanding of the nature and mechanism of the oestrous cycle is coming forth. There is hope that eventually some measure of control may be obtained over the aberrations of the cycle and possibly, also, the normal course of events, therein.

A brief historical statement of the more recent development in the field indicates that rather limited phases of the problem have been attacked in a fairly definite order. It has been through such a systematic study by many individual workers that the understanding of the oestrous cycle has become what it is today.

Many outstanding contributions have been made regarding the morphological changes in the ovaries and the genital tract during the normal oestrous cycle in many different species. Notable among these outstanding contributions on laboratory animals might be mentioned the work of Stockard and Papanicolaou (1917) and Courrier (1923) on the guinea pig, Long and Evans (1922) on the rat, Allen (1922) on the mouse, Hartman (1923) on the opossum, and Evans and Cole (1931) on the dog.

Considerable attention has also been paid to working out the cyclic changes in the non-pregnant female of farm animals. Among the investigators who have worked with these animals might be mentioned Seaborn (1925) and Aitken (1927) working on the mare; Marshall (1903) working on the sheep; Hammond (1927), Murphey (1926), and Cole (1930) on the cow, and Corner (1921), McKenzie (1926), and Wilson (1926) on the sow.

With the increased knowledge of the normal changes during the oestrous cycle, there has been an increase also in the number of studies that have contributed greatly to an understanding of the regulatory mechanism of the cyclic changes. Extra-ovarian characteristics of oestrum were produced in ovariectomized mice and rats by means of ex-

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tracts of liquor folliculi by Allen and Doisy (1924). By means of this and other investigations it has been proved that the ovary is the controlling organ over the genital tract in producing the oestrous changes. The relation between the corpus luteum and the genital tract has been demonstrated by various workers, notably Hisaw and Leonard (1930) and Corner and Allen (1929).

Zondek and Aschheim (1927) and Smith and Engle (1927) proved that the function of the ovary was directly dependent on the anterior pituitary. The stimulation of the activity of the ovary and in turn activity in the genital tract were due to anterior pituitary hormones. Claus (1931) has separated extracts from the anterior pituitary into two fractions, indicating that there may be two anterior pituitary hormones, the one stimulating follicular development and the other causing luteinization of the follicles.

Many facts have been brought more recently into evidence regarding the reciprocal relationship between the anterior pituitary and the gonads by Moore and Price (1930), Meyer, Leonard, Hisaw, and Martin (1930), and Martins and Rocha (1931). Numerous others have contributed to the understanding of this general relationship.

Briefly, recent studies on the oestrous cycle might be recounted as the determination of: first, the changes in the normal oestrous cycle; second, the relationship of the ovary to the genital tract; third, the relationship of the anterior pituitary to the ovary; and fourth, the reciprocal relationship between the ovary and the anterior pituitary.

There are some fertility problems of especial economic significance which confront the sheep breeder. Ewes often fail to conceive when bred at the first oestrum of the breeding season. Furthermore, ewes of one flock seldom enter into the breeding season at a uniform time. Such occurrences are undesirable as early lambs of uniform age are usually the most profitable. The incentive for production of lambs to go on to the market earlier in the year has led some producers to try to find means of changing the time of the breeding season from fall to spring. Moving the flock from a plains region into the hills during early summer, changing the amount and character of the feed, and removal of all feed and water from the flock for a day or so are all methods which have been used in attempting to induce oestrum in an artificial manner.

This study represents a portion of a larger project having to do with the factors affecting fertility in the female domestic animal. It is first necessary to learn the normal conditions existing in the reproductive system of a female before the effect of any factor on fertility can be determined. Observations on the cyclic changes in the genital tract of a non-pregnant ewe should give evidence as to the normal sequence of events in preparation for a successful pregnancy in that animal. To this end a histological study has been undertaken.

The length of the oestrous cycle in the ewe has been cited as from a week to 30 days. Very few accurate observations appear to have been made. McKenzie and Phillips (1930) reported a study of 116 cycles in Hampshire, Shropshire, and Southdown breeds of sheep, in which they found the average length of cycle to be 16.6 days. Seventy-nine per cent of the cycles observed were found to be between 14 and 16 days. The duration of oestrum, as shown by the length of time that the ewes were receptive to teaser rams, was found to average 26.8 hours in a study of 247 periods. Seventy-four per cent of all of the periods were between 18 and 36 hours. The duration of oestrum varied significantly in different breeds of sheep and between ewe lambs and older ewes. There seemed to be no such variations in the length of the oestrous cycle.

Ovulation was not observed before 23¾ hours following the first appearance of oestrum by Allen, McKenzie, Kennedy, and Beare (1931). In their recovery of tubal ova from bred ewes, unsegmented ova were recovered between 23¾ and 50 hours following the beginning of oestrum. Cole and Miller (1932 a) observed, with Rambouillet ewes, that in some cases ovulation had occurred 25 hours after first acceptance of the ram. They appear not to have observed it before that stage.

Marshall (1903) has reported that the histological changes in the uterus of the sheep during the oestrous cycle relate primarily to the blood vessels. He described these changes as periods of rest, growth, destruction, and recuperation. The period of rest corresponded to dioestrum. The growth phase occurred in early prooestrum and was characterized by increase in size of the deeper blood vessels and increase in number of nuclei in the stroma immediately next the cotyledon. He described the increase in number of nuclei as being brought about by simple division without mitosis. The period of destruction occurred in late prooestrum or early oestrum. It was characterized by the breaking down of some of the capillaries in the superficial stroma and extravasation of blood into the stroma. The extravasated blood usually remained just beneath the epithelium and was thought to give rise to pigmentation during the recuperative phase. This last phase might begin in late oestrum but usually corresponded to metoestrum.

Cole and Miller (1931) (1932 b) have reported that in general the cyclic changes in the vaginal smear of the ewe are more apparent macroscopically than microscopically. There was a profuse flow of mucus accompanying oestrum, especially during the first half. The vaginal contents appeared to be of a cheesy consistency during metoestrum and dioestrum, changing from a rather dry to a more fluid form in the latter stage. The prooestrous smear was a scant amount of thick mucus.

Cornified cells were never entirely absent from the smear. Many leucocytes were present during the day before oestrum and the first day of oestrum. Large numbers of leucocytes were again present for about a day some time during the metoestrous period.

The report of Darlow and Hawkins (1931) with regard to the vaginal smear in the ewe appears to be in very close agreement with that of Cole and Miller.

MATERIAL AND METHODS

The data to be presented have been gathered from nine ewes. With the exception of one animal (No. 1251 from the University breeding flock), all were purchased on the Kansas City market and hence their previous history was unknown. They were brought to the University Farm approximately one month before any began coming into heat.

It is realized that from the standpoint of uniformity the animals used were not entirely satisfactory. However, the variations which existed in the group, such as age, breed, weight, and condition as to fatness, were believed not to affect greatly the type of physiological changes, the evidences of which were being studied and are here reported.

With the beginning of the sexual season, the ewes were taken off of bluegrass pasture and placed in dry lots. There they were fed alfalfa hay ad libitum and a grain ration of 1 pound per head per day, consisting largely of oats 3 parts and bran 1 part, by weight.

Table 1 shows the breeding of each animal as nearly as could be determined and also the approximate age. The weight together with a notation as to condition at the time of slaughter is given for each animal.

TABLE 1.—BREEDING, AGE, SLAUGHTER DATA

Ewe No.	Breed	Approx. Age, Yrs.	Weight at Death, Lbs.	Condition
1251	Shropshire, Pure Bred	4	170	Fat
45	Western Scrub	2	110	Medium
22	Hampshire, Grade	5	116	Thin to medium
25	Shropshire-Hampshire, Grade	5	164	Medium to fat
53	Shropshire-Southdown, Grade	3	130	Medium to fat
23	Hampshire, Grade	6	130	Medium
28	Hampshire, Grade	6	120	Medium
38	Hampshire, Grade	2	120	Thin to medium
35	Hampshire, Grade	2	138	Medium

The animals were kept under observation particularly as to the recurrence of oestrum. All animals were observed through two or more complete oestrous cycles before slaughter. Oestrus was determined by the willingness of the ewe to accept a ram. Teaser rams wearing aprons were used in making this determination. In some cases the teaser rams were painted so that they would mark the ewes upon mounting and were allowed to run with the ewes at all times. In other cases they were turned with the ewes only at the time observations were being made. Both practices were followed to a certain extent.

In general the observations for determining the length of the recurring oestrous cycles were carried on 4 times per day. The determinations made from such observations are given in Table 2. It may readily be seen that the determination of the length of each recurring cycle might be in error ½ to ¾ day. The determinations of the beginning of the last oestrum before slaughter for those animals killed at an early stage in the cycle, were based either on continuous observations or observations at 2-hour intervals.

TABLE 2.—RECENT OESTROUS HISTORY

Ewe No.	Length in Days of Cycles Observed Previous to Slaughter	Length of Time from Beginning of Last Oestrum until Slaughter
1251	17½; 16½; 17	1 hour
45	16; 16; 16¼	1 day (23 hours)
22	17½; 17¼	2 days (48 hours)
25	34½; 16¼; 16¼; 14¾; 15¾	4 days
53	10¾; 10¾; 32½; 7	5½ days
23	14¼; 14; 15½; 10½	7½ days
28	16½; 17½; 17; 16¼; 16¼; 17	9 days
38	16¼; 16½; 16; 15; 17	11 days
35	15½; 16¼	14 days

The nine animals were slaughtered at the end of intervals following the beginning of the last oestrum which are indicated in Table 2. The genitalia thus secured represent various successive stages of the oestrous cycle and are the material upon which this study is based.

Ewes 53, 38, and 35 had been operated on (laparotomy) approximately two months before the time of slaughter. At the time of operation, the ovary, Fallopian tube, the upper portion of the uterine horn, all on the same side, had been removed. It was observed at the time of slaughter that the animals had completely recovered from the operation.

The ewes were killed by bleeding to death after cutting the throat. The skin was removed from the belly and an opening made along the median-ventral line. The entire genital tract was then removed. Sections were removed from the tract for histological study as follows: 1. *Vagina*—section from the ventral wall at a point midway between the external urethral orifice and the external os of the cervix. 2. *Uterine horn*—cross section of the horn taken at the level of the apparent external bifurcation. 3. *Fallopian tube*—(a) Mid-region of the tube—cross section taken at a level mid-way between the tubo-uterine junction and the fimbriated end of the tube; (b) Fimbriated end of the Fallopian tube-entire fimbriated end. Sections were usually cut from a region approximately 1 mm. from the ovarian end. From 15 to 85 minutes elapsed from the time animals were stuck until the tissues were fixed. The body cavity was usually opened approximately 10 minutes after the animal was stuck. Bouin's fluid was used as a routine fixative. The tissues were dehydrated, embedded in paraffin, and sectioned (8-10 micra). Delafield's hematoxylin, Mayer's hemalum, orange G, and eosin were used for routine staining.

HISTOLOGICAL OBSERVATIONS

1. **Vagina.**—The epithelium of the vagina was of the stratified squamous type. Numerous epithelial buds jutted down into the underlying stroma and stromal papillae protruded into the overlying epithelium in the region between the buds. The height of these buds and papillae was rather variable, even in the same individual in different regions (epithelial buds, 220 to 525 micra; papillae, 80 to 400 micra).

The greatest average height of the papillae occurred in the 4-day, 5½-day, 7½-day, and 9-day stages (127-208 micra). However, there was great variation in the height of the papillae in these stages (Figure 1).

Congestion of the blood vessels of the upper stroma was difficult to estimate. It might be said, however, that in the 1-hour stage these blood vessels were greatly dilated and gorged with blood. Such an extreme condition was not noted at any other stage.

Edema, as measured by the density of the stroma, was greatest in the 1-day and 2-day stages; it was least in the 11-day, 14-day, and 1-hour stages. During the interval from the 4-day to 9-day stages inclusive an intermediate condition was noted.

Leucocytes were never entirely absent in any of the stages studied. The number present varied a great deal in different individuals and in different areas from sections from the same individual. Different areas of the stroma were more variable in the number of leucocytes present than different areas of the epithelium. In spite of the great variability it might safely be said that the smallest number of leucocytes in both the stroma and epithelium occurred in the 1-day stage (Figure 2). The stages when the largest number were present were not so definite. As best as it could be determined, the stages from 5½ days to 11 days showed the greatest number. The number in the stroma appeared to be greatest in the 9-day stage. There was some variation in the number in the epithelium during the period previously mentioned. Cells of the lymphocyte type appeared to predominate at all stages (Figure 1).

Cyclic changes in the height of the vaginal epithelium, if they occurred, appeared to be obscured by the variation in thickness of the lining epithelium in different regions of the same individual. This was true not only of measurements in micra but also in number of cell layers.

There was some evidence of cyclic changes in the average height of a single epithelial cell layer in the supra-papillary areas. This average height was greatest in the 11-day, 14-day, and 1-hour stages (10-11 micra).

Mitosis was observed most frequently in the stages from the second to eleventh day inclusive. It was most frequently observed in the second or third cell layer above the basement membrane. The place of cell division in most cases appeared to be parallel with the basement membrane (Figure 1).

Perhaps one of the most striking characteristics of sections from the animals in oestrum appeared in the germinal layer of the epithelium. There the nuclei were very much crowded, their long axes being parallel with each other and perpendicular to the basement membrane. This crowded condition showed up somewhat in the 14-day stage. It appeared to reach its maximum in the 1-day stage, when the nuclei elongated and were very slender. There was a slight crowding present in the 2-day stage, but thereafter the nuclei were more or less plump and tended to lie at all angles with the basement membrane (Figures 1 and 2).

Fairly clear-cut cyclic changes were observed in the staining affinity of the cytoplasm of the epithelial cells. The greatest affinity for acid stains appeared in the 14-day, 1-hour, and 1-day stages. There was a predominance of basic stain in the cytoplasm in the interval from the first to the fourteenth day. Varying amounts of acid tingeing occurred during the interval, but never enough to predominate. The staining reactions just described occurred in the cell layers above the germinal; the germinal layer seemed to be more nearly constant in the staining affinity. The nuclei of the epithelial cells had the greatest affinity for the basic stain from the 2-day stage to the 7½-day stage inclusive.

The physical appearance of the cytoplasm of the epithelial cells showed differences in the successive stages which would appear to be cyclic. The two extremes of appearance might be described as fibrillar on the one hand and globular on the other. The most distinctly fibrillar condition was noted in the 1-day stage. An increasing amount of the globular condition occurred in the lower layers above the germinal layer in the 2-day, 4-day, and 5½-day stages. This condition reached its height in the 7½-day stage when one-half to two-thirds of the layers just above the germinal showed large globules, especially near the cell boundaries. Following this the fibrillar condition began to be noted on the eleventh day and reached its height in late oestrum (Figures 1 and 2).

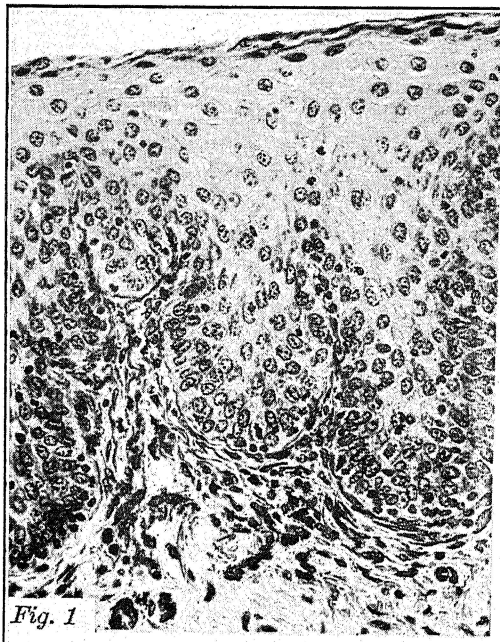
Fig. 1

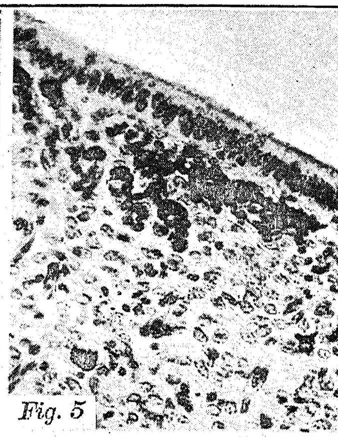
Fig. 5

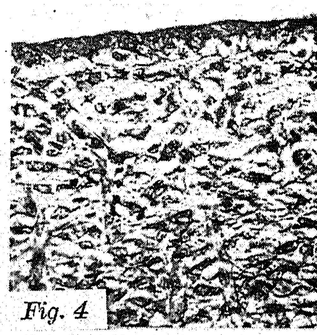
Fig. 4

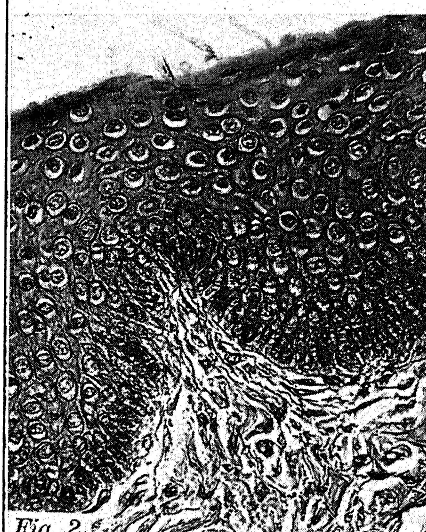
Fig. 2

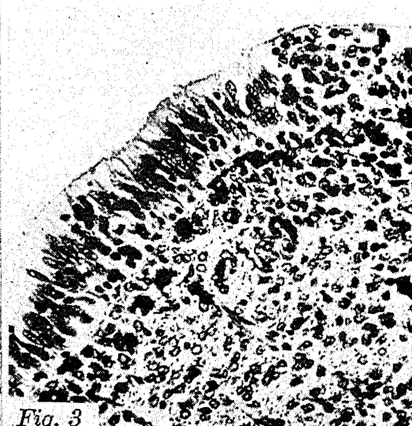
Fig. 3

PLATE I.—FIGURES 1 TO 5, INCLUSIVE
(See Opposite Page)

Figure 1.—Section of the vagina cut at 10 micra. From No. 23, 7½ days since beginning of last oestrum. Mayer's hemalum and Orange G, x 300. Note presence of leucocytes scattered in stroma and epithelium; rather high stromal papillae; clear definition of cell membranes; rather globular appearance of the cytoplasm of the cells; lack of crowding of the nuclei in the germinal layer; mitotic figures in the lower regions of each of two epithelial buds; three cornified layers; fewness in number and small size of the vacuoles surrounding the nuclei.

Figure 2.—Section of vagina cut at 10 micra. From No. 45, 1 day since beginning of oestrum. Mayer's hemalum and Orange G, x 300. Note fewness of leucocytes in both epithelium and stroma; rather low stromal papillae; lack of definition of cell membranes; rather fibrillar appearance of the cytoplasm of the cells; extreme crowding of the nuclei in the germinal layer; absence of mitosis; large number and large size of the vacuoles surrounding the nuclei. From 4 to 6 cornified layers are present, but cannot be distinguished in the photograph.

Figure 3.—Section of uterine horn showing cotyledon, cut at 10 micra. From No. 23, 7½ days since beginning of last oestrum. Mayer's hemalum and Orange G, x 300. Note the tall columnar epithelium; the large number of leucocytes in the damaged epithelium.

Figure 4.—Section of uterine horn showing cotyledon, cut at 10 micra. From No. 22, 2 days since beginning of last oestrum. Mayer's hemalum and Orange G, x 300. Note the low epithelium; the vacuolization of the proximal ends of the epithelial cells and the crowding of the nuclei to the distal ends; the prominence of the blood vessels and the edema of the stroma.

Figure 5.—Section of uterine horn showing cotyledon, cut at 10 micra. From No. 28, 9 days since beginning of last oestrum. Mayer's hemalum and Orange G, x 300. Note the very low simple columnar epithelium with the large nuclei; fewness of leucocytes. The large black masses just below the epithelium are pigment.

The clearness of definition of the epithelial cell boundaries was most marked from the 4-day to 11-day stages. In the 14-day and 1-hour stages the boundaries were clearly defined only in scattered regions. For the most part the boundaries were not visible in the 1-day stage; they were clearly defined only in scattered regions in the 2-day stage (Figures 1 and 2).

Another condition which was never entirely absent at any stage was a form of vacuolar degeneration. The nuclei of the cells involved became more or less shrunken, while the cytoplasm almost disappeared, leaving a clear vacuolar space of varying size surrounding the nucleus. The staining affinity of the nucleus of such cells appeared to be altered only when the nucleus was extremely shrunken. A definite cyclic change in the amount of vacuolar degeneration was not entirely apparent. The greatest number and the largest vacuoles occurred in the 1-day stage; the smallest number and the smallest vacuoles were in the 9-day stage. Varying numbers and varying sizes of vacuoles appeared in the animals slaughtered at stages intermediate to the two mentioned. In no case was the size and number so great as in the 1-day stage nor so small as in the 9-day stage (Figures 1 and 2).

An extreme amount of cornification never appeared in the sections from any of these animals; neither was cornification ever entirely absent from the picture. The greatest amount of cornification appeared in the 1-day, 2-day, and 4-day stages; there were from four to six cornified layers in these stages. Three to four cornified layers were present in the 5½-day stage. One to four cornified layers were present from the 7½- to 14-day stages inclusive. In the 1-hour stage there were areas in which no cornified layers were present. In other areas as many as three cornified layers were present and one to two such layers prevailed for the most part (Figures 1 and 2).

Desquamation was most pronounced in the 1-day, 2-day, and 4-day stages. In the 2-day stage it appeared to be occurring over practically all of the surface, at least as far as the sections observed were concerned. In stages other than these three, occasional individual cells were seen to be desquamating.

2. **Uterus.**—The epithelial lining of the uterus was typically simple columnar. Very often, however, the presence of two to three rows of nuclei gave the epithelium a pseudostratified appearance. The basement membrane was usually fairly well defined. Coiled tubular glands which were branched in some instances passed far down into the underlying stroma. These glands opened onto the surface only in the inter-cotyledonary areas. Deeper portions of the glands were found below the cotyledons, but they did not pass up through the cotyledons. The glandular epithelium greatly resembled that covering the inter-cotyledonary areas. It averaged about the same in height as the surface epithelium in the upper portions of the glands but was somewhat lower in the deeper portions.

The stroma of the cotyledons was much more densely cellular than that of the inter-cotyledonary regions. Large numbers of very small blood vessels were seen passing up through the cotyledonary stroma. The number of cells in the inter-cotyledonary stroma was usually much greater just below the epithelium than in the deeper regions. The number in the upper regions often approached, but seldom equalled that in the cotyledonary regions.

Variable amounts of pigment were present just below the epithelium. When present it was usually much more abundant just below the supra-cotyledonary epithelium than below the inter-cotyledonary epithelium. From observations of non-pregnant uteri from several breeds of sheep, it seemed possible that there was a breed difference in the amount of pigment present.

The uterine stroma showed the least edema in the 9-day and 11-day stages. There was then an increase in the amount of edema in the 14-day, 1-hour, and 1-day stages. By far the greatest amount of edema occurred in the 2-day stage. There was then a decreased amount in the 4-day, 5½-day, and 7½-day stages.

There was a marked congestion of the larger blood vessels in the lower regions of the stroma near the muscular layers in the 2-day and 4-day stages. Some blood could always be detected in the lower blood vessels in the other stages, but the vessels were as a rule less distended and showing no signs of congestion. Much more blood could be seen in the capillaries of the cotyledons in the 2-day stage than in any other stage.

The average height of the inter-cotyledonary and glandular epithelia did not show definite cyclic changes. There were great variations in the average height of the epithelium in different individuals, but there was also extreme variation in the height of the epithelium in different areas of a section from a single individual. The latter variation amounted to as much as 6-19 micra. Likewise, the external and internal diameters of the glands did not show definite cyclic changes.

There did, however, appear to be some increase in the amount of coiling and branching of the glands during certain successive stages, namely, 5½-day, 7½-day, and 9-day stages. This condition was judged by the appearance of a larger number of cross sections of glands in a given field of the stroma and also by the presence of a larger number of cross-sections of branched glands.

Leucocytes, chiefly of the lymphocyte type, seemed to be always present, both in the epithelium and the stroma. Fewer leucocytes were present in the 1-hour and 1-day stages than in any other. Large numbers of leucocytes were invading the epithelium in the 5½-day and 7½-day stages. They were often present in large clumps in the more damaged portions of the epithelium (Figure 3).

The changes in the epithelium covering the cotyledons were somewhat different than in the inter-cotyledonary epithelium. No section through a cotyledon was studied for the 1-hour stage. In the 1-day stage, the epithelium was of the simple columnar type and rather tall (28-31 micra). The nuclei were very large. The epithelium was rather low columnar, almost cuboidal in the 2-day stage, the typical height being 11-17 micra. The proximal ends of the cells were very much vacuolated and the nuclei seemed to be crowded to the distal ends of the cells (Figure 4). There was a slight increase in height of the cells in the 4-day stage, being 17-22 micra. The proximal ends of the cells were very much vacuolated and the nuclei seemed to be crowded to the distal ends of the cells (Figure 4). There was a slight increase in height of the cells in the 4-day stage, the height being 17-32 micra. The epithelium was simple columnar and the nuclei were very large, appearing to occupy the greater part of the interior of the cells. In the two succeeding stages,

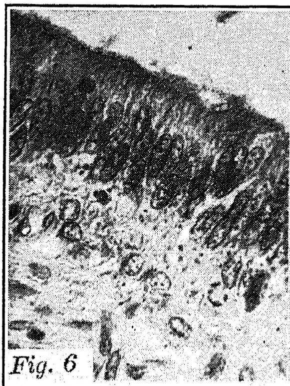
Fig. 6

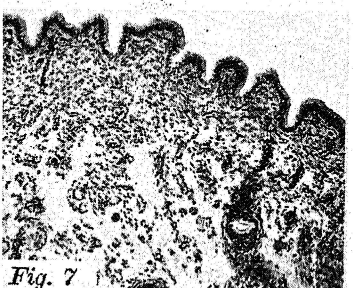
Fig. 7


Fig. 8


Fig. 9


Fig. 10

PLATE II.—FIGURES 6 TO 10, INCLUSIVE

(See Opposite Page)

Figure 6.—Section of uterine horn, cut at 10 micra. From No. 45, 1 day after the beginning of last oestrum. Delafield's hematoxylin and eosin, x 410. Note the irregular dark staining granules in the epithelium just above the basement membrane; also, the nucleus near the basement membrane in which the chromatin is just breaking up into granules (karyorrhexis).

Figure 7.—Section of uterine horn, cut at 10 micra. From No. 28, 9 days since beginning of last oestrum. Mayer's hemalum and Orange G, x 68. Note the folding of the epithelial surface. No cotyledon appears in this section.

Figure 8.—Section through the Fallopian tube, cut at 10 micra. From No. 25, 4 days since the beginning of last oestrum. Mayer's hemalum and Orange G, x 300 Note the long prominent cilia on the high epithelial cells; large size and vacuolization of the cells of the connective tissue stroma.

Figure 9.—Section through the Fallopian tube, cut at 10 micra. From No. 35, 14 days since the beginning of last oestrum. Mayer's hemalum and Orange G, x 300. Note the low epithelium and cytoplasmic projections with dark staining nuclei extending into them on the surface of the epithelium.

Figure 10.—Section through the Fallopian tube, cut at 10 micra. From No. 1251, 1 hour after the beginning of oestrum. Mayer's hemalum and Orange G, x 300 Note the rather high cytoplasmic projections from the surface of the epithelium.

5½-day and 7½-day, the cells again became very tall (39-53 micra). While the nuclei were rather elongated and small in both stages, they were arranged so as to give a pseudo-stratified appearance in the former stage and a rather uniformly single layer in the latter. Leucocytes were present in both stages, but in the latter the epithelium was becoming seriously damaged (Figure 3). The cells were again arranged in low simple columnar fashion in the 9-day stage and they had very plump nuclei (Figure 5). The cells were 22-28 micra in height. Tall epithelium (28-39 micra) showing an increasing amount of pseudo-stratification was present in the 11-day and 14-day stages. The nuclei were somewhat elongated in shape and medium sized in these stages.

A tendency toward vacuolization was present in the distal ends of the epithelial cells in the 14-day stage. A slight amount of this appeared in the 11-day stage and was yet present in amounts diminishing from the maximum in the 1-hour, 1-day, and 2-day stages. No such tendency was evident in the remaining stages.

A goodly number of nuclei showed evidence of degeneration in the 1-hour, 1-day, and 2-day stages. The chromatin seemed to have broken up into irregular, deep-staining particles. This regressive change might probably be described as karyorrhexis. The condition was most prevalent among the nuclei lying in the region next to and immediately above the basement membrane. The 1-day stage showed a greater amount of this type of degeneration than the other two stages named (Figure 6).

Several mitotic figures were observed in the 2-day, 4-day, and 5½-day stages. None was observed in other stages. These figures were usually located in the distal ends of the cells, well above the general level of the nuclei in the epithelium.

The epithelial surface was considerably folded in the 9-day and 11-day stages. In cross sections this gave a rather grossly corrugated effect (Figure 7).

Observations of sections from the mid-region of the cervix showed there was a secretory phase to the cyclic changes in that organ. This phase seemed to reach its height in the 14-day stage. Some secretory activity was evident in the 9-day stage, and there was rather intense secretory activity in the 11-day stage. There was very little secretion evident in the other stages observed.

3. **Fallopian Tube.**—(*a*) *Mid-region of the tube.*—The mucosa of the Fallopian tube was thrown into thin longitudinal folds or plicae. These were usually more or less branched. Each fold contained a certain amount of cellular connective tissue in its center as it appeared in cross section (stroma). The epithelium was chiefly columnar and ciliated. The pseudo-stratified condition was often noted. True glands did not occur in the Fallopian tube.

Definite cyclic changes appeared to take place in the height of the tubal epithelium. During the period represented by the 1-day to the 5½-day stages, the increased height of the epithelium was apparent. The peak in height existed in the 2-day and 4-day stages. In those stages the height typically varied from 25 to 36 micra. Succeeding stages showed a decreased height until on the 14th day; 14-22 micra represented the typical height. This was followed by some increase in the 1-hour stage.

The cilia on the epithelial cells appeared to be more prominent and to be much longer in the 4-day stage than in any other. Attempts at measuring their length in this stage showed them to be from 8 to 14 micra, whereas, in other stages they were 6 to 9 micra for the most part (Figure 8).

The connective tissue in the plicae appeared to be very much increased in amount in the 4-day and the 5½-day stages. This observation was confirmed somewhat by actual measurements taken of the width of the stromal areas. In these stages many of such measurements were 60-140 micra. In the other stages, none was greater than 50 micra and the majority were 30 micra or less (Figure 8).

The individual cells of the connective tissue appeared to be very much swollen and vacuolated. This condition probably might be referred to as hydropic degeneration.

The phenomena of cytoplastic projections were observable on the tubal epithelium during certain successive stages. Cytoplasmic projections were observed in the 9-day, 11-day, 14-day, and 1-hour stages.

The number of such projections was much greater in the last two named stages than in the first two. Deeply staining nuclei were usually seen extending into the cytoplasmic projections in the first three stages named. With the staining technique used, very few nuclei could be detected in the projections in the 1-hour stage. The cytoplasmic projections were very tall (8-14 micra) as compared to the three preceding stages (4-8 micra). With the increased length of the projections, there was a tendency for them to become pedunculated and only a slender stalk could be seen connecting the body of the projection with the distal end of the cell. These projections at first inspection often appeared as globules lying free on the epithelial surface. It was impossible to distinguish whether these projections arose from ciliated or non-ciliated cells. The number of cytoplasmic projections in the 1-hour stage appeared to be much larger than the number of non-ciliated cells which could be distinguished in other stages (Figures 9 and 10).

The clearest definition of cell membranes occurred in the 2-day to 7½-day stages inclusive. There was especially clear definition in the 4-day stage. In the remaining stages the cell membranes were more or less obscure, the greatest obscurity occurring in the stages where there were cytoplasmic projections.

(*b*) *Fimbriated end of the tube.*—The fimbriated ends of the Fallopian tubes were not obtained from the animals killed in the 1-hour stage and the 5½-day stage. The series then included only the seven remaining stages.

The greatest height of epithelium at this level was shown in the 1-day, 2-day, and 4-day stages. The typical height in these stages varied from 28 to 36 micra. The typical height in the 7½-day, 9-day, and 11-day stages varied from 22 to 31 micra, whereas, in the 14-day stage it was 19 to 22 micra.

Cytoplasmic projections were present in variable numbers throughout the cycle. The greatest number and the greatest height of cytoplasmic projections appeared in the 4-day and 5½-day stages. Their height in those stages was typically 6 to 11 micra. Although the number of cytoplasmic projections varied in the other stages, they never appeared so numerous as in the two previously mentioned stages and in general their height was lower (3 to 8 micra).

Many of the cytoplasmic projections in the 4-day stage were found to contain nuclei. The number of projections showing nuclei in the 7½-day, 9-day, 11-day, and 14-day stages was variable but in general they seemed much fewer than in the 4-day stage. Very few if any nuclei appeared to be present in the cytoplasmic projections in the 1-day and 2-day stages.

THE OVARIES

Data for this section were furnished by Dr. Mary J. Guthrie and Miss Virgene Warbritton of the Department of Zoology. The observations were made upon the ovaries of the same animals whose genital tracts were considered in the preceding sections. A later publication will deal with the ovaries in detail.

A cyclic change was demonstrated in both size and color of corpora lutea. A correlation did not appear to exist between the size of the follicles and the apparent oestrous cycle (Table 3).

TABLE 3.—CYCLIC CHANGES IN THE OVARIES

	Corpora Lutea				Follicles	
	Present Cycle		Previous Cycle		Size in mm.	
Stage	Size*	Color	Size*	Color	3 largest	Mean D.**
1-hour			405 700	Opaque pink	8, 4, 4	3.32
1-day	64	Blood red	504	Cream	5, 5, 3	2.73
2-day	72 101	Blood red	252 210	Cream	4, 4, 4	3.03
4-day	200 422	Translucent pink	125 101	Cream	5, 5, 5	3.37
5½-day	720	Translucent pink	20	Cream	7, 4, 3	3.68
7½-day	1188 810	Pink Deep pink	10 12	Cream	8, 7, 5	3.67
9-day	1200	Pink	100	Yellow	6, 5, 4	3.48
11-day	560	Pink	27 27	Yellow	7, 7, 2	5.93
14-day	891	Opaque pink			5, 5, 3	3.53

*Product of three dimensions in millimeters.
**Mean diameter is calculated on follicles 2 mm. or larger and equals the cube root of the average of cubes of diameters of individual follicles.

The corpora lutea reached their maximum size at about the middle of the cycle of their formation. A decrease in size followed during the remainder of that cycle and continued through the succeeding cycle. Occasional vestiges of very old corpora lutea two or more cycles in age were found. The color changes graded from a blood red in very young corpora through translucent pink, opaque pink, cream, and finally yellow in old corpora.

There was evidence of a gradual increase in mean diameter of the follicles 2 millimeters or larger during the first one-third of the cycle.

Following that time there was an irregular but fairly well sustained mean diameter. There were follicles just as large at approximately the mid-period of the cycle as there were just prior to ovulation.

TABLE 4.—TABULAR SYNOPSIS

	Oestrus		Metoestrus				Dioestrus		Prooestrus
	1 hour	1 day	2 days	4 days	5½ days	7½ days	9 days	11 days	14 days
VAGINA									
Epithelium									
Suprapapillary cell layer	Highest							---Highest---	
Mitosis				-----------Most frequent-----------					
Germinal layer nuclei	-------Crowded-------								Crowded
Staining affinity	---Acid---		---------Predominately basic---------						Acid
Appearance of cytoplasm	-Fibrillar-		---Increasing globular---				-Increasing fibrillar-		
Cell membranes				-------Clearest definition-----					
Vacuolar degeneration		Most				Least			
Cornification		-------Most-------				----------Least----------			
Desquamation		-------Most-------							
Leucocytes		Few			----------Most----------				
Stroma									
Papillae				--------Highest---------					
Congestion of blood vessels	Most								
Edema		---Most----							
UTERUS									
Epithelium									
Mitosis			---Most frequent---						
Leucocytes	---Fewest---				---Most----				
Vacuolization	----Decreasing----							--Increasing--	
Nuclear fragmentation	-----Present------								
Cotyledonary epithelium		Tall	Low Vacuolated	Low	---Tall---- -Leucocytes-		Low	---Tall----	
Inter-cotyledonary epithelium							--Folded--		
Stroma									
Edema			Most				---Least---		
Congestion of blood vessels			---Most---						
Coiling & branching of glands					-------Most-------				
Cervical glands							-Increased secretion-		
FALLOPIAN TUBE									
Mid-region									
Epithelium		---------Highest---------							
Cell membranes		------Clearest definition------							
Cytoplasmic projections	Highest						-----Present------		
Stroma of folds				--Swollen--					
Fimbriated end									
Epithelium		-----Highest------							
No.& ht. of cytoplasmic proj.				-Greatest--					
OVARY									
Corpora lutea		Blood red				--Largest--			Opaque pink
Follicles-Mean D		-------Increasing-------							

DISCUSSION OF DATA

Any division of the stages which were studied into the conventional phases of the oestrous cycle is of necessity more or less arbitrary. The ewes slaughtered in the 1-hour stage and the 1-day stage were definitely receptive to the ram and therefore in oestrum. For purposes of discussion, the 2-day, 4-day, and 5½-day stages are considered as in metoestrum; 7½-day, 9-day, and 11-day stages in dioestrum; 14-day stage in prooestrum.

A consideration of the histological changes in the genital tract in relation to the vaginal smear of the ewe as reported by Cole and Miller (1931) (1932b) is of considerable interest. Cornified cells were never completely absent from the smear. Some cornification in the superficial layers of the vaginal epithelium, likewise, never was entirely absent. While desquamation was most pronounced in late oestrum and in the greater part of metoestrum, slight amounts were observed in all other stages.

Polymorphonuclear leucocytes were reported in the vaginal smear. Only a very few such cells were ever noted in the histological sections; lymphocytes were present for the most part. There was a reduction of the number of leucocytes in the vaginal smear during the last half of oestrum. This is in accord with the small number of leucocytes observed in the sections during late oestrum. The reported presence of leucocytes in the smear for about a day some time between the second and seventh day metoestrum and the frequent encountering of smears similar to metoestrous smears during dioestrum may be in accord with observed variations of numbers of leucocytes in different individuals and in different areas in sections from the same individual during late metoestrum and early dioestrum.

The levels of the genital tract which were studied histologically do not throw a great deal of light on the inflow of mucus which was observed in the smears during late prooestrum and early oestrum. It may be, however, that the secretory activity which was noted in the mid-region of the cervix during much of dioestrum and which reached its height in prooestrum, may be the source of this mucous flow. Such an explanation would be based on a certain amount of time being required for the mucous flow to reach the regions studied in the vaginal smear.

The breaking down of some of the capillaries in the superficial stroma and extravasation of blood into the stroma, as noted by Marshall (1903) was not generally observed in this study. In a very few isolated instances, small numbers of red blood cells were identified in the stroma but no significance was attached to them.

Certain of the cyclic changes noted at the different levels of the genital tract are similar to changes reported in other species by other investigators. The formation of vacuoles surrounding the nuclei in the epithelium of the vagina is similar to the condition noted by Wilson (1926) in the sow. The end-product of such vacuolization seemed to be more apparent in the sow, however, than was observed in the ewe. Although vacuoles of varying size and number were present in sections from all individual ewes studied, there was no evidence that they played any such part in cell removal as was observed by Wilson in the sow. His observation was that these vacuoles coalesced to form larger vacuoles containing remnants of degenerating cells. The larger vacuoles came to lie near the surface, their linings eroding eventually and their contents escaping. Only in one or two isolated instances was anything similar to this process observed in the ewe. In these cases some vacuoles may have coalesced to form a large vacuole deep down in the center of a large epithelial bud. In no case did they appear to be coming to the surface and eroding.

The greater affinity of the cytoplasm of the epithelial cells lining the vagina for the acid stains at the time of oestrum has been noted in other species. Evans and Cole (1931) stated that by the fourth to the sixth day of oestrum in the dog, the entire epithelium, with the exception of four or five basal layers, took the eosin stain deeply, although there was still a marked distinction between the most superficial true cornified cells and those beneath them. It was noted in the sow also by McKenzie (1926) that in oestrum, and soon after, the epithelial cells took more of the eosin. This was in contrast to the time just before oestrum when the more superficial cells took the blue hematoxylin.

Very few leucocytes were present in the vaginal epithelium of the ewe in late oestrum. The absence of leucocytes in the vaginal epithelium at the time of oestrum has been pointed out by Long and Evans (1922) in the rat, Allen (1922) in the mouse, Hartman (1923) in the opossum, Wilson (1926) in the sow, and is implied by Evans and Cole (1931) in the dog. Leucocytic invasion began in late oestrum in the guinea pig as pointed out by Stockard and Papanicolaou (1917). Cole (1930) reported that the leucocytic infiltration was more intense in some areas of the vagina of the cow than in others. An intense infiltration of leucocytes and lymphocytes occurred in the region just anterior to the external urethral orifice at the time of oestrum, whereas the number in the area just posterior to the cervix was small and did not present marked fluctuations at different periods of the cycle.

Many polymorphonuclear leucocytes have been reported in the leucocytic invasions in the above mentioned species. This condition is different from that observed in the ewe in which the great majority of the cells were the small mononuclear lymphocytes.

A distinct cornified layer has been described as present during oestrum in the guinea pig, rat, mouse, opossum, and dog. No actual cornification was observed in the sow. A very low epithelium was described in the cow at the time of oestrum and the superficial layer consisted of mucus secreting cells. One or two cornified layers were present in the ewe in early oestrum and this had increased to four to six layers in late oestrum.

The folding of the epithelial surface of the uterus during dioestrum which was noted in the ewe is similar to the condition observed in the uterus of the sow by McKenzie (1926). He described the epithelial surface as appearing quite hillocky during dioestrum.

Coiling and branching of the uterine glands in the ewe in late metoestrum and early dioestrum is somewhat similar to, although probably not as marked as a condition described in some other species. Cole (1930) described glandular hypertrophy as becoming very marked in the cow at eight to eleven days postoestrum. A maximum degree of complexity of the uterine glands was also observed as present in the dog from the eighth to the fourteenth day of metoestrum by Evans and Cole (1931). An extreme condition of the uterine glandular development was described and pictured by Hammond (1925) as occurring in the rabbit during pseudo pregnancy.

In general the cyclic changes in the height of the tubal epithelium of the ewe are very much like the changes in the sow. Snyder (1923) reported for the latter animal that the epithelium was more than twice as high one to three days after ovulation than during the second week after ovulation. The greatest height in the ewe was observed in late oestrum and in metoestrum. As was pointed out by Snyder, this period represents the time when the egg is passing through the tube.

Snyder further noted that the fluid content of the connective tissue stroma of the tubes in the sow appeared to be altered periodically. The stroma was most swollen and edematous when the epithelium was high. A condition similar to this was observed in the ewe. The connective tissue stroma appeared very much swollen at about the time the epithelium was the highest. There may be a difference in the two cases, however, as the edema in the tube of the ewe appeared to be mostly intracellular. The individual cells were very much swollen and vacuolated.

Cytoplasmic projections were described by Snyder as occurring in the epithelium of the sow's tube. They made their appearance as the epithelium became lower during the second week after ovulation and began to disappear during the third week after ovulation. Their disappearance did not seem to have been complete until the time of ovulation. He further observed that the length of the projections varied inversely with the height of the epithelium.

In general the time of appearance of the cytoplasmic projections in relation to the height of the epithelium in the ewe correspond to that observed in the sow. The projections were not present when the epithelium was the highest. The inverse relationship between the height of the projections and the height of the epithelium did not appear to hold in the ewe. The highest projections appeared at a time when there had been some increase in the height of the epithelium.

Allen (1922) reported nuclear extrusions from the epithelial cells of the tube of a mouse. This process reached its height during the late metoestrous and early dioestrous stages.

Snyder (1924) observed cytoplasmic projections from the epithelium of the human tube. Such a condition was present in the premenstrual stage. The greatest height of epithelium existed in the intermenstrual stage.

Among others, Courrier (1924) and Bourg (1931) appeared to refer to cytoplasmic projections and nuclear extrusions as "intercalary cells". They suggested that this phenomenon might in reality be a holocrine type of secretion.

Although the preparations of the sheep tubes did not give evidence as to the functions of these projections or extrusions, it seems that the suggestion of a holocrine type of secretion, i. e., a means of cell removal, is as reasonable as any. It is interesting to note that these projections were at about their maximum development in early oestrum when insemination would occur in the natural state and sperm would be passing up through the tract.

SUMMARY

Nine ewes were killed at known stages of the oestrous cycle. Their genital tracts were removed and sections fixed for the study of the cyclic changes in the mucosa of different regions of the tract.

A. Changes in the mucosa of the vagina:

1. Greatest height of papillae in late metoestrum and early dioestrum.

2. Greatest edema present in late oestrum and early metoestrum; least edema in late dioestrum and prooestrum.

3. Leucocytes never entirely absent. Fewest number present in late oestrum; greatest number present from late metoestrum throughout dioestrum.

4. Greatest average height of a single epithelial cell layer in the supra-papillary areas from late dioestrum to early oestrum.

5. Cell division most frequent in metoestrum and dioestrum.

6. Crowding of the nuclei in the germinal layer in prooestrum and oestrum.

7. Greatest affinity of the cytoplasm of the epithelial cells for acid stains during prooestrum and oestrum.

8. Greatest affinity of the nuclei of the epithelial cells for the basic stains during metoestrum and early dioestrum.

9. Fibrillar appearance of the cytoplasm of the epithelial cells from late dioestrum up to and including oestrum.

10. Globular appearance of the cytoplasm during metoestrum and large part of dioestrum.

11. Clearest definition of epithelial cell boundaries during latter part of metoestrum and dioestrum.

12. Presence at all times of a certain amount of vacuolization surrounding the nuclei of the epithelial cells in certain regions; greatest number and largest vacuoles during oestrum; fewest number and smallest vacuoles during dioestrum.

13. Some cornified layers present at all stages. Greatest amount of cornification in late oestrum and greater part of metoestrum; desquamation most pronounced in the same stages.

B. Changes in the mucosa of the uterus:

1. Greatest edema of uterine stroma in early metoestrum; least edema in dioestrum.

2. Greatest congestion in deeper blood vessels of uterine stroma during metoestrum.

3. Coiling and branching of uterine glands in late metoestrum and large part of dioestrum.

4. Leucocytic invasion in epithelium in late metoestrum and early dioestrum.

5. Cell division most frequent during metoestrum.

6. Folding of the epithelial surface in large part of dioestrum.

7. Karyorrhexis of some of the nuclei just above the basement membrane of the epithelium during oestrum and early metoestrum.

8. Tendency toward vacuolization of the distal ends of the epithelial cells from late dioestrum to early metoestrum, most pronounced in prooestrum.

9. Successive changes in epithelium covering the cotyledons: tall columnar during oestrum; low columnar in early metoestrum with vacuolization of proximal ends and crowding of nuclei to distal ends of cells; followed by normally appearing low columnar; again tall columnar in late metoestrum and early dioestrum; invasion of leucocytes in early dioestrum after which the epithelium is again low columnar; again tall columnar in late dioestrum and prooestrum.

10. Secretory activity in the cervical glands throughout the greater part of dioestrum and especially marked in prooestrum.

C. Changes in the mucosa of the Fallopian tube:

1. Greatest height of epithelium in late oestrum and throughout metoestrum.

2. Longest and most prominent cilia in the middle of metoestrum.

3. Cells of the connective tissue stroma of the folds swollen and vacuolated throughout greater part of metoestrum.

4. Cytoplasmic projections from epithelial cells throughout most of dioestrum, in prooestrum, and in early oestrum. Projections highest in early oestrum.

5. Clearest definition of cell boundaries in metoestrum and early dioestrum.

6. Greatest height of epithelium in fimbriated end of Fallopian tube in late oestrum and metoestrum.

7. Cytoplasmic projections from epithelial cells of fimbrated end present throughout cycle in variable numbers. Greatest number and greatest height of projections present throughout greater part of metoestrum.

D. Changes in the ovary:

1. Corpora lutea of maximum size encountered at about the middle of the cycle of their formation.

2. Color changes in the corpora lutea graded from a blood red to opaque pink during the cycle of their formation and from an opaque pink to a yellow during the succeeding cycle.

3. The mean diameter of the follicles 2 millimeters or larger appeared to increase during the first one-third of the cycle.

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