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# Histomorphological changes in the tubular genitalia of the sow (Sus scrofa domesticus) as influenced by age

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# HISTOMORPHOLOGICAL CHANGES IN THE TUBULAR GENITALIA OF THE SOW (SUS SCROFA DOMESTICUS) AS INFLUENCED BY AGE

by

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# INTRODUCTION

The structure of normal tissues and organs of domesticated animals has long been of prime interest to a Veterinary Anatomist. It is very difficult to give a definition of "normal" to a tissue structure and form or to differentiate between the so-called normal tissue from tissue which might appear pathological unless studies of such tissues from birth to senility are made. Growth of an organism begins immediately after fertilization and is a continuous dynamic process. The periods of growth can be subdivided into Prenatal and Postnatal periods. The Prenatal growth period extends from the time of fertilization or conception till birth and is defined as the period of gestation. Growth after birth passes through the stages of maturity, followed by senility and terminates in death. During this life cycle of an animal, tissues and organs cannot be expected to assume a static morphology when the whole organism is continuously being subjected to physiological stresses and strains.

In order to study the normal tissues and organs of a few domestic animals, a comprehensive gerontologic program was envisioned and instigated by Dr. R. Getty, Head of the Department of Veterinary Anatomy, at the Iowa State University, Ames, Iowa. Since the year 1957, a colony of purebred beagles has been maintained by the Department of Veterinary Anatomy and purebred hogs from the Iowa State University Farm have also been raised and sacrificed at pre-determined time intervals. The genetic history, diet records, time of whelping or farrowing, number in litter, etc. have been known and this information is filed in the Department. According to Getty

(1963) one cannot appreciate those changes that take place from birth to senility correctly and completely, the so-called pathologic or physiologic changes, unless studied scientifically in chronological order.

To date, morphological and physiologic changes, as influenced by age, in tissues have been studied mainly in man and some laboratory animals. Information is lacking in current literature on the tissue changes brought about by senescence in the domestic animals. The current revised editions of histology textbooks report the comprehensive form and structure of the tissues and organs but do not mention any age period to which these morphological descriptions relate.

First reports on the age changes on the reproductive tract of the sow are furnished on the ovary by Hadek and Getty (1959a)and on the uterus by the same authors (1959b).

The present studies on morphological changes in the uterine tubes, uterus, cervix and vagina of the sow as influenced by age have been undertaken as one of the gerontologic projects in this department. The breeding of the domestic pig (Sus scrofa domesticus) is a profitable and a rewarding enterprize for a farmer. It is hoped that the present studies might reveal more information on the functional nature of the genital tract, regarding the most productive period or age interval and also the age period of decline in reproduction. Significant morphological changes are expected from birth till the age of puberty in the tubular genitalia of the sow. This is the most important growth period. At the onset of puberty cyclic changes in the epithelium, tunica mucosa (endometrium in the uterus) and tunica muscularis (myometrium in the uterus) are a natural phenomenon influenced by gonadotrophic hormones of the adenohypophysis and the ovary. Changes in the vasculature of the tubular genitalia regarding their morphological characteristics have been observed. The changes so far reported in the arteries of the domestic pig by French et al (1965), Lüginbuhl (1965), and Getty (1965a) appear to be identical to those seen in the arteries of man. It is hoped that the information derived from the present investigation will add further to the application of comparative studies of this nature particularly as related to the reproductive tract. In addition, the establishment of the so-called normal microscopic anatomy of the tubular genitalia in the different age groups studied, should also serve as a base line of information for the endocrinologist and the obstetrician. Also observed are the mesonephric remnants in close relation to or incorporated in the tubular genitalia in the different age groups studied. These embryonic remnants may be of some significance to a pathologist who may be interested in the neoplastic growths of mesonephric origin whose characteristics are not very clear. It is hoped that the information derived as a result of these studies would be useful to not only breeders, but also to the histologists, comparative anatomists, physiologists, pathologists, and diagnosticians.

# LITERATURE REVIEW ON TUBULAR GENITALIA OF THE SOW

## Development

The tubular genitalia of the sow consists of uterine tubes or Fallopian tubes, the uterus, cervix, and vagina. This part of the genital tract is derived from the embryonic Mullerian ducts. The urogenital sinus is a derivative of the cloaca.

Mullerian ducts develop in both sexes (Arey, 1965). As they appear close to and lateral to the mesonephric ducts at the cranial pole, they are also called paramesonephic ducts. In the human embryos of 10mm length (six weeks), they appear as grooves in the thickened epithelium of the urogenital ridge. According to Patten (1948) the Mullerian ducts can be located and carefully dissected out in the pig embryo of 30 to 40 mm size.

As described by Arey (1965) the extreme cranial end of the groove remains open, while more caudally, the lips of the groove close and create a funnel-like tube. Starting as an epithelial inrolling the Mullerian ducts grow caudally with its blind end. The female duct is intimately related to the mesonephic duct which could induce the growth of the Mullerian duct.

On approaching the cloaca the two urogenital ridges swing medially thus reversing their original relationship with the mesonephric ducts which assume a lateral position in relation to the mesonephric ducts. In the human embryos of 9 weeks the portion of Mullerian ducts coursing within the genital cord fuse to end blindly at Muller's tubercle (Arey, 1965). The potential lumen of the fused Mullerian ducts is packed with epithelial cells similar to those lining cranial part of the ducts. The cells lining the lumen of the urogenital sinus grow back to merge with the cells of the Mullerian ducts. This combined cell mass constitutes the Mullerian tubercle which projects into the lumen of the urogenital sinus (Patten, 1958). In all higher mammals, the vagina and the caudal part of the uterus are derived by fusion of the Mullerian ducts. The uterus thus opens into the vagina via the unpaired cervix. Cranially from the cervix there is a great degree of variation in the fusion encountered in the different groups. In the sow the fusion occurs a short distance cranial to the cervix forming the body of the uterus while the rest of the unfused ducts give rise to the uterine horns and the uterine tubes.

Partial fusion of the Mullerian ducts results in the formation of the uterus with horns and complete fusion in the single uterus of the primates. The fallopian tubes do not fuse and always remain separate. Near the cephalic end , toward the ovaries, a funnel shaped opening develops - Ostium tubae abdominale. In the sow a pouch-like dilation invests the ovary. In the human a fringed funnel shaped ostium opens towards the ovary (Patten, 1958).

## Phylogenetic Development

The phylogenetic origin of the Mullerian ducts can be traced to their development in the Elasmobranchs in which they originate by the lengthwise splitting of the pronephric ducts. Half of the split tubular duct becomes the oviduct and the other remains as the mesonephric duct. The oviduct appropriates the peritoneal funnels to serve as its coelomic opening called the ostium (Hyman, 1947).

The Mullerian ducts or oviducts in the majority of vertebrates open separately into the cloaca. The uterine opening into the cloaca is separate in the monotremes or egg-laying mammals. In the marsupials the terminal portion of the uterus is differentiated as a vagina.

In mammals above the marsupials a single vagina is derived as a result of fusion of the two vaginae. Fusion of uteri also takesplace to a varying degree. The fused caudal portions are called the body of the uterus and the separate portions are the horns. In man and other primates complete fusion of the uterus occurs, thus forming a single uterus (Hyman, 1947).

## Types of Uteri

Kent (1954) has classified 4 types of uteri as follows:

1. Duplex uterus: These types of uteri are found in monotremes and marsupials. As described in the preceding paragraph there is no caudal fusion of the Mullerian ducts; hence the uteri are completely paired. In marsupials there is a partial fusion of the ducts, but the passageways remain paired. Some rodents exhibit a similar condition.

2. Bipartite uterus - In higher mammals, the uteri unite caudally but there are many degrees of union. The body of the uterus has two lumens.

3. Bicornuate type - In this type the two separate uterine horns open into a median chamber, the uterine body.

4. Simple uterus or uterus simplex - This is the uterus of apes and man where the horns of the uterus have been eliminated and the oviducts open directly into the body.

According to Davies and Kusama (1962) the human vagina is developed entirely from the urogenital sinus, the uterus and intermediate part of the cervix being of Mullerian origin. Bulmer (1957) has stated that the vagina in the human arises as a proliferation of the epithelium in the caudal part of the pars pelvina of the urogenital sinus and is therefore endodermal in origin. The pars pelvina of the sinus is a narrow and elongated part distal to the bladder and is succeeded at the level of Bartholin's glands by the pars phallica of the sinus. The elongation of the sinus proliferation displaces the fused Mullerian ducts in a dorsocranial direction. The Wolffian or mesonephric ducts remain caught up in the sinus proliferations close to the pars pelvina and are later incorporated into the region of the hymen. Canalization of the solid sinus (vaginal) proliferation takes place between the 18th and 22nd weeks. The junction between the Mullerian and urogenital (vaginal) epithelium was tentatively identified in a 17 mm fetus before the cervix was marked off from the vaginal canal. The fusion of Mullerian ducts forms a canal lined by stratified columnar epithelim. The epithelium when traced in the direction of the vagina appeared to form a stratified squamous epithelium. The smooth muscle differentiating in the wall of the Mullerian canal extended caudally to a level slightly above the transition zone, being absent at the level of the zone. On this basis Bulmer (1957) judged the transition zone to lie at the site of the future cervix.

## Macroscopic and Microscopic Structure

As described by Montane, Bourdelle and Bressou (1964), the oviducts of the sow extend from the ovaries to the cornua of the uterus following a flexuous course. They are 20 to 25 cms in length. The infundibulum with its large opening is attached to the internal layer of the ovarian bursa. This attachment continues to reach the cranial border of the ovary and descends forward to reach the cranial border of the broad ligament which merges with the extremity of the cornua of the corresponding side. Its caliber, which is sufficiently small in the cranial part, increases gradually towards the uterine end.

The fallopian tubes of the sow are 15 to 30 cm in length and are less flexuous than in the mare (Sisson and Grossman, 1953).

The uterus has a body 5 to 6 cms continued caudally with a thick ill-defined neck from the cervix and vagina. Cranially the uterine horns are long, thin and flexuous and are separated from each other at an angle. The uterine horns are attached to a thin mesothelial layer which extends between the horns as an intercornual ligament (Montane et al., 1964). According to these authors the caliber of the uterine horns is almost uniform and in the nongravid uterus they are continuous with the oviduct without any clear demarcation. The horns are rounded from side to side. The free margin or the greater curvature and the attached border or the lesser curvature are similar to the ruminants. In the aged and those animals which have undergone frequent pregnancies, large muscular bands are seen running parallel in a longitudinal direction on the surfaces and borders of the horns.

As described by Sisson and Grossman (1953), the horns of the gravid uterus present dilatations, the ampullae, which contain the fetuses and are separated by constrictions. The gravid uterus lies on the ventral abdominal wall, and toward the end of gestation extends forward to the stomach and liver.

The neck of the uterus is 10 cm long and continued by the vagina without forming any intravaginal projection. When slit open peculiar rounded prominences are seen in the interior and their interdigitations appear to occlude the cervical canal (Sisson and Grossman, 1953). Montane, et al. (1964) describe these irregular elevations as arranged in 2 or 3 parallel rows.

The texture of the uterus in the neck region is very thick due to the superficial muscular fibers forming longitudinal muscular bands.

The broad ligaments contain a large amount of unstriped muscle. A large lymph node is seen near the ovary enclosed by the broad ligaments (Sisson and Grossman, 1953). The sublumbar insertion or the attachment of the broad ligament extends to the median line to reach the area just behind the kidneys.

In the upper part of the ligament the muscular tissue forms a rounded band termed the round ligament, 12 to 15 cm long in the form of a falciform fold which attaches to the superior inguinal ring (Montane et al. 1964). According to Sisson and Grossman (1953), the anterior end of the round ligament forms a blunt projection and posteriorly ends in the subserous tissue at the internal inguinal ring. The internal inguinal ring described by Sisson and Grossman (1953) is referred to as the superior inguinal ring by Montane et al. (1964).

The vagina is 10 to 12 cms long and narrow. It is ill-defined from the uterus both externally and internally. It is invested by a layer of peritoneum in its cranial two thirds. The plicated mucous membrane is intimately united with the muscular coat. The tunica muscularis comprises a thin circular layer located between the longitudinal layers. In the wall of the vagina, as in the cow, two canals of Gartner open on either side between the vagina and the vestibule by small narrow orifices. These orifices lie in a small fossa incompletely surrounded posteriorly by a mucous fold. (Montane *et al*. 1964).

## Uterine Tubes

The wall of the uterine tube is composed of mucosa-submucosa. The muscularis mucosae is lacking. The epithelium consists of simple columnar to pseudostratified epithelium in ruminants and swine (Trautman and Fiebiger 1957). Novak and Everett (1928) have reviewed the historical aspects of investigations by numerous authors of the characteristics of the epithelium lining the uterine tubes of women. Frommel (1886) first refuted the traditional concept that the tubal epithelium comprises a single layer of columnar cells. This observer called attention to the fact that two types of cells constituted the tubal epithelium. He also observed another type of a compressed cell "the stiftchezellen" with a flat nucleus and no cytoplasm. Subsequent investigators called this a "peg cell". Frommel's studies were made upon the uterine tubes of cats, dogs, sheep and monkeys.

The first American investigator to report his findings on the epithelia of the fallopian tubes was Gage (1904). His findings on the uterine tubes of the mouse and the bat indicate that the tubular mucosa is lined with cylindrical epithelium which is chiefly nonciliated, though some ciliated cells are present in young mammals.

Voinot (1900) studied the entire length of the fallopian tubes, i.e. Infundibulum, Isthmus and Ampulla. In humans, he found cilia absent in intrauterine life though appearing just before birth. Up to the age of puberty two types of cells were observed. Ciliated cells predominated at the fimbriated extremity of the infundibulum. Chiefly non-ciliated cells were observed in the isthmus and ampulla. Transitions among nonciliated cells include the Stiftchezellen or the peg cells.

Snyder (1923) in his studies on the tubal epithelium of the sow has described these cells as small, round cells with a deep staining nucleus. Novak and Everett (1928) stated that the peg cell was considered a transitional phase of nonciliated cells. According to Tröscher (1917) Stiftchezellen are degenerating epithelial elements. He did not observe mitosis in the ciliated cells.

Hadek (1955) in his studies of tubal epithelium in sheep refers to the peg cells as rod cells which he observed during metestrus of the cycle. They are as high as ciliated and non ciliated secretory cells and appear to be produced from secretory cells by the extrusion of the nucleus. There is a gradual transition from a secretory to a rod cell. In early diestrus the nuclei of some cells move closer to the free surface or distal extremity of the cell creating an impression of wandering out. A constriction appears in the thin elongated nuclei. After being extruded from the cell they appear round or oval measuring 2-5 $\mu$ in diameter. In late diestrus the nuclei are to be seen in the lumen of the fallopian tubes. At this time the cells from which they originate appear thin, rod-like and without nucleus. The rod-like remnants disappear by the onset of the next cycle.

Casida and McKenzie (1932), McKenzie _et al_. (1933) and McKenzie and Terril (1937) have expressed their opinion that the presence of nuclei in the projections of secretory cells represents a holocrine secretion. Novak and Everett (1928) have indicated that the secretory cells project beyond the ciliated cells and show a bulbous herniation into the lumen of the tube carrying the nucleus with it in the premenstrual phase or luteal phase in the woman. They did not interpret their significance. In spite of the great loss of cells, mitosis was rarely observed in the tubal epithelium. In pregnancy they observed that the epithelium is lower and flatter than in the menstrual stages.

Weeth and Herman (1952) have also reported nuclear extrusions from the surface epithelium of the fallopian tubes of the cow during 2 to 4 days postestrus period. Goblet-like cells were seen in the epithelium and the nuclear protrusions appear related to the cell in two different groups. In one group, the projections are still in contact with the cells and form finger-like processes projecting into the lumen of the oviduct. The other group includes those cells which lie free in the lumen of the oviduct. Projections of both groups give a positive Feulgen reaction and have a nucleus. Those projections which are still in contact with the cells possess in addition a cytoplasmic covering which gives a faint reaction with pyronin. The cells lying free in the lumen do not have this feature. The appearance of these cells coincides with the cessation of secretion and are possibly undergoing a form of degeneration.

Fredricsson (1959) observed the presence of two types of epithelial cells lining the tubal mucosa in the fallopian tubes of woman and rabbit. The secretory cells which are taller project over the ciliated cells like a dome. The secretory cells are tall during the follicular phase of the estrus cycle and short during the luteal phase. Hadek (1955), in his studies on the sheep oviduct, has stated that no variation in height between secretory and ciliated cells in any phase of the estrus cycle exists. There is variation of height of the epithelium during the cycle but these variations affect both secretory and ciliated cells equally. Clyman (1966) studied the human fallopian tubes with the electron microscope. He observed increase in size of the secretory cell, an increase in the number of mitochondria and increase in the size of Golgi apparatus in the estrogen phase. The endoplasmic reticulum increased and under the influence of progestational steroids became dilated and filled with a fine amorphous substance.

Fredricsson (1959) made the following observations on histochemical studies of the fallopian tubes. His findings indicate the presence of fine granules of lipids and vacuoles, Alkaline phosphatase, PAS reactive and diastase resistant material, and non-specific esterase located in the cytoplasm of the supranuclear region of the secretory cells. Glycogen was also observed in the infranuclear region of the ciliated cells and a low amount of glycogen was present during pregnancy, the post menopausal period and puerperium.

Fawcett and Wislocki (1951) observed the presence of alkaline phosphatase in the ciliated cells of the human fallopian tubes.

Clyman (1966) has reported two types of secretory granules in the secretory cells studied with the electron microscope. (1)A dark electron dense type appeared after the 11th day of the cycle and increased in number during day 15 and 16 of the cycle; (2) larger secretory light grey granules appeared and increased in number during day 17 and 18 of the cycle. Appearance of glycogen granules in late proliferative and early secretory phase has been reported.

Snyder (1923) studied the cyclic changes in the epithelium of the fallopian tubes of the sow. He reported the epithelium to be composed of ciliated and monociliated cells which are equally distributed. A few small rounded cells were seen between the cylindrical epithelium and have a deeply staining nucleus.

During estrus the cells reached a maximum height of 25 micra as measured from the basement membrane to the level of the basal granules of the ciliated cells. The height of the epithelial cells falls to 10 micra during diestrus or the luteal phase about the second week after ovulation. During the third week the height of the epithelium increases gradually reaching a maximum height. After the 2nd week of ovulation cytoplasmic protrusions appeared in the nonciliated cells projecting beyond the ciliated cells. Deeply staining nuclei may be seen not infrequently in the protrusions which become pedunculated and detach.

The stroma is edematous in the first and third weeks when the epithelium is high. Closely packed strands were seen after ovulation when the epithelium is low. Wandering cells, plasma cells, eosinophils, and other cells appear that fluctuate irregularly without any apparent relation to the cycle.

In pregnancy the epithelium is similar to the luteal phase of the cycle.

According to Trautman and Fiebiger (1957) there are no glands in the mucosa-submucosa. The mucosa forms large primary and secondary folds which increase greatly towards the ampulla and ostium abdominale. The tunica muscularis is rich in elastic tissue and gives off numerous radial strands into the submucosa. The inner part of the tunica muscularis has circularly arranged smooth muscle fibers but isolated longitudinal and oblique bundles may also be seen. It becomes thicker towards the uterine end and blends into the circular uterine muscle layer. A thin outer longitudinal muscle layer is present which is somewhat separated from the inner circular layer by a vascular layer.

Kipfer (1950) described, in the uterine tube of women, three different dispositions of the muscle fibers in relation to the longitudinal axis of the organ. The muscle fibers run in three different distinct directions. At the pars intramuscularis, the loss of external longitudinal musculature is compensated by a strong inner longitudinal component. The middle circular layer exhibits the most complicated structure in which each of two bilaterally symmetrical components penetrate the wall of the tube in a spiral manner, from outside inwards. The spiral course causes compression of the muscular elements as well as the longitudinal axis and also simultaneous coil-like penetration of the middle layer. The muscle fiber groups, according to Kipfer, run in a spiral course.

Toni and Maccaferri (1951) observed, in the Fallopian tubes of women, that the internal longitudinal layer was disposed in an oblique fashion, the middle circular layer was observed running in a spiral fashion in the ampulla and the isthmus. The external layer consisted of an oblique fascia.

The ultrastructure of the smooth muscle of the fallopian tubes of women was studied by Clyman (1966) with the electron microscope. He observed that the elongated smooth muscle cells have a double membrane - an outer plasma type membrane and an inner more electron dense membrane. Two types of smooth muscle cell bridges are described. A common type nexus is a fusion of the outer membranes and a closer approximation of the inner membranes; a protoplasmic bridge is less frequently seen. Interspersed bundles of myofilaments, scattered mitochondria and sparsely scattered ribosomes were present in the cytoplasm. The intercellular space is filled with collagen fibrils. No difference was observed between the smooth muscle cells of the fallopian tubes and other human smooth muscle.

The tubo-uterine junction

High concentrations of sperm, after coitus and artificial insemination, at the tubo-uterine junction of the sow has aroused considerable interest in its function and structure.

Anderson (1928) indicated that the circular muscle of the tube and uterus are continuous and no thickening at the tubo-uterine junction was observed. A sphincter at this site was lacking. The mucosa of the tube and that of the uterus are joined together by a band of transitional mucosa bearing the villi. These villi appeared short,wide and flat often appearing to be fused with a common base but retaining individual tips. They become larger and narrower towards the ovarian end. Functionally the villi may absorb uterine material as they are richly vascularized. They may also act as one-way valves in the event of pressure from the uterine direction. According to Corner (1921) no glands are present in the villi of the tubo-uterine junction.

A comparative anatomical feature of the tubo-uterine junction of the guinea pig, mouse, rat, rabbit, cat, dog, lion, and the pig have been reported by Lee (1928).

In the tubo-uterine junction of the sow, large polyp-like projections guard the tubal ostium and are distributed irregularly. They are more numerous near the opening of the tube and become gradually less peripherally in the uterine cavity. The average length of these polyps was 2 mm. in length. The epithelium covering the polyps was columnar with greater cytoplasmic content simulating, in general, the epithelium of the uterine tubes. The stroma of the body of the polyps was loose but compact at the base. Uterine glands were generally present at the base of the polyps. Large vessels and lymphatics occupied the stromal layer. The musculature as observed by Lee was slightly greater at this junction than that of the adjoining part of the tube.

According to Rigby and Glover (1965) there is a significant decrease in the lumen of the tube in the first centimeter from the uterine ostium. The narrow lumen continues about 6 cm. and widens out into the ampullary region. Characteristic diverticulae were seen in the first few milimeters. These appear as branches of the lumen, are directed cranially and may be of relevant functional significance.

Age changes in the fallopian tubes

In senile women (ages 60 to 95 years) the epithelium varied from a cuboidal to flat type except in the interstitial portion where it is still cylindrical, (Voinot, 1900). Cilia may be retained even when the epithelium has been flattened. Tröscher (1917) had also observed that the epithelium in senile women became cuboidal or almost flat. The mucosal folds become rounded.

Novak and Everett (1928) found that no cilia were present in the tubal epithelium of a seven month old fetus. With the exception of the cyclic changes, the tubal epithelium of the newborn infant is similar to the adult.

After menopause there is no change in the morphology of ciliated and secretory cells until the age of 60 years. Above this age the epithelium becomes cuboidal to flat. No cilia are seen on the flattened cells. The tubal folds become more or less rounded. No distinction can be made between ciliated and secretory cells. More recently Pinero and Foraker (1963) studied the age changes in the fallopian tubes of women (15 to 56 years). These investigators observed increasing amounts of plical fibrous tissue after menopause. No generalizations as to vascular changes with age appear possible. They further supported these observations by planimetric studies. Sections for their studies were taken from the fimbriated end of the uterine tube. Foraker and Crespo (1962) found no change in histochemical reaction pattern related to age in the fallopian tubes.

On the effect of age on the musculature of the fallopian tubes, Toni and Maccaferri (1951) observed that the tubal muscular coat increases in thickness in the age range of 20 to 50 years. There is a gradual decrease in thickness of the musculature after 40 to 50 years age. The regression or atrophy of the musculature of the fallopian tubes approximately corresponded with the physiological state of the genital tract, i.e. menopause.

## Uterus

A detailed review of literature on the historical aspects of investigation of uterine studies has been dealt with by Fabian (1961). He also studied in great detail the height of endometrium of swine under the influence of different phases of the estrus cycle.

<u>Microscopic anatomy</u>: Schmaltz (1911) described the morphological manifestations of the uterine epithelium of the sow as many layered. The endometrium of the uterus of the domestic animals bears a columnar epithelium of which some cells are ciliated. The secretory epithelium is high in woman and the mare and low in the carnivores. In the sow and ruminants the epithelium is stratified (Trautman and Fiebiger, 1957). Detailed investigations of the microscopic anatomy of the endometrium and the changing morphology under the influence of the estrus cycle were carried out by Corner (1921). Cilia are lacking on the surface epithelium of the sow and goblet cells are absent. The stroma and the mucosa is a fluid filled gelatinous tissue in which glands, blood vessels and lymphatics are seen. Cellular elements in the mucosa consist of fibroblasts, plasma cells, and macrophages. The stroma is condensed under the surface epithelium forming a narrow subepithelial connective tissue zone of which the most superficial fibroblasts are flattened against the epithelium to form a basement membrane. Corner divides the mucosa of the sow into two parts. The superficial zone of the mucosa has few glands and the basal zone is packed with twisted glands. The glandular cells of the superficial zone are 15 to 25 microns high as against 12 to 18 microns in the glands of the basal zone. The

glandular cells of the superficial zone contain more cytoplasm and the cells lining the superficial tubules appear larger and clearer than those of the smaller and deeply situated glands. One fourth of the gland cells are ciliated and ciliated cells are fewer in the basal zone.

The blood vessels pass through the muscularis giving off branches to plexuses in and between the muscular layers and then form a network of large channels near the base of the stroma. From this network long loops ascend toward the lumen to end in a delicate capillary plexus in the subepithelial tissue, just below the epithelial cells.

According to McKenzie (1926) the epithelium of the sow's uterus is simple columnar in type, but pseudostratified conditions are characteristic of this tissue for about a third of the estrus cycle, namely from the proestrus stage till nearly a week after heat. McKenzie's observations are similar to those of Corner regarding the absence of cilia on the surface epithelium and its presence on the epithelial cells of the crypts and glands. Green (1950) stated that the uterine epithelium of the sow varies from pseudostratified to cuboidal during the cycle. Palmer et al. (1965) have indicated that the uterine epithelium appeared to be degenerate during the immediate postpartum period. Regeneration starts 7 days after parturition and is completed at the 21st day. After this period the epithelium remains low columnar or pseudostratified during lactation. On the 3rd or 4th day after weaning, increase in epithelial height and glandular development are noticed.

Snyder and Corner (1922) further substantiated the earlier observations of Corner (1921) that cilia are not present on the surface epithelium at any stage of the estrus cycle but are present in the uterine glands. Contrary to this observation, Lovell and Getty (1968) observed that many ciliated cells are present on the surface epithelium of the cephalic end of the uterine horns near the utero-tubal junction of the sow at 8½ and 27 hours after natural service. The ciliated cells were so numerous that they were obviously not part of a glandular orifice.

Cyclic changes: Swezy (1935), Hooker (1945) and Robson (1947) have reported the influence of ovarian hormones on the cyclic histology of the laboratory animals.

Murphy (1924) and Cole (1930) studied the histological variations of the cow's uterus during the estrus cycle. Casida and McKenzie (1932) made cyclic histological observations of the genital tract of the ewe. On the sow Corner (1921) reported the following changes during the estrus cycle.

Estrus: The epithelium is stratified and mitotic figures are evident. Mitotic figures are also seen in superficial glandular tubules. Corner also reports a vacuolar degeneration in some epithelial cells in early periods of estrus. Epithelial cells appear to be 25 to 30 $\mu$ tall. The endometrial stroma is edematous. McKenzie's (1926) observations of endometrial histology during estrus are similar to Corner's. According to McKenzie the vacuolar degeneration starts in proestrus and subsides till late estrus when vacuoles are absent. Fabian (1961) described the foamy appearance of the uterine epithelium at the basal part of the cell. The oval nuclei lie very irregularly in the basal to the middle third of the cell. Some nuclei move upwards and are located more towards the lumen. Cell mitosis is observed in the glandular epithelium which is ciliated superficially. Fabian describes two types of edema in the mucus membrane of the uterus. One type of edema consists of large vacuoles and the other is a diffuse type. He also observed erythrocytes in addition to the wandering leucocytes.

Primary and secondary folds of mucous membrane are described in the cleft-like uterine lumen. According to Green (1950) several layers of fibroblasts are seen lying close together. Some lymphocytes and polymorphs lie close to the epithelial surface. Edema as described by Green is most pronounced in the sub-epithelial region. The coiling of glands is not so pronounced at estrus.

Postestrus or early luteal phase: During this stage of the cycle the epithelium is 35 to 50 microns high (Corner, 1921). According to McKenzie's data the epithelium steadily increases in height and varies from 16-34 to 33-45 microns.

The epithelium becomes simple columnar. The glandular and epithelial cell division ceases after 6 to 9 days. Edema is reduced and some eosinophilic leucocytes are seen in the stroma. On the 5th day following heat Green (1950) has stated that the glandular growth fills the endometrium. On the 6th day serrations in the surface epithelium are evident. On the 7th day pseudostratified appearance of the epithelium is lost and changes to simple columnar and the serrations of epithelium appear more enhanced.

Fabian (1961) presents this period of the cycle as corpus luteum phase I, i.e. 4th to 11th day post estrus. The mucus membrane, according to his measurements, is 5-10 mm thick which is the mean value with a range variation of 1.86 mm. A homogeneous layer covering the epithelium is described. He also reports the presence of stiftchenzellen cells or pin cells. These cells have a long narrow very intensely stained nucleus. Some wandering cells are seen close to the glands. Regression of edema is evident. The subepithelial cellular layer in which many wandering cells are found is indicated as a narrow strip.

In the late luteal phase, 10 to 15 days postestrus, the surface epithelial cells appear in a low columnar form measuring 15 to 20 microns.

Cytoplasmic processes ranging from 3 to 8 micra in height protrude from the surface of each cell. A complex hillocky surface is no longer seen but the eosinophils appear to fade from the uterine stroma. Glandular epithelium returns to the usual size (Corner, 1921). During the period, according to Green (1950), the serrations of epithelium disappear and is replaced by low columnar epithelium. The major folds of epithelium recedes slowly with the onset of edema. The glands decrease in diameter and seem to be less numerous because the stroma expands as a result of the edema.

During the 15th to 20th day post estrus Corner (1921) observed the degeneration of the corpora lutea with simultaneous development of new graafian follicles. The cells of the surface epithelium begin to be arranged into a pseudostratified epithelium.

McKenzie (1926) saw slight edema of the endometrial stroma in late diestrus which increased gradually until proestrus. The edema continued in a lesser degree until ovulation but disappeared immediately after heat. Leucocytes were present in the stroma at all times though their number was greatly augmented from late diestrum until 4 days following heat.

Green's observations indicated that glands tend to uncoil because of the thickening of the stroma, and by the end of these three days there is an active disintegration of gland cells (Green, 1950). Observations of Fabian (1961) do not differ very much from those of Corner (1921), McKenzie (1926), and Green (1950). Fabian describes the cellular morphology of the epithelial cells in greater detail.

In spite of the cyclic changes in the uterine endometrium which is influenced by the ovarian hormones, there is an interaction of uterine as well as ovarian secretions. Anderson et al. (1963) have investigated the effect of functional properties of endometrium on the corpus luteum in the sow. According to these authors, a sufficient quantity of functional endometrium appears necessary to provide a luteolytic stimulus to bring about the involution of the corpus luteum. The inference drawn from their experiments is that direct neural pathways from the uterus are not essential for supplying a luteolytic stimulus during the estrus cycle in the gilt.

Some 15 enzymes have been investigated in the mouse uterus by Thiery and Willinghagen (1963). They located alkaline and acid phosphatase, 5 nucleotidase, adenosinetrophosphatase, B glucoronidase NAOD, NAOPD in the endometrial epithelium. These enzymes were found to show cyclical variations in intensity and localization which parallel the estrogen impregnation of the animals. Cyclical variations in intensity were found in the endometrial stroma for the activity of alkaline phosphatase, adenosin triphosphatase, and B-glucoronidase. Analogous variations in the myometrium are reported for alkaline phosphatase and B glucoronidase. These authors suggest a relationship between functional activity of fibroblasts and the intensity of the activity of alkaline phosphatase in the subepithelial connective tissue. The high phosphatase activity of the macrophages may be attributed to storage of organic substances derived from the breakdown of the endometrial epithelium. In the endometrial epithelium and stroma, sudden increase of amino peptidase activity at the termination of the estrus cycle may be the expression of the intense catabolic phenomenon characteristic of this phase.

Okey et al. (1930) investigated variations in lipids of the uterine mucosa of the sow. They found that lecithin (phospholipid) content of the uterine mucosa increased significantly during the cycle with little change in cholesterol. The increase of lecithin coincides with the phase of maximal glandular proliferation under the influence of the corpus luteum and with the lowest levels of oxygen utilization and the greatest deposition of glycogen in the mucosa.

Nellor (1963) injected progesterone to castrate normal cycling and pregnant heifers and gilts. Differentiation and mobilization of connective tissue plasma cells into cells that resemble immature and mature lymphocytes were seen under the influence of progesterone. The cells invade the subepithelial stroma and epithelium of the vagina, uteri and fallopian tubes, penetrate the lumina of the fallopian tubes in large numbers, enter the vaginal lumen to a limited extent and are rarely seen in the lumina of the uteri.

Lymphocytosis, preceded by acidophilic granulation of the cytoplasm and fragmentation of the nuclei appears to be the fate of the wandering cells. These lymphocytes appear to have been mobilized from the endometrial stroma rather than the circulating blood because no detectable change in total leucocytic count or differential count was noticed.

Diethylstilbesterol treatment inhibited induced progestational lymphocytosis of the genital tract while increasing the numbers of polymorphonuclear leucocytes in the tissues. Progestational lymphocytosis of the genital tract is a physiological phenomenon recognizable during the normal estrus cycle and at specific stages of pregnancy in swine and cattle.

Corner (1921) observed eosinophils 7 days after ovulation in the endometrial stroma of the sow. Baker et al. (1967) associated the presence of eosinophils in the endometrium with peroxidase activity. Lovell and Getty (1968) recently reported on the fate of semen in the uterus of the sow during 27 hours after natural service. They have described that polymorphonuclear leucocytes were beneath and in the epithelium of the uterus, 10 minutes after service. After 30 minutes the smears of the seminal fluid which was considerably reduced contained 25% leucocytes and 75% spermatozoa. Many leucocytes could be seen in the epithelium at this time. After 1 hour and 45 minutes no seminal fluid was present in the uterine lumen except a foamy moisture. Smears of this material contained 50% leucocytes and 50% spermatozoa. Leucocytes were observed to have phagocytosed spermatozoa totally or partially. At 8½ hours there was no fluid in the uterine lumen; the smears from the moist surface contained 75% leucocytes and 25% spermatozoa. Leucocytes and epithelial cells from the endometrium were closely associated with clumped spermatozoa. After 27 hours smears from the uterine mucosa contained mainly old degenerating leucocytes and epithelial cells and only rarely were spermatozoa observed. It appears from the above findings that the neutrophils attack and remove the spermatozoa-like foreign bodies.

Myometrium: It is divided into a thick inner circular layer and a thinner outer longitudinal layer by the stratum vascularis, which contains numerous large vessels and nerves in most of the domestic animals. This layer is not very well developed in swine and man (Trautman and Fiebiger, 1957). The serosa, the external longitudinal layer and the stratum vascularis, are all continuous with the broad ligament.

The differentiation potentialities of the smooth muscle cell have been recognized by Wissler (1968). This cell in the arterial media is a multifunctional cell in the sense that it fabricates elastic fibers, collagen fibers, mucopolysaccharides and myosin. The postnatal differentiation of the smooth muscle of the rabbit myometrium was investigated by Yamamoto (1961). He has reported that the uterine horns of newborn rabbits has epithelium, mesenchymal tissue and serosa. Two layers of the mesenchyme can be distinguished, one being the inner layer surrounding the epithelium with a network of fibers arranged more closely than in the outer layer. The two layers become apparent in a 30 day old embryo and continue up to 12 days postnatal of the newborn. In the newborn of 9 to 12 days of age, a small number of spindle-shaped cells are found loosely arranged beneath the epithelium. From this pattern Yamamoto presumes that the endometrial cells differentiate. At 14 days postnatal a circular layer of muscle appears. The outer longitudinal muscle layer is not observed in rabbits of 4 and 5 weeks of age. In addition to the light microscopic observations, Yamamoto also studied the differentiation of the inner circular layer of the myometrium with the electron microscope. The appearance of myofilaments is associated with a decrease in the Palades particles at 6 days postnatal. At nine days of age, the filamentous particles were observed in the matrix of the cytoplasm. At this stage these calls somewhat resemble a smooth muscle cell. According to Yamamoto these cells could be called myoblasts. The definite myofilaments are seen at 12 days postnatal.

An electron microscopic study of the uterine smooth muscle was undertaken by Mark (1956). He indicated that the smooth muscle fiber of the human and rat is bounded by a cell membrane which resembles the sarcolemma of striated muscles in thickness and its association with external connective tissue fibrils. The nucleus exhibits one or two nucleoli with dense tortuous filaments. Mitochondria, endoplasmic reticulum, cytoplasmic matrix, and a vesicular component of the cytoplasm have been described.

The myofilaments lacking in membrane attachments are oriented parallel to the tapering portions of the cell where individual filaments terminate at many points adjacent to the border. This arrangement, together with the close relationship of various parts of the membrane to external connective tissue folds, suggests that forces during contraction may be exerted over a great area of the cell surface in a rather concentrated way at the pointed ends.

Languens and Lagrutta (1964) in their studies of the smooth muscle of the myometrium of the human uterus with the E.M. described three types of cellular contact. They considered that these regions provide a morphological basis for the spread of the excitatory impulses between muscle cells.

Similar observations were also made by Silva (1967) in the myometrial cells of the rat. According to Silva, the most common type of cellular contact consists of opposing plasma membranes separated by a gap of 100 to 300 Angstroms containing an electron lucid material across which filamentous structures extend obliquely. In the second type, opposing plasma membranes appear to be in direct contact. The third and least frequently seen type consists of an intercellular bridge with cytoplasmic continuity between adjacent cells.

Bergman (1968) studied the effect of estrogen on the uterine smooth muscle of the castrated rats. After 2-5 days treatment with estradiol or diethylstilbesterol, the nucleoli became highly developed and tend to be located near the nuclear membrane. Centrioles are seen near the nuclei although no mitosis was observed. Golgi apparatus and ribosome studded sarcoplasmic reticulum are prominent features of hypertrophying muscle fibers. In the absence of estrogen the uterus is electrically and mechanically quiet and there is no evidence of well-defined cellular junctions like those found in physiologically active tissue.

Bird and Willis (1965) have observed that the endometrial stroma of the adult human uterus is capable of differentiating into smooth muscle under the following 3 conditions: (1) Tumors of the endometrial stroma; (2) hyperplasia of the uterus secondary to ovarian hormonal influence; and (3) the normal premenopausal uterus. In the normal premenopausal uterus at the endometrial myometrial junction, histological distinction between the deep stromal cells and the adjoining muscle is often difficult or impossible and there appears to be a gentle transition from the one to the other. They suggest that myogenesis may be a normal function of the endometrial stroma.

Green (1950) and Sternberg et al. (1954) have expressed their views that the endometrial stroma retains, in the adult life, a latent capacity for differentiation not possessed by other Mullerian derivatives. This is strikingly apparent when neoplastic growth occurs.

Age changes in uterus

Rolle and Charipper (1949) have described in sequence the age changes in the ovary, uterus and vagina of the golden hamster. No hyalinization was noted in the aging ovary, uterus or vagina. In the uterus between 6 and 15 months of age, there is a progressive increase in connective tissue between the smooth muscle fibers of the circular muscle layer. The connective tissue fibers also increase in the stroma but the subepithelial stroma is more cellular than the outer portion. In the 18 to 21 month age group the outer stroma fibers appear thicker and more dense. The circular smooth muscle decreases in amount while no change is evident in the longitudinal muscle layer. By 24 months the circular muscle layer is further reduced while the longitudinal muscle layer is still well preserved. The fibers of the outer and inner stroma have more connective tissue. The diameter of the uterus increases at 3 months, remains static to 6 months, and then there is a gradual rise to 24 months with the exception of a drop at 10 months.

In studying the human uterus and its vessels, Lapina (1957) observed a slight increase in uterine size from age 18 to 30, a continuing growth of the uterine artery in this period and a decrease in the size of the uterus and its vessels beyond age 55.

According to Speert (1949) cystic glands varying in size and number constituted one of the most consistent as well as one of the most interesting findings in the aged women. The cysts have a predilection for the superficial stratum where they are covered by the thinnest layer of surface epithelium. Their similarity to the glands of a hyperplastic endometrium is only superficial, the chief characteristic common to both types being the swiss cheese pattern presented by the cystic glands of varying size. The epithelium of the post menopausal cysts is flat and inactive looking, mitotic figures are rare in contrast with the histologic picture of cystic hyperplasia seen in younger women. Increased fibrous change and hyalinization are seen especially near the surface. Thin walled veins are prominent under the surface epithelium whereas spiral arteriolar apparatus could not be demonstrated. In contrast to the basophilic color assumed by youthful endometrial stroma, the senile mucosa is predominently eosinophilic, but considerable individual variation exists. The lymphoid elements of the stroma are replaced after the menopause by a less cellular fibrous structure with fibrocytes. The matrix appears homogeneous and hyalinized near the surface. Parks et al. (1958) investigated changes in the endometrium of 335 normal postmenopausal women. They confirmed the findings of Speert (1949) in observing the increased cystic pattern with advancing age. In serial sections no communications of isolated cystic glands with the ducts were found. The progressive increase in the incidence of cystic pattern with advancing years after the menopause and the failure to demonstrate communications with a duct suggest that these cystic changes are merely a slowly progressive distension phenomenon and are not an aftermath of "swiss cheese" hyperplasia during the climacteric. These authors have also indicated that myomas decreased with postmenopausal advancing years. That of endometrial polyps did not change nor did that of adenomyosis, endometriosis, or ovarian tumors.

Collagen in old age

In the mouse, Loeb et al. (1939) reported a progressive deposition of collagen in the uterus throughout life as estimated histologically. Wolfe et al. (1942) observed similar increase of collagen in the rat.

Woessner (1963) indicated three age related changes in the uteri of women. The wet weight collagen and elastin increased to a maximum by age 30. These parameters remained constant for the next 20 years, then declined to levels of about ½ of the maximum values in the period of 50 to 65 years. After the menopause there is a resorption of the large part of the uterus accompanied with a reduction in wet weight collagen and elastin. Collagen decreased by about the same proportion as wet weight (53%). Therefore the uterus does not become more fibrous in old age, but there is a tendency for it to become slightly less fibrous. Uterine collagen was digested by bacterial collagenase at all ages, but the rate of digestion became much slower in old age. Another investigation by Woessner (1963) in pregnancy revealed that collagen and elastin may increase 600% within 9 months. This excess of collagen is completely resorbed within a few weeks in the post partum period (Woessner and Brewer, 1963). These collagen and elastin properties which are considered inert from the viewpoint of metabolic turnover, may behave in an exceedingly dynamic fashion in the uterus. These rapid changes in collagen metabolism suggest that uterine collagen may differ from other collagens in its physical and chemical properties.

In the aging uterus of the white rats, Schaub (1964-65) found that the collagen content increases and its concentration becomes three-fold The collagen of virginal uterus shows similar age changes as that of other organs. The percentage of thermolabile collagen decreases from 10-14% of young animals to 5% in 30-40 month old ones. The newly formed collagen in the pregnant uterus and in placenta is biologically young collagen. In the post partum uterus only young collagen is removed and the aged cross-linked collagen resists degradation. After the involutionary period the percentage of labile collagen is at the same low level as in old animals. Mochow and Olds (1966) investigated relation between age and number of calvings on total uterine thickness, muscle thickness, number of gland cross sections per square millimeter and gland diameter. They concluded that 30% of the variation in muscle thickness was due to age, independent of the number of calvings. Likewise, variation in gland diameter was associated with individual cow differences.

Dhindsa *et al*. (1967) determined the time and trans-uterine migrations of pig embryos after ligating the uterine tubes of one side. Migration of embryos from one horn to the other usually occurred first on day 8 or 9 of gestation. The uterus was occupied completely after day 15. Rate of migration and distribution of embryos was not affected by number of embryos, number of corpora lutea or by uterine length. Uterine length did not change between days 6 and 15.

Dhindsa and Dziuk (1967) have also reported partial doubling of uterine horns in 5 out of 500 gilts. This anomaly does not interfere with sperm transport, fertilization, pregnancy or may not hinder parturition. This anomaly which was observed in genetically related gilts suggests a genetic influence.

Yamauchi (1964) studied the oviducts and uteri of cows aged 28, 21, and 17 years. A common feature in all of the three animals was the cystic distension of the uterine glands. Cystic uterine glands frequently contained secreted material. Sometimes, a hyaline substance was present in the epithelium of the cystic gland. In two cases granulosa cell tumors were present in the ovaries of the animals studied. The cysts in the uterine endometrium could be attributed to the granulosa cell tumors.

According to Trautman and Fiebiger (1957) the blood vessels enter the uterus from the broad ligament. These vessels are thick, numerous and follow a tortuous course in the stratum vasculare giving off branches to the other layers. The subepithelial and periglandular capillaries arise from the mucosal twigs. No valves are present in the veins. In animals that have undergone pregnancy, the arteries and veins show cushion-like thickenings in the tunica intima and in all layers of the vascular wall, increased amount of elastic tissue is seen. This elastic tissue forms clumps in old subjects.

Lymphatics are numerous and form a subserous network; the nerves end partly in the muscle and partly in the mucosa.

## Cervix

The cervix or the neck of the uterus of the pig is remarkable for its length (10 cm) and it is directly continued by the vagina without forming any intravaginal projection. On opening the cervix peculiar, rounded prominences appear on the interior and some of these dovetail to occlude the cervical canal. They are continuous caudally with folds of the mucous membrane of the vagina (Sisson and Grossman, 1953).

The mucosa of the cervix is folded and there are secondary folds. In the cow the surface epithelium consists of columnar cells which are mucigenous, they secrete increasing quantities of mucus during estrus and pregnancy and continue caudally into epithelium of the vagina from the external uterine orifice. Hollow cone-like epithelial cords extend into the underlying tissue. The collagenous propria contains some elastic fibers. Large venous plexuses are present in the deeper layers of the cervical propria in the mare. The cervical musculature is very well developed and is rich in elastic fibers. In the sheep, goat, and swine the muscular layer forms annular folds of the body and also of the portio vaginalis uteri in the cow and the mare. In the zone of transition from the vagina to the uterus the arrangement of the musculature varies according to the development of the cervix. In the ewe and the sow there is no demarcation between the uterine and vaginal muscles except that the thickening of the muscle layers is observed in the region of the cervical prominences (Trautman and Fiebiger, 1957). In their studies of the human cervix musculature, Danforth and Evanston (1954) stated that it is composed predominently of fibrous connective tissue. The so-called cervical musculature consists of isolated alternate strands of smooth muscle fibers embedded in a collagenous matrix. These strands vary in different cervices and are irregularly scattered except in the most peripheral areas of portio supravaginalis where they tend to be concentrated. These strands of cervical muscle are in continuity with the muscle of the corpus and are reflected inferiorly into the vaginal vault. Although they bear strong resemblance to the muscle of the upper vagina, they bear no evident similarity to the heavy closely packed collagen sparse bundles of the corpus. The contractile ability of the cervix in vitro studies is negligible.

Krantz (1959) has dealt with the microscopic anatomy of the human cervix. According to his description the epithelium covering the portio vaginalis is stratified squamous type. Five epithelial zones that cannot be demarcated clearly have been described. These are (1) stratum cylindricum, basal cell or germinal layer composed of a single row of somewhat cuboidal cells with relatively large dark staining nuclei and a high nuclear cytoplasmic ratio. Mitotic figures appear occasionally in this layer. (2) Stratum spinosum - the second layer consists of polyhedral cells joined together with the so-called intercellular bridges of the light microscope. (3) The third zone or stratum spinosum has somewhat flattened cells which have glycogen rich frequently vacuolated clear cytoplasm. (4) The fourth zone is less well defined in the cornified type but is a distinct, independent zone or stratum granulosum in keratinized epithelium. (5) The outer or the fifth zone, stratum corneum is composed of several layers of flat pyknotic nuclei. The thickness of this zone depends on previous cervical trauma or the phase of the menstrual cycle.

The epithelium of the portio vaginalis rests directly on the fibrous substance of the cervix, no submucosa or glands being present.

The endocervical canal and its glandular components are covered by a mucous membrane composed of tall columnar cells, some of which are ciliated (Dougherty and Low, 1958) and (Ham, 1965). The epithelial cells appear uniform and are arranged in a single row. These cells actively secrete mucin and stain deeply with P.A.S. and H and E stains. The squamous columnar junction or the meeting of the two types of epithelia has been classically described as abrupt. Flühmann (1957) reported that the sharply differentiated squamo-columnar junction occurs rather rarely. He was able to find an abrupt junction in only 29 percent of his specimens. In this area a continuing process of epithelial breakdown has been observed. The process of evolution of cells in this area into mature epithelium has been variously termed squamous metaplasia, epidermization, reserve cell hyperplasia and squamous prosoplasia.

The basement membrane underlying the epithelium of cervix is a subject of controversy (Krantz, 1959). A basement membrane implies a specialized layer of intercellular substance between the underlying connective tissue and the epithelial surface. On the results of his studies, Dougherty (1961) indicated that a basement membrane could not be observed consistently under the epithelium of the normal cervix or carcinoma in situ specimens in specially stained paraffin sections with the light microscope. A structure resembling a basement membrane can sometimes be seen in position under masses of carcinoma tissue using the same method of examination. With the electron microscope Dougherty observed an exomembrane consisting of an inner protomembrane and an outer perimembrane separating the epithelium and the connective tissue. The exomembrane delineates some, but is missing from most, carcinoma epithelium in electron microscope preparations of the cervix. Younes et al. (1965) also made correlative studies of the basement membrane with the light as well as the electron microscope. Under the electron microscope, the light microscope basement membrane was shown to be composed of reticular connective tissue fibers in the 0.4 to 0.5 micron area of the stroma immediately subjacent to the electron microscope basement membrane. Incubation of the sections with collagenase did not stain the basement membrane with the PAS stain. Warren et al. (1966) are of the opinion that the use of the term "basement membrane" and associated phrases such as "extension through" and "disruption of" in describing criteria for diagnosing invasive uterine carcinoma is ill advised. From their studies they have deduced that the existence of a basement membrane in the human cervix is more apparent than real.

Classically the cervical glands have been described in the human cervix as a branching racemose mucous type with the glandular epithelium similar to the surface epithelium. Fluhmann (1957, 1958) has challenged this concept and has stated that compound tubular racemose glands do not exist in the cervix. With plastic reconstructions from serial microscopic sections and thick cleared preparations he has demonstrated that the basic epithelial structure of the cervical mucosa is a cleft or groove. These clefts run in an oblique, transverse or longitudinal direction never crossing another cleft, although they may bifurcate and extend downward as two branches. Occasionally they become occluded so that an area becomes obliterated as a tunnel or blind tube. It is in these tunnels that Nabothian cysts are presumed to be formed (Flühmann,1961). With these studies it becomes apparent that the epithelium of the cervical glands or clefts is identical with that of the surface.

Presence of lymph follicles has been reported in the cervical mucosa by Demetrakopoulos and Greene (1958). According to their observations, a positive correlation between the presence of lymph follicles and the presence of moderate or heavy chronic inflammation exists. It was concluded by these investigators that the lymph follicles in the cervical mucosa are not congenital structures but appear after birth and are usually associated with and result from chronic inflammation. No correlation was found between lymph follicles in the cervix and age, parity, gravity of the patient or menstrual phase of the endometrium.

Embryonic mesonephric duct remnants had been recognized in the cervix uteri by Meyer (1924). These mesonephric remnants according to Huffman (1948) are, for the most part, located in the middle portion of the cervical wall or near the mucosa in the region of the internal os. These structures consist of small tubules or canaliculi lined by low columnar, non secretory epithelium consisting of cuboidal cells with translucent pale cytoplasm and large ovoid, or round nuclei and termed mesonephric duct remnants.

In the human uterus these mesonephric remnants can rarely be demonstrated since the duct infrequently penetrates the uterus above the external os. Generally these vestiges are patchy and fragmentary in the cervix and are probably not found in more than 1 per cent of specimens (Novak *et al.*, 1954).

Cyclic changes in the cervix

Hamilton (1949) studied the cervical mucosa of the monkey. Her findings indicate that the height of the epithelial cells varies with the phases of the cycle, more particularly with the titre of blood estrogens. Three peaks in cell height followed by three declines in cell height correspond to the waves of the rise and fall in blood estrogens. The peaks in cell height mark the degree of accumulation of mucus in the luminal end of the cells, which slough off when no longer supported by the circulating estrogen.

Previously Markee and Berg (1944) suggested the following interpretation of the cause of three peaks in the estrogen level. The first peak with the onset of a new menstrual cycle is caused by several ovarian follicles which begin to develop and secrete estrogen. This is followed by the atresia of all the follicles except one resulting in a drop in blood estrogen and a lowering of the cervical epithelium.

The second and the highest peak is due to the secretory activity of a single follicle destined to ovulate. With the rupture of this follicle a marked drop in estrogen output occurs followed by complete sloughing of the mucus tops of the cells. This mucus is the midinterval or ovulating

With the maturation of the corpus luteum estrogen along with progesterone is secreted by the ovary causing the third accumulation of mucus in the cervical cells. With the premenstrual involution of the corpus luteum there is again a return to the low columnar or even cuboidal stage.

Hartman (1962) is of the opinion that while no clear-cut and orderly sequence of cyclic changes has yet been demonstrated in the human cervix, enough stages appear in well illustrated microphotographs in literature on the subject to indicate that there actually is a cycle in the human endocervix. This has not yet been clearly demonstrated because of the difficulties in securing undamaged specimens for study as has been possible in the rat, the guinea pig, the cow and the monkey.

Definite recognizable cyclic changes in the cervix of the guinea pig have been investigated by Jurow (1943). These alterations follow very closely the variations present in the other parts of the genital tract, especially in the vagina. Most characteristic findings are present in the lower cervix, although they are by no means absent in the upper part.

Hamilton (1947) reported on the cyclic changes in the cervix uteri of the rat. Two parts of the cervix, the upper and the lower part, have been described. The lower part of the epithelium has horny scales which become part of the genital discharge and appear as an element of vaginal smear in estrus. The upper part of the cervix has a layer of columnar epithelium.

In their studies of the morphological matrix in the uterine cervix of the mouse, Leppi _et al_. (1968) have observed that injections of estradiol benzoate and Releasin in the ovariectomized mice activated the fibroblasts, characterized by extensive dilated rough surfaced endoplasmic reticulum and a prominent Golgi.

Graham (1968) induced epidermization in the mouse uterine cervix by implanting 10 per cent diethylstilbesterol pellets into 3-4 month old mice. He compared his results with untreated control animals and with 1 month old diethylstilbestrol treated animals of his previous experiment. It was observed that new stratified epithelium appeared in the cervices of older animals after shorter periods of treatment than in younger animals. No evidence of metaplasia was detected and it was concluded that there is an age factor influencing the development stilbestrol induced stratified epithelium since evidence of metaplasia is associated with this process only in the young animals.

The cervix of the cow and its cyclic changes have been studied by a number of investigators. According to Hammond (1926) the epithelium near the external os uteri of the cow was thin and the superficial layers consisted of tall columnar mucous secreting cells with many goblet cells interspersed between them. Roark and Herman (1950) described the mucosa of the cervix in the form of many plica that had a central core of cell-poor connective tissue. These plica were lined by a single layer of mucoid epithelium which formed simple sacculated and branched tubular glands. The character of the epithelium varies considerably in different stages of the cycle. Secretory activity by the columnar epithelium of the cervix was formed at all stages of the cycle.

Herrick (1951) indicated four annular folds of mucous membrane arranged spirally. Throughout the length of these folds, the mucosa is thrown up in secondary, tertiary and often quarternary folds. These folds are lined with simple columnar, nonciliated mucus producing epithelial cells. Each cell constitutes a unicellular gland. Due to the folding of the mucous membrane of the cervix, the epithelium is arranged so that it appears to contain many branched tubular glands, Stratified epithelium appears in the base of each fold. The squamous epithelium of the vagina joins the columnar epithelium of the cervix near the portio vaginalis.

Cyclic changes in the cervix of the cow

Estrus: The findings of Roark and Herman (1950) indicate that the cells were tall, columnar, with basally crowded and elongated nuclei, with a height of 15-24 to 10-16 microns. They stained intensely with mucicarmine. Lymphocytes were noted in both the stroma and the epithelium but polymorphonuclear leucocytes were lacking. Stroma was edematous. Herrick (1951) noted that the nuclei were absent in large numbers of cells. Edema in lamina propria and increase in size of vessels was observed.

Metestrus: Loaded stage of mucous cells disappear and desquamation of epithelium is seen. Cells at distal ends of the folds were the first and those at the base of the crypts the last to empty their contents into the lumen of the cervix. Considerable regenerative activity of the epithelium is seen near the basement membrane. Less congestion and collapsed vessels were seen in the loose connective tissue propria (Herrick, 1951). Roark and Herman (1950) also gave a similar account of the cervix in metestrus.

Diestrus: For diestrus their identical views are as follows: Diestrus is marked as a morphologically inactive period. Epithelial cells were reduced in size and stained lightly with mucicarmine at the periphery. The epithelium at this stage of the cycle was characteristic of the larger part of the interestrual period. The height of the cells varied from 12 to 20 microns.

The lamina propria appeared compact with tight bundles of connective tissue fiber. The larger vessels appeared collapsed and the smaller ones indistinct.

Proestrus: Evidence of epithelial regeneration with change in form from cuboidal to columnar is seen. Gradual increase in mucus secretion is manifested by intense staining of epithelial cells with mucicarmine. The connective tissue stroma appeared loose with slight edema and congestion as a result of increase in diameter of the vessels.

## Vagina

The vaginal mucosa has been studied in the pig, cow, guinea pig, mouse, and rat by many investigators for cyclic morphological changes and for the diagnosis of pregnancy.

The wall of the vagina consists of a mucosa submucosa, a tunica muscularis and an adventitia or cranially, a serosa with a muscularis serosa. The mean length of the vagina according to Cowan and Macpherson (1966) is about 20 cm in normal sows, and slightly over 15 cm. in pubertal and prepubertal gilts. Longitudinal and circular folds are seen in the vaginal mucosa which bears a stratified epithelium whose appearance is altered in various species by the sexual cycle (Trautman and Fiebiger, 1957). The lamina propria does not form pa pillary bodies and a rich cellular area is seen under the epithelium. The submucosa is loose.

The tunica muscularis usually consists of a thick inner circular layer and a thin outer longitudinal layer. In the sow, according to Trautman and Fiebiger (1957), a thin layer of longitudinal muscle is found inside the circular layer. There is no demarcation between the uterine and the vaginal muscles except that the circular layer thickens considerably in the region of the cervical prominences, whose basis it forms.

The collagenous adventitia contains large vessels, nerves and ganglia.

Glowinski (1958) described clear cells in the mucosa of the fallopian tubes, normal and pregnant uteri and the vagina of the human. He suggests a hormonal function of these cells with a more or less broad spectrum (endocrine, paracrine, and enzymatic). In many organs this function was demonstrated histochemically. In other organs clinical and laboratory findings justified the assumption of this concept for the explanation of the mechanism of hormonal action on effectors.

In addition to the clear cells, Hoffmeister and Rupec (1968) have demonstrated Langerhan's cells in the epithelium of the normal human vagina with the electron microscope. These cells are found in different levels of the stratum spinosum. The cell has an indented, often V or X shaped, nucleus and is cytoplasm poor in contrast. Desmosomal connections with neighboring cells are not present. Rod shaped organelles with a periodicity of 90-100 Angstroms were seen as characteristic of this cell. Membrane bound granules corresponding ultrastructurally to the promelanosomes were present. Golgi apparatus, mitochondria, numerous vesicles and the lack of tonofibrils agree with the previous descriptions of the cells of Langerhan.

The human vagina also reveals anovulatory cycles many months prior to menarche (Wied and Keebler, 1967). The presence of a completely atrophic cell pattern generally indicates that normal menarche will not occur within one year. The presence of highly proliferated cell types in early childhood i.e., over 4 years prior to expected menarche, warrants examination of the individual for the possible presence of luteoma or other abnormal hormonal producing conditions.

Cyclic changes in the vaginal mucosa

Proestrus: The vaginal epithelium corresponding to the proestrus stage of the cycle consisted, on an average of 10 or more layers of cells (Bal *et al.*,1969). The findings of McKenzie (1926), Wilson (1926), and DeBois *et al.* (1965) were similar. According to Done and Heard (1968) the epithelium proliferates rapidly, reaching a depth of 16 to 20 cells during late estrus with numerous crypts. Palmer *et al.* (1965) observed 12 to 15 layers of cells 3 to 4 days after post weaning of the sows and also reported invasion of the epithelial layer by neutrophils. They believe that this state is similar to proestrus in the sow.

Estrus: Cornification of the vaginal epithelium during estrus has been reported in the guinea pig by Stockard and Papanicolaou, (1917), Courrier (1923), Burges and Wislocki (1956) and Leonardelli (1961). Long and Evans (1922) made similar observations on the rat and Allen (1922) on the mouse. Casida and McKenzie (1932) have stated that some cornification is present in all stages of the late estrum and metestrum accompanied by pronounced desquamation. Brown (1944) stated that the vaginal epithelium of the sow thickens during estrus and described columnar cells on the superficial layer. No distinct cornification was observed in the epithelium of the sow by McKenzie (1926), DeBois *et al.* (1965), and our own observations (Bal *et al.*, 1969).

Metestrus: This phase can be regarded as the initial degenerative stage of the vaginal epithelium in the sow with an increasing tide of leucocyte-like cells. Done and Heard (1968) have stated that the depth of the epithelium decreases, sharply at first and then more slowly to 3 or 4 cells during metestrus and diestrus.

Diestrus: The epithelial layers are somewhat reduced or the same as the previous stage of the cycle except that there is no cornification. Wilson (1926) describes vacuolar degeneration just a week after estrus and states that by the second week after estrus the vacuolar degeneration ends. No intraepithelial leucocytes are reported.

Nellor and Brown (1966) regard the leucocyte-like cells (hitherto described by most of the authors as neutrophils) to be degenerating plasma cells. Gansler (1954) suggests that these cells arise from the genital tract itself and do not migrate from the vascular system. Recently a plasma cell cycle in the hamster vagina has been reported by Devargus (1968). Cyclical variation of the plasma cells parallel to the level of estrogen exists in the mucosa under normal and experimental conditions. At estrus the lamina propria shows a small number of plasma cells. At diestrus the plasma cells increase in number and accumulate beneath the epithelium. A few plasma cells may be found between the epithelial cells. The concentration of plasma cells and their migration are greater still after castration. Administration of estrogen to castrated animals provides a remarkable decrease in the number of plasma cells situated in the lamina propria and their disappearance from the epithelium. He suggests that these phenomena may be related to an immunological response situated in the vaginal mucosa.

Zupp (1924) reported on the estrus flow in the pig. The amount and nature of secretion varies, mucus being most abundant at heat, while just before heat there is only enough to moisten the walls. During heat the epithelial cells are small, regular, stain well and show a distinct nucleus. They soon increase in size and show degeneration and cornification. The leucocytes are normal at first but soon show degeneration and are present for only a few days.

Howe (1967) studied the leucocytic response to spermatozoa in segments of the ligated rabbit vagina. The rabbit semen induced a leucocytic response in the vagina, uterus, and oviduct. There was active phagocytic engulfment of spermatozoa in all ligated segments 8 to 16 hours after artificial insemination accompanied by a progressive decrease in the number of spermatozoa. Although there was a complete absence of spermatozoa in all smears obtained 24 hours after injection a high leucocyte response was still present in all 48 hour smears.

Vaginal epithelium of the sow in pregnancy

DeBois *et al*. (1965) have observed in their study that the vaginal epithelium of the sow is reduced to 2 layers during pregnancy. This phenomenon, according to these authors, provides the basis for the histological pregnancy test.

Walker (1967) indicated that the typical pregnancy epithelium of the sow is regular and flat with a fairly straight basement membrane, has a height of about 12 to 15 microns and characteristically shows nuclei arranged in clear rows of two or three parallel to the basement membrane. In many biopsies pregnancy epithelial cells show a ready tendency to become detached. The height may be as low as five microns or as high, especially in late pregnancy, as the cyclic type of epithelium. Cells may be polyhedral, columnar, or flattened and all types may be present in a single biopsy. Some low resting diestrus epithelium can be easily confused with the pregnant state when a high proportion of nuclei show degenerative changes.

According to Done and Heard (1968) the vaginal epithelium of the pregnant sow comprises two, or at the most, three layers of cells arranged regularly. These cells are at first cuboidal, but by the third month of gestation they are often flattened with densely staining nuclei. A cellular pattern of pregnancy becomes apparent 14 days after mating in some sows, but uniformity for accurate diagnosis is established by about 30 days. During the last three weeks there may be some increase in the number of cells and some irregularity in their arrangement. This may be due to circulating estrogens of placental origin. Raeside (1963b) estimated high levels of oestrones nearing the end of the gestation period in the sow.

Loeb et al. (1938) investigated the effect of age and estrogen on the vagina of the mouse. Under normal conditions an increase in the amount of collagen in the stroma of the vagina, cervix and uterus of mice begins in the first few weeks of life and from then on progresses more definitely in older mice, especially after cessation of the sexually active period. The differences found at different age periods are relatively not great as compared with those experimentally produced through the administration of estrogen. Injections of estrogens over long periods increase the amount of fibrous hyaline substances which act as foreign bodies and cause the formation of epitheloid and giant cells and an ingrowth of connective tissue.

# MATERIALS AND METHODS

The tissue samples of the tubular genitalia were obtained from sows bred at the Swine Farm, Iowa State University, through the Department of Animal Sciences. The animals were in normal health as observed by the usual standards of antemortem examination.

Investigation of age changes would not be meaningful if the animals to be studied were not bred in the same environment and raised on a regular, controlled diet. Therefore the breeding, age, and dietary records of the sows used in this study have been maintained in the Department of Veterinary Anatomy. The need for such controlled studies have been emphasized by Cammermeyer (1963). Weights of the animals and most of their organs were recorded before killing. The pigs were killed by electrocution. A heavy rubber cord cable was attached to two electrodes. One electrode was attached to the upper lip and the other inserted in the anus to be attached to the anal mucosa. Shocks from 110 volts alternating current were applied for about one minute. The animals were exsanguinated immediately after electrocution by exposing and cutting the axillary artery and vein.

As soon as the bleeding ceased, the animals were eviscerated and tissues from the tubular genitalia were collected in the following manner:
Fallopian tubes: Tissue samples were taken from all three regions: the infundibulum, the ampulla, and the isthmus.
Uterus: Tissues were taken from the middle of the uterine horns and close to the body of the uterus.

Cervix: Tissue samples were taken from cranial and caudal parts (portio vaginalis) of the cervix.

Vagina: Sections of the vagina were taken cranial to the urethral opening. The tissues were fixed in 10% buffered formalin solution within 15 minutes after the death of the animals.

Sixty-six pigs were utilized in this investigation. Age and breed of the sows are presented in Table 1. Blocks of the tissues from Fallopian tubes, uterus, cervix and vagina were made by embedding them in paraffin according to standard histological techniques. Sections 6 microns in thickness were prepared. In the case of the cervix and the Fallopian tubes, serial sections of 6 microns were cut to study these areas. From birth to six months tissue sections were stained with the following stains:

1. Hematoxylin and eosin
2. Crossman's modification of Mallory's triple stain (Crossman, 1937)
3. Getty's Liver technique using the combination of Weigert's, Van Gieson's and Heidenhain's stains.

From the age of one year the following stains, in addition to the above mentioned stains, were used:

4. Verhoeff's elastic stain. Picro-indigo carmine and Van Gieson were used as counter stain (Pearse, 1961).
5. Von Kosa's stain for calcium (Armed Forces Institute of Pathology, 1960)
6. Oil-Red-O as modified by Bell (1959).

References regarding the detailed procedures of the staining techniques employed in this work have been cited and are available in the Library,

Table 1. Age and breed of sows

| Identification no. of the pig | Breed | Age | Number |
|---|---|---|---|
| 2 | Yorkshire | 1 day | 1 |
| 3 | Yorkshire | 1 day | 1 |
| 4 | Yorkshire | 1 day | 1 |
| 1243 | Yorkshire | 1 week | 1 |
| 1260 | Yorkshire | 1 week | 1 |
| 1263 | Yorkshire | 1 week | 1 |
| 1320 | York and Ch.White | 2 weeks | 1 |
| 1322 | York and Ch.White | 2 weeks | 1 |
| 1329 | York and Ch.White | 2 weeks | 1 |
| 1652 | Duroc | 1 mo. 1 week | 1 |
| 1653 | Duroc | 1 mo. 1 week | 1 |
| 1662 | W.D.D.P.L. | 1 mo. 1 week | 1 |
| 68 | Ch.White | 2 mos. | 1 |
| 69 | Ch.White | 2 mos. | 1 |
| 92 | Ch.White | 2 mos. | 1 |
| 627 | Pc-York.,L.R. | 2 mos. | 1 |
| 1651 | Duroc | 2 mos. 2 weeks | 1 |
| 1680 | Duroc | 2 mos. 2 weeks | 1 |
| 1682 | Pl.China.Landrace | 2 mos. 2 weeks | 1 |
| Y650 | Yorkshire | 3 mos. | 1 |
| 708 | Ch.White | 3 mos. | 1 |
| 716 | Ch.White | 3 mos. | 1 |

Table 1. (Continued)

| Identification no. of the pig | Breed | Age | Number |
|---|---|---|---|
| 2250 | Pc-York.,L.R. | 4 mos. | 1 |
| 9713 | Pc-York.,L.R. | 4 mos. | 1 |
| 1292 | Yorkshire | 6 mos. | 1 |
| 9310 | Pc-York.,L.R. | 6 mos. | 1 |
| 3430-S | York., L.R. | 1 yr 2 mos. | 1 |
| 5930-S | York., L.R. | 1 yr. 2 mos. | 1 |
| 2944 | York., L.R. | 1 yr. 3 mos. | 1 |
| 2021-S | York., L.R. | 1 yr. 5 mos. | 1 |
| 6333 | York., L.R. | 1 yr. 7 mos. | 1 |
| 3521 | Yorkshire | 1 yr. 8 mos. | 1 |
| 6153 | York., L.R. | 2 yrs. | 1 |
| 6154 | York., L.R. | 2 yrs. | 1 |
| 2442 | York., L.R. | 2 yrs. 2 mos. | 1 |
| 2443 | York., L.R. | 2 yrs. 2 mos. | 1 |
| 2156 | Poland China | 2 yrs. 6 mos. | 1 |
| ISU951 | Landrace | 2 yrs. 6 mos. | 1 |
| 632 | Landrace | 2 yrs. 6 mos. | 1 |
| 3202 | York., L.R. | 2 yrs. 7 mos. | 1 |
| 3196 | York., L.R. | 2 yrs. 9 mos. | 1 |
| 1040 | Chester White | 2 yrs. 10 mos. | 1 |
| 1361 | York., L.R. | 3 yrs. 1 mo. | 1 |
| ISU-39 | Landrace | 3 yrs. 7 mos. | 1 |
| 4876 | Landrace | 3 yrs. 7 mos. | 1 |

Table 1. (Continued)

| Identification no. of the pig | Breed | Age | Number |
|---|---|---|---|
| 4878 | Landrace | 3 yrs. 7 mos. | 1 |
| 5335 | Chester White | 3 yrs. 7 mos. | 1 |
| 4915 | Chester White | 4 yrs. | 1 |
| 4919 | Chester White | 4 yrs. | 1 |
| ISU-4966 | Landrace | 4 yrs | 1 |
| BB-1 | Farmer's hybrid | 4 yrs. 6 mos. | 1 |
| BB-2 | Farmer's hybrid | 4 yrs. 6 mos. | 1 |
| BB-3 | Farmer's hybrid | 4 yrs. 6 mos. | 1 |
| 392 | Landrace | 4 yrs. 7 mos. | 1 |
| 5898 | Yorkshire | 4 yrs. 11 mos. | 1 |
| 6015 | Yorkshire | 4 yrs. 11 mos. | 1 |
| 5895 | Landrace | 6 yrs. | 1 |
| 4583 | York., L.R. | 6 yrs. | 1 |
| 6043 | Landrace | 6 yrs. | 1 |
| 5815 | Landrace | 6 yrs. 3 mos. | 1 |
| 221 | Landrace | 6 yrs. 5 mos. | 1 |
| 312 | York., L.R. | 6 yrs. 9 mos. | 1 |
| 13 | Chester White | 7 yrs. 9 mos. | 1 |
| 1 | Chester White | 7 yrs. 9 mos. | 1 |
| LJF 1 | Yorkshire | 8 yrs. | 1 |
| 54-1175 | Landrace | 8 yrs. | 1 |
| | Total | | 66 |

except for Getty's Liver technique, the detailed procedure of which is as follows.

Dr. Getty used the following three variations of this technique on the liver.

1. Weigert's, Harris' Hematoxylin, and Van Giesen's.

2. Weigert's, and Van Giesen's.

3. Heidenhain's, and Van Giesen's.

Weigert's (modified) Elastic Tissue Stain:

Crystal violet ----------- 2 grams
Dextrin -------------------0.5 grams
Resorcinol ---------------- 4 grams
Distilled water ----------200 cc.

Bring to boil in enamel dish. Add 25 cc of 29% aqueous solution of Ferric Chloride. Stir and boil 2 - 5 min. Cool and filter. Discard filtrate and dry precipitate. Return filter paper and ppt. to dish. Add 200 cc of 95% alcohol and heat. Stir constantly. Remove filter paper when ppt. is dissolved. Cool. Filter and add 95% alcohol to make 200 cc. Add 8 cc of 4% HCL.

Mordanting solution -----------------------------5% Iron Alum

Heidenhain staining solution:

Water ----------------------- 80 cc.
Hematoxylin ----------------- 1 gram
Glycerin -------------------- 10 cc.
Alcohol --------------------- 10 cc.

The most convenient method for doing this is to prepare a 10 percent solution of hematoxylin in alcohol and then dilute this with the glycerin in water immediately before use. This stain is very slow if used cold, so that it is customary to heat both the mordanting and staining solutions to about 50 degrees centigrade before use.

Differentiating solution:

| | |
|---|---|
| Alcohol | 65 cc. |
| Water | 35 cc. |
| Picric acid | 0.5 gram |

Van Giesen's picric acid fuchsin stain:

| | |
|---|---|
| Acid fuchsin 0.1% aqueous solution (Acid fuchsin 0.5 gram, distilled water 100 cc.) | 5cc. |
| Picric acid, saturated aqueous solution about | 100cc. |

The following Heidenhain's iron hematoxylin may be used instead of the above Regaud's Heidenhain but slides must be left a longer time in Sol. 1. Ferric alum - average time 1 hour - 3 hours. (Can be left overnight.) After slides are removed from ferric alum they should be washed thoroughly in water (10 min to 1 hr.). Excess Fe-Alum injures Sol. 2 and turns hematoxylin black. Stain in Heidenhain hematoxylin 1 hr., 12 hrs., or overnight. Differentiate in another solution of Fe-Alum 10 to 30 seconds. To determine the time inspect the slide. A dull grayish hue is correct. Quick method - Destain in saturated solution of picric acid. Wash in running water 1 hour.

Heidenhain's iron hematoxylin:

| | | | |
|---|---|---|---|
| Sol. I. | Ferric alum | 2.5 gm. | (Ammonium ferric sulfate) |
| | Distilled water | 100 cc. | |
| Sol. II. | Hematoxylin | 0.5 gm. | |
| | Alcohol 95% | 10 cc. | |
| | Distilled water | 90 cc. | |

<u>Staining procedure:</u>

1. Xylol ------------------------------------------ 2 minutes

2. Absolute alcohol ------------------------------ 2 minutes

3. Alcohol 95% ------------------------------------ 2 minutes

4. Alcohol 70% ------------------------------------ 2 minutes

5. Running tap water ------------------------------ 2 minutes

6. Distilled water -------------------------------- 5 minutes

7. Weigert's (modified) elastic tissue stain------ 45-60 minutes

8. Wash quickly in 3 changes of 95% alcohol ------

9. Running tap water ------------------------------ 2 minutes

10. Distilled water ------------------------------- 5 minutes

11. Transfer the slides to the mordanting solution (5% Ammonium ferric sulfate) for -------------- 30 minutes at 50 degrees centigrade or overnight at room temperature.

12. Rinse each slide in distilled water to avoid carrying over too much of the mordant into the staining solution.

13. Transfer the slides to the Heidenhain staining solution for ------------------------------ 30 minutes at 50 degrees centigrade or overnight at room temp.

14. Transfer the slides to distilled water and wash until no more stain comes away.

15. Dip each slide up and down in the differentiating solution until it appears to be partly differentiated and transfer to tap water until no more color comes away. (A dull grayish hue is correct.) You may examine the slide under the scope. If further differentiation, continue to repeat the process.
16. Transfer all the slides to tap water until they have turned blue. If the tap water becomes yellow from traces of picric acid, it should be changed or differentiation will continue. If tap water won't blue slides, a pinch of sodium bicarbonate should be added to a coplin jar containing the tap water.

17. Van Giesen's --------------------------------- 15 to 30 minutes

18. Alcohol 95% (Wash quickly) ----------------------

19. Absolute alcohol - 2 changes

20. Xylol - 2 changes

21. Mount cover slip.

It was observed that the tunica muscularis of the Fallopian tubes and the uterus is not differentiated after birth. In order to determine the growth pattern, measurements of the different layers of the uterine wall were taken with a Bausch and Lomb micrometer. The micrometer was calibrated to a Bausch and Lomb binocular microscope. The measurements of the different layers of the uterine wall taken about the middle of the uterine horn are presented in Table 2. The layers of the uterine wall (endometrium, inner circular layer and outer longitudinal layer) were measured at least at five places around each layer and mean values were recorded. While drawing the growth curves (Figures 1, 2, and 3) mean thickness of each layer of the different age groups were plotted. In order to correlate the growth curve of the uterus from birth to 6 month with the ovaries, the ovarian weights of the animals studied have also been shown in Table 2.

It has already been mentioned that tissues from the Fallopian tubes were collected from three different regions viz. the infundibulum, the ampulla and the isthmus. Most of the sections were taken from the ampulla. Between the ages of 37 days to 75 days, only one tissue sample from the region of the ampulla was taken and the rest of the tissue was collected from the infundibulum and the isthmus. In spite of this missing link in the region of the ampulla, mean measurements of the muscular tunic of the ampulla of the rest of the age groups is presented in Table 3.

# OBSERVATIONS

## Uterine Tubes

One day postnatal (Pig nos. 2, 3 and 4)

In a day old gilt, the tunica mucosa-submucosa (as muscularis mucosae is absent) is lined mostly with tall, simple, columnar epithelial cells. In some of the areas, however, the epithelium becomes pseudostratified. Cilia are seen projecting from some of the epithelial surfaces. The cells measured on the average 11-12 microns in height. It also appears that there is an apocrine secretory activity in the epithelium (Figure 5). Basophilic globules (nuclear fragments) are seen separating from the distal extremity of the epithelial cells which are non-ciliated secretory cells. Some large clear cells are seen between the surface epithelium. The nuclei of these cells are centrally located and a clear area represents the cytoplasm which is either unstained or dissolved during staining procedures. Lamina propria: it consists of cellular elements well supplied with a rich bed of blood vessels. Long finger-like villi or fimbria are seen projecting into the lumen-covered by epithelium. Some of the large fimbria appear to be branching.

The external tunic: It appears that this outermost tunic of the Fallopian tubes consists of dense cellular elements. Their nuclei have a condensed chromatin. The intercellular substance appears to be constituted of collagen mostly. In the adult sow, this layer is composed of smooth muscle cells forming the tunica muscularis. No definite pattern of cells is seen nor was there any evidence of mitosis in these cells. One is therefore inclined to assume that these cells have not reached a final stage of differentiation into a muscular coat (Figure 4).

The tunica media of the largest arteries, seen towards the mesenteric border (mesosalpinx), was composed of one to two cells of smooth muscle. The internal elastic lamina of these arteries was distinct and the lumen of these arteries was lined with endothelium.

<u>One week post-natal: (pig nos. 1243, 1260 and 1263)</u>

The following changes were observed from the one day old age group:

1. Mucous membrane: the fimbria of the uterine tubes grow longer and their branching increases. The lamina propria appears more vascularized. A few lymphocyte-like cells are observed in the propria.
2. The external tunic exhibits a regular arrangement of densely packed cells in a circular pattern forming a thick layer. Outside this circular layer a thin layer of undifferentiated cells appear to be arranged in a longitudinal manner. Tunica serosa invests the whole of the uterine (Fallopian) tubes.

The arteries in the tunica serosa are small, their walls consisting of one to two layers of smooth muscle. A distinct internal elastic lamina is observed. The cells of the tunica media are not arranged in any definite pattern. The tunica adventitia of these vessels is composed mostly of collagenous fibrous tissue.

The veins appear as sinuses lined with endothelium, around which is the connective tissue of the lamina propria as shown also in Figure 12.

The largest vessels appear between the inner circular layer and the outer longitudinal layer of the tunica muscularis.

Two weeks to one month post natal (pig nos. 1320, 1322, 1329, 1652, 1653 and 1662)

In this age group, the following conspicuous changes in the Fallopian tubes are seen as compared to the previous age groups described above.

1. Increase in the connective tissue of the tunica mucosa which also extends into the core of the villi or fimbria.
2. Further growth of the fimbria forming anastomotic connections with each other.
3. Enlargement and growth of the tunica muscularis.
4. Increase in number and growth of the vessels in the tunica muscularis.
5. Enlargement of vessels in the mesosalpinx (Figure 6) supplying the uterine tubes.

Two months to three months post natal (pig nos. 68, 69, 92, 627, 1651, 1680, 1682, 1650, 708 and 716)

In this age group increased infiltration of mononuclear leucocytes is seen in the lamina propria. Some mononuclear cells with a granular eosinophilic cytoplasm were also noticed.

The lymphatic and venous sinuses appear enlarged in the mucous membrane of the uterine (Fallopian) tubes. The fimbria grow in size forming anastomotic connections with the adjoining processes (Figure 7).

Further growth of the tunica muscularis is evident which is thicker towards the isthmus where the fimbria become short (Figure 8).

Four months to six months post natal (pig nos. 2250, 9713, 1292 and 9310)

The gilt reaches puberty at this age interval. The remarkable change seen in this age group is that the lamina propria increases in thickness forming the core of the villi or fimbria (Figure 10). Many plasma cells are seen in this layer. Some mononuclear leucocyte-like cells are seen in the epithelium.

One of the specimens, sow No. 1292, 6 months old, appeared to be in the estrus phase of the cycle at the time of its sacrifice. Congestion and edema was seen in the lamina propria. The epithelial cells are tall with a vesiculated nucleus. Ciliated, secretory and some peg-like cells with long thin nucleus with condensed chromatin are seen.

In the tunica muscularis, small arterioles are seen in increasing numbers, whereas in the younger age group only small capillaries are seen. The cells of the tunica muscularis increase in size. The nucleus of these differentiated smooth muscle cells, which had condensed chromatin in the younger age groups, appear vesiculated and less dense.

The main arteries coming through the mesosalpinx are of normal adult size (Figure 11).

One year two months (pig nos. 5930, 3430-S, and 2944-S)

The mucous membrane has many fimbria which are extensive at the ampulla and gradually diminish in length and size towards the isthmus or the tubo-uterine junction (Figure 8). The epithelium is mostly ciliated, simple, columnar epithelium and also appears pseudostratified in some areas. In specimen No. 3430-S there appeared to be an apocrine secretory activity. Eosinophilic cytoplasmic globules and nuclear fragments dissociated from the cell surfaces were observed as described in younger age groups.

The mucosa-submucosa is very thin. There is no muscularis mucosae. Most of the mucosal connective tissue is seen in the fimbria. Specimen

No. 2944-S appeared to be in estrus. Some clear cell infiltration is seen and hyperplasia and congestion of mucosal connective tissue is evident. Epitheloid cells, plasma cells, and mononuclear leucocytes were observed in the tissue of the mucosa. In addition to these cells, fibrocytes with deep basophilic nuclei and a few multinucleated giant cells were present.

The tunica muscularis is very thin at the infundibulum and ampulla. It consists of inner circular and an outer longitudinal layer of smooth muscle. Most of the arteries are seen between the two muscle layers. A tunica serosa invests the tunica muscularis and continues as the mesosalpinx.

Vessels The arteries at the border of the mesosalpinx appeared normal with a distinct internal elastic laminae and tunica media, lymphatic and venous sinuses are present in the mucosal tissue (Figure 12).

One year 5 months to one year 8 months (pig nos. 2021-S, 6333 and 3521)

In this age group, the epithelium of the uterine tubes varies from simple columnar to pseudostratified columnar epithelium with ciliated and secretory cells. A few signs of apocrine secretion were seen. The connective tissue of the mucosa was rich in basophilic fibroblasts and oblong type of plasma cells. It appears that the plasma cells may be derived from the fibroblasts as postulated by Nellor (1963). In the region of the isthmus, the size of the fimbriae is reduced in length and they look more like villi simulating those of the intestines. These small fimbria appeared slightly wider. The connective tissue forming the lamina propria was infiltrated with plasma cells.

Arteries appeared normal in the wall of the tube and the mesosalpinx (Figure 13).

Two years two months of age (pig nos. 6154, 2442, 6153, and 2443)

In the region of the ampulla and upper part of the isthmus, long branching fimbria are present. The connective tissue of the mucosa has mononuclear and polymorphonuclear leucocytes. Detached cytoplasmic globules which stain eosinophilic were suggestive of apocrine secretion. Epithelium appeared similar to the previous age groups.

The tunica muscularis gradually thickens from the infundibulum towards the isthmus and the tubouterine junction. Unlike the epithelial surface on the upper part of the tube, apocrine secretion appears absent or fades towards the tubouterine junction.

Small arteries in the tunica muscularis at the points of their branching have thickened intimal cushions (Figure 14). The arteries at the mesosalpinx border appeared normal.

The walls of the larger veins have smooth muscle with concentrically arranged layers of elastic fibers. Venous and lymphatic sinuses are present as in previous age groups.

Two years six months of age (pig nos. ISU 951, 3202 and 632)

Sections at the ampulla show the same features as in the lower age groups such as long narrow fimbria. Apocrine secretion and a thin tunica muscularis are evident. In the sections at the lower region of the isthmus, the fimbria become smaller, thicker and comparatively reduced in number. More connective tissue is seen in the lamina propria. Apocrine secretion appears to diminish. The tunica muscularis gradually increases towards the uterus. In this age group some of the arteries at the salpingial border had a thickened tunica intima. The internal elastic lamina was somewhat interrupted and the smooth muscle in the tunica intima was arranged in a longitudinal pattern.

Two years ten months to three years one month of age (pig nos. 1040, 319b and 1361)

The salient changes in this age group were as follows as compared to the lower age groups. Arteries coming through the mesosalpinx are affected with thickening of the tunica intima. Subendothelial hyperplasia appears evident. Some arteries in the tunica muscularis also show slight thickening of the tunica intima (Figures 15, 16). Elastic fibers are seen in the fibrous walls of the veins.

Three years and seven months of age (pig nos. ISU 31, 4876, 4878 and 5335)

In this age group epithelial ingrowths into the lamina propria was seen. These ingrowths at this stage simulate glandular alveoli. The lamina propria appears somewhat thickened. Arteries at the border of the mesosalpinx appear affected with intimal thickening. The pattern of intimal thickening is similar to the arteries of two years six months of age group.

Four years of age (pig nos. 4915, 4966 and 4919)

The remarkable change in this age group is the growth of connective tissue in the lamina propria of the fimbria. Secondly, the epithelial ingrowths or invaginations from the surface epithelium are enhanced forming small cysts in the mucosa (Figure 17). The cystic invagination of the epithelium reaches deep into the tunica muscularis. Some small epithelial cysts are seen in the subepithelial mucosa as well as the tunica muscularis.

A few arterioles in the lamina propria and tunica muscularis indicated thickening of the tunica intima (Figure 18). Most of the arteries supplying the fallopian tubes at the salpingial border have thickened tunica intima which is more enhanced than in the previous age groups (Figure 19).

Four years six months of age (pig nos. BB1, 392, BB[ and BB3)

In this age group some clear cells were observed in the epithelium. Some epithelial cell nests and small cysts are seen in the tunica propria. Stray micro-cysts also appear in the tunica muscularis.

In the larger arteries seen in this section the tunica intima was thick.

Five years of age (pig nos. 5898 and 6015)

The veins in these specimens of the uterine tubes appear tortuous (Figure 20). Some of the veins show plaques in the tunica intima.

The arteries of the mesosalpinx show persistent thickening of the tunica intima.

Six years of age (pig nos. 5895, 6043, 4583 and 5815)

From the ovarian end of the uterine tubes down to the tubouterine junction, the following morphological changes are evident in this age group. The epithelium lining the fimbria and the base of fimbria have a tendency to invaginate into the underlying connective tissue of the tunica mucosa-submucosa (Figure 21). Small cysts and epithelial cell nests are noticed (Figure 24). In some tissue sections the epithelial cystic structures are seen in the deep tunica muscularis (Figure 24).

Arterioles affected with intimal thickening are seen in the tunica mucosa-submucosa as well as in the tunica muscularis.

The external longitudinal layer of the smooth muscle of tunica muscularis continues into the mesosalpinx enclosing the vessels and nerves supplying the uterine tubes. The arteries in the salpingial border which supply the uterine tubes are seen to be affected with intimal thickening to a considerable extent. The veins that drain the uterine tubes appear to follow a tortuous course.

Six years five months to six years nine months (pig nos. 221 and 312)

In this age group many small nests of epithelial cells invaginate into the tunica propria and the tunica muscularis (Figure 25). Some cyst formation is seen. Increased fibrosis is also seen in the tunica propria. Within the cystic cavity lined with the epithelial cells small fimbria-like processes are seen. This appears to be an attempt to form new fimbria with advancing age. Some clear cells are seen in the surface epithelium.

A number of arteries in the tunica propria and tunica muscularis exhibit with intimal thickening. Increased fibrosis is also seen in the tunica muscularis.

Seven years nine months to eight years three months (pig nos. 13, 1, L.J.F. 1 and 1175)

Most salient features of the morphological changes in this age group are as follows:

1. Increased number of epithelial nests and cysts in the tunica propria (Figure 30).
2. These cysts appear to invade the deeper layers of the tunica muscularis (Figure 31).
3. Increased fibrosis in the mucosa-submucosa.
4. Increased fibrosis in the tunica muscularis.
5. Thick intimal plaques also appear in the veins (Figures 28, 29).
6. Large arteries of the Fallopian tubes are severely affected with intimal thickening as compared to smaller arterioles in the tunica propria and the tunica muscularis (Figures 26, 27 and 32).

## Uterus

One day postnatal (pig nos. 2, 3 and 4)

In a cross section of the uterine horn, it is seen that the epithelium is pseudostratified columnar type to what appears as stratified epithelium. Near the body of the uterus (where the two horns fuse) the epithelium tends to become a simple columnar type.

In the region of the uterine horn, the epithelium appears in small folds. At the depressed area between the folds, the epithelial cell appears to be differentiating into glandular type of epithelium and beginning to invaginate into the subepithelial connective tissue. These epithelial cells appear somewhat vesiculated and their nuclear chromatin was granular, unlike the more condensed chromatin of the neighboring cells. There was more euchromatin in the nucleus. These areas can be looked upon as the points from which the glands of the uterine endometrium begin to develop and grow towards the myometrium (Figure 33).

Subepithelial layer of endometrium This layer of dense staining nuclei is composed of more cells than intercellular fibrous elements. Fine collagenous fibrils are present. Very few capillaries are seen in this layer. The glands are not seen in this layer, which appears to be composed of cells simulating fibroblasts or mesenchymal cells. These cells do not appear to be in an advanced stage of differentiation.

Primordial muscular layer or myometrium The subepithelial cellular layer blends into this layer without a distinct demarcation. This layer, corresponding to the myometrium of the adult sow, is densely packed with cells that appear different from the cells of the endometrium as seen in sections stained with Mallory's connective tissue stain. Nevertheless, they do not resemble the typical smooth muscle cell of the adult myometrium. The whole layer is composed of cells arranged in a circular pattern in cross-section. No distinct outer longitudinal layer is seen (Figure 33).

Surrounding this layer externally are cells that can be considered as cells of the subserous layer at this stage. These cells, forming about 2-3 layers, appear to be arranged in a longitudinal pattern and somewhat at a right angle to the circular layer of the myometrium. As these cells do not resemble typical smooth muscle of the adult, it can be assumed that they have not completely differentiated into smooth muscle cells of the myometrium.

Small vessels are seen in the subserous layer. The tunica media of the arteries was composed of one to two cell layers of smooth muscle. A thin serous coat invests the muscular layer or myometrium.

At the mesometrial border, a small remnant of the mesonephric duct is seen to persist in all of the specimens observed. This duct is lined by stratified cuboidal epithelium. A small amount of subepithelial connective tissue is seen which is surrounded by a muscular coat. This duct lies in very close approximation to the myometrium of the uterine horn.

Small arteries, of the size described above, and nerves are present descending towards the uterus in the folds of the serous membrane which is the broad ligament of the uterus. A day old uterus appears to be very well innervated, as shown in Figure 35.

<u>One to two weeks postnatal (pig nos. 1243, 1260, 1263, 1320, 1322 and 1329)</u>

<u>Epithelium</u> Epithelial lining of the uterine lumen appears in folds. As in the day old pig, glands are seen to be developing at the depression of the folds and appear more deeply invaginated in the subepithelial tissue (Figure 36). Mitotic figures are seen in the invaginating glandular epithelial tissue (Figure 36). The nuclei and cytoplasm of the primordial glandular cells appear vesiculated with no discernible heterochromatin.

Subepithelial layer of endometrium A layer of cells with dark staining nuclei is seen close to the epithelium. These cells can be easily differentiated from the other cells of the subepithelial endometrium whose nuclei do not stain so darkly. Mitotic figures are seen in these cells. The subepithelial cellular layer appears to be differentiating as a connective tissue layer of the endometrium.

Myometrium or muscular layer This consists of cells which crowd towards the periphery in a concentric or circular manner. The cytoplasm of these cells is more eosinophilic and their nuclei are dark and spindle shaped. These cells appear to be differentiating into an inner circular muscle layer.

Outside this circular layer is a layer of cells forming a thin subserous layer (Figure 37). Small vessels are seen between the circular layer of cells and the subserous layer. The vessels consist of small arteries, lymph capillaries or sinuses and venous sinuses. The lymph and venous sinuses are lined with endothelium surrounded by connective tissue. These vessels do not have a wall. Some larger vessels and nerves are present in the mesometrium towards the broad ligament. In sections of the uterus near the body, the mesonephric ducts with a muscular coat persist in this age group (Figure 35).

One month one week postnatal (pig nos. 1652, 1653 and 1662)

In the sections studied in this age group, all the layers constituting the uterine tube appear to be quite distinctly differentiated.

Endometrium The epithelium consists of a layer of simple columnar epithelial cells mostly with a tendency to become pseudostratified in a few regions. The subepithelial connective tissue or lamina propria consists of areolar connective tissue. Mononuclear lymphocyte-like cells with granular eosinophilic cytoplasm are also seen. The uterine glands which are present in the horns of the uterus are completely developed and extend deep to the inner circular layer of the myometrium (Figure 40). Near the body of the uterus where the two horns fuse, the glands tend to disappear and the mucous membrane appears in deep folds.

Myometrium This layer appears well differentiated into an inner circular layer of smooth muscle and an outer longitudinal layer (Figure 39). The outer longitudinal layer of smooth muscle is intermingled with more connective tissue and fibrocytes.

Vessels Vessels of the size of arterioles are seen between the two muscle layers of the myometrium. The tunica media (wall) of the arteries at the mesometrial border are composed of three to five layers of smooth muscle. Small veins and venous sinuses, along with lymphatics and nerves, are present in this region.

Two to two and one half months postnatal (pig nos. 68, 69, 92, 627, 1651, 1680 and 1682)

In the specimens of the tissue sections studied in this age group, it was observed that the endometrium is greatly developed as compared to the myometrium. The endometrium formed 2/3 of the wall thickness of the uterus. The mucosal folds appear deeper in this age group.

Increased number of mononuclear leucocyte-like cells appear in the endometrium. They are seen to be penetrating the surface epithelium as well as the glandular epithelium. In the surface epithelium they appear as clear cells (Figure 41). Eosinophil-like cells are also present. The nuclei of these cells are somewhat eccentric. In addition to the above mentioned cell types, fibroblasts with condensed nuclear chromatin are seen.

Myometrium In the myometrium, the largest of the vessels lie between the inner circular and outer longitudinal layer. Many lymphatic and venous sinuses are also seen in this area.

At this age the external longitudinal layer appears more developed and extends into the mesentery of the uterus forming the broad ligament. Between the folds formed by the longitudinal smooth muscle in the broad ligament, main vessels and nerves approaching the uterus are seen (Figure 42).

The mesonephric duct lined by cuboidal epithelium and invested with a thick muscular coat persists at this age.

Three months postnatal (pig nos. Y650, 708 and 716)

The following changes are seen:

1. There is an overall growth in the size of the uterus.
2. Most of the epithelial lining of the lumen is pseudostratified columnar to simple columnar with a few ciliated cells.
3. Mononuclear eosinophilic cells are seen in greater numbers than the previous age group. Some of these cells are binucleated.
4. The uterine glands are numerous and well developed.
5. In the connective tissue of the myometrium, mitotic figures are seen.

These could be dividing fibroblasts.

6. The mesonephric duct is seen to persist in this age group.

Four months postnatal (pig nos. 2250 and 9713)

A significant difference observed in this age group is that the larger size arterioles appear at the base of the endometrium adjoining the inner muscular layer of the myometrium (Figure 43). Vessels of this size are not seen in the younger age group.

Mononuclear eosinophilic cells are present but in lesser numbers.

In the endometrium large venous sinuses slightly engorged with blood can be seen. Lymphatic sinuses similar in structure to the venous sinuses are also present. A patent mesonephric duct was not observed in the sections of this age group.

Six months postnatal (pig nos. 1292 and 9310)

The uterus appears very well developed. The epithelium is pseudostratified columnar type. In specimens of this age group, the endometrium exhibits a granular layer of lymphocytic cells beneath the epithelium. Slightly intermingled with the lymphocytic cellular layer were fibroblasts with a basophilic nucleus having condensed chromatin.

Mononuclear cells with eosinophilic granules appear to be a normal feature of the endometrium.

In specimen no. 1292, the endometrium appeared edematous. Large veins, venous sinuses and lymphatics were engorged (Figure 44). This animal was probably in estrus.

In the myometrium an increased amount of connective tissue is seen. Large vessels and nerve bundles appear between the two muscle layers.

In the folds of the broad ligament into which the external longitudinal layer of the myometrium continues, well grown normal arteries (Figure 45), veins, venous sinuses and nerve bundles are present.

<u>One year two months of age (pig nos. 3430-S, 2944-S and 5930-S)</u>

Pig no. 3430-S was pregnant. The glands of the endometrium appeared with tall columnar cells.

The subepithelial layer consists of cellular elements as lymphocytes, fibrocytes and a few plasma cells. The fibrous elements are denser as compared to the rest of the endometrium which is constituted of areolar connective tissue in which are contained glands, arteries, lymphatics and venous sinuses (Figures 46, 47, and 65).

The myometrium consists of a thick outer longitudinal layer and a somewhat thinner inner circular layer. Tunica serosa and a subserosal connective tissue invests the myometrium. The outer tunica serosa along with the outer longitudinal layer of myometrium continues as the broad ligament of the uterus. On the whole, regular invaginations are formed by the myometrium. Specimen 3430-S was found to be pregnant (Figure 55). It was observed that the inner circular layer of the myometrium had accumulated a considerable amount of collagenous connective tissue which appears to be a normal function in pregnancy (Figure 56).

<u>One year five months to one year eight months (pig nos. 2021-S, 6333 and 3521)</u>

In this age group most of the epithelium lining the uterus is pseudostratified columnar epithelium. Clear cells are seen in the epithelium and the uterine glands exhibit low cuboidal epithelium, suggesting their inactive stage.

Some arteries in the endometrium have a thickened tunica intima at this age group (Figure 48). The internal elastic lamina is distinct but not the internal limiting membrane. In the larger endometrial arteries elastic fibers were seen intermingled in the cells of thickened tunica intima. In some of the affected arterioles the thickened intima is about half the cross sectional diameter of the vessel. Cells of the intima were arranged in a longitudinal pattern.

Veins have fine elastic fibers in their walls. It may be proper to call these veins elastic veins. The walls of these veins were well vascularized (Figure 49).

The large arteries supplying the uterus from the mesometrial border appear normal.

Two years two months of age (pig nos. 6153, 6154, 2442, and 2443)

Sections of specimen no. 6153 exhibit the manifestation of the proestrus stage of the cycle. The uterus appears rejuvenating with the following changes. The epithelium is somewhat taller with a subepithelial layer of the denser connective tissue with fibroblasts and mononuclear leucocyte-like cells. This layer would somewhat correspond to the granular layer described in age groups below 6 months. Clear cells are present in the epithelium similar to ones shown in Figure 42.

The upper part of the endometrium in this section is edematous. The vessels appear engorged with blood.

The glandular epithelium of the endometrium shows basophilia and are of cuboidal shape. A few clear cells appear in the glandular epithelium.

Studies of the vaginal epithelium of specimen no. 6154 are suggestive that the animal is in the post-estrus stage of the cycle. The endometrium appears shrunken. The glands of the uterus are closely packed together.

The common feature observed in the sections of this age group is that the endometrial arteries have thickened tunica intima (Figure 52). In some vessels increased amounts of collagen would suggest an intimal sclerosis. The elastic fibers are somewhat concentrically arranged in the tunica intima. Helical arteries are also seen in the myometrium.

Two years and six months of age (pig nos. ISU-951, 3202 and 632)

Sections of specimens ISU 951 and 632 represented a morphological picture of early proestrus. The more superficial part of the endometrium shows edema but the lower part toward the myometrium was denser. The cytoplasm of the glands appear basophilic.

The common feature in the specimen sections of this age group was the thickened tunica intima in the endometrial arteries (Figure 53). In the affected arteries considerable collagenous and elastic tissue was present. These arteries could be said to have intimal sclerosis.

In specimen no. 3202 the affected arterioles exhibited the intima reaching half the cross-sectional diameter of their walls (Figure 54).

Two years ten months to three years one month of age (pig nos. 1040, 3196 and 1361)

The epithelium lining the uterine cavity varies from simple columnar to pseudostratified columnar epithelium. The condition of the endometrium on the whole varies according to the stage of the cycle. Many mononuclear leucocytes resembling clear cells were seen. These cells have a clear halo around them and they also look like lymphocytes. Many lymphocytes were observed in the vessels and the source of these cells could be the hemopoietic tissue.

Almost all the endometrial arteries have a thickened intima in this age group (Figure 57). Collagenous and elastic tissue are seen in the intima.

Three years seven months of age (pig nos. 3196, 4878, 4876, and 5333)

The salient age change in the uterus of this age group is that all the arteries, large and small, show varying degrees of intimal sclerosis. There is also an increase of collagenous connective tissue in the tunica media of the affected arteries. It appears that veins are also affected with intimal thickening.

In the severely affected arterioles, the tunica intima formed 2/3 of the entire arterial wall in cross section.

Four years of age (pig nos. 4919 and 4966)

The uterine cavity is lined with simple columnar epithelium alternating with pseudostratified columnar epithelium. Clear cells, as usual, are seen in the epithelium.

Some increase in the connective tissue of the myometrium is seen. A few glands in the endometrium appear cystic and a considerable number of lymphocytes were observed. Arteries with thickened tunica intima are present. The cellular elements of the thickened tunica intima in some vessels appear to be synthesizing more elastic tissue and collagen as opposed to the tunica media which synthesizes only collagen.

In a few arterioles, the whole of the tunica media appears to have been infiltrated by elastic and collagenous tissue. This condition can be described as arteriosclerosis.

A few veins had a thickened tunica intima. A plaque in the vein appeared like a homogeneous mass of tissue devoid of cellular elements.

Four years 6 months of age (pig nos. BB1, BB2, BB3 and 392)

In sections of specimen no. BB2 taken close to the body of the uterus the endometrial glands appear to decrease. At this region a few endometrial arterioles show thickened tunica intima.

In specimens approaching estrus eosinophils were seen in the endometrium.

In smaller endometrial arteries, elastosis of the entire arterial wall was observed and elastic fibers were also seen to spread into the tunica adventitia.

The mesometrial arteries show thickening of the tunica intima. The walls of the veins contained elastic fibers.

Four years eleven months (pig nos. 5898 and 6015)

In the specimens studied in this age group the following changes were noted:

There was increased thickening of the tunica intima in the mesometrial arteries; in the endometrium entire walls of the arterioles appeared affected with elastosis. In the severely affected arterioles the intima formed two thirds of the cross sectional diameter of the arterial wall. The arterioles closer to the epithelium appeared more affected with intimal thickening (Figure 58).

Six years of age (pig nos. 5895, 6043, 4583 and 5815)

The epithelium is typical of lower age groups. The endometrium contained eosinophils, plasma cells, and lymphocytes with a halo around them simulating clear cells. The arterioles exhibit thickened tunica intima which formed 1/2 to 2/3 of the cross sectionaldiameter of the arterial wall.

The intimal thickening was seen in almost every artery in this age group (Figure 59). The tunica media contained more collagenous tissue as compared to the younger age groups. The sclerotic media then is a mixture of smooth muscle and collagen.

Increased elastosis was seen in the tunica media of the veins. Elastic fibers appear mixed with the collagenous fibers in the fibrous walls of the veins (Figure 60). Whether elastic fibers are elaborated by smooth muscle, elastoblast or fibroblast is questionable because not much smooth muscle was observed in the walls of these veins. (See discussion on blood vessels.)

Six years five months to six years nine months (pig nos. 221 and 312)

A salient morphological change observed in the age group was the presence of glandular cysts in the endometrium in close proximity to the myometrium. Compared to the younger age groups the intimal thickening in the vessels was more enhanced.

Seven years nine months to eight years three months (pig nos. 13, 1, L.J.F. 1 and 1175)

The endometrium shows the presence of eosinophils, and lymphocytes simulating clear cells. At the base of the endometrium the uterine glands became cystic (Figure 62). The epithelium lining the enlarged cystic cavity was flattened to form a simple squamous epithelium.

More elastosis was seen in the entire wall of the arterioles and it is difficult to identify the tunica media from the tunica intima. In very small arterioles, elastosis appeared to be spreading into the tunica adventitia (Figures 61, 64).

The mesometrial arteries were also severely affected (Figure 63). Increased collagenous and elastic fibrosis was seen in the veins.

Specimen no. L.J.F., 1 appeared to be in the early stages of pregnancy. Formed fetal membranes were observed apposing the uterine epithelium. Arterioles in the endometrium of this animal had thickened tunica intima but the elastic tissue in the intima appeared to have faded.

## Cervix

<u>One day postnatal (pig nos. 2, 3 and 4)</u>

The cervix after birth of the gilt does not appear to be well developed. The epithelium lining of the cervical canal consists of simple columnar epithelium to pseudostratified columnar epithelium. The mucous membrane appears in regular folds; no glands are present (Figure 66). Dark staining smaller cells having basophilic nuclei with condensed chromatin form a kind of subepithelial granular layer.

Most of the wall of the cervix is composed of fibroblast-like cells of the mesenchymal origin. The intercellular substance consists of collagenous fibers. Very few smooth muscle fibers are seen scattered towards the outer surface of the wall. In sections stained with Mallory's connective tissue stain, it was observed that some of the fibroblast-like cells appear to be differentiating into smooth muscle fibers.

The wall of the cervix is invested with areolar connective tissue in which small arteries, veins, lymph vessels and nerves are present.

The walls of the arteries supplying the cervix at this age is constituted of two to three layers of smooth muscle cells forming the tunica media (Figure 67). The paired embryonic mesonephric ducts can be observed in the wall of the cervix (Figure 66). Their structure is the same as described in their association with the uterus.

One to two weeks postnatal (pig nos. 1243, 1260, 1263, 1320, 1322 and 1329

The mucous membrane is lined by simple columnar epithelial cells. Many mitotic figures appear in the epithelium. A subepithelial granular layer described in one day old specimens also persists in this age group. No distinct demarcation appears between the lamina propria and tunica muscularis. The whole wall of the cervix is composed of undifferentiated dense fibroblasts of mesenchymal origin. Some cells appear to be differentiating into smooth muscle fibers which stain pink with Mallory's connective tissue stain.

Mononuclear eosinophil-like cells are seen in every part of the wall of the cervix. Some of these cells have a dumbbell shaped nucleus and a few others are binucleated. Toward the peripheral area of the wall the differentiating smooth muscle cells assume a circular pattern. Outside this circular layer, a layer of cells arranged in a longitudinal pattern are present. In two weeks old gilts small arteries are present in this layer along with veins.

The cervical wall is enveloped by areolar connective tissue in which arteries, veins and nerves are seen. Small ganglia are present in the adventitial layer (Figure 68). The nerve cell bodies of these ganglia have eccentric nuclei and are surrounded by satellite or capsule cells. Arteries supplying the cervix at this age have a distinct internal elastic lamina and the tunica media of the largest arteries consists of 4 to 6 cells of smooth muscle (Figure 69).

Veins have thin walls composed of a few cells of smooth muscle intermingled with elastic fibers.

Mesonephric ducts are patent lying embedded in the outer part of the wall of the cervix.

One month to two and one half months postnatal (pig nos. 1652, 1653, 1662, 68, 69, 92, 627, 1651, 1680 and 1682)

In this age group further growth of mucous membrane folds is seen. The epithelium is simple columnar to pseudostratified ciliated columnar lining the cervical canal. Basophilic fibroblasts with condensed nuclear chromatin and mononuclear cells form a subepithelial layer. The mucosa-submucosa which is composed of mostly areolar connective tissue blends with the tunica muscularis, which at this age is well differentiated into an inner circular and an outer longitudinal layer of smooth muscle fibers (Figure 71). Lymph nodules appear to be in the subepithelial connective tissue.

The arteries increase in growth as compared to the previous age group. They have a distinct internal elastic lamina, a tunica media and adventitia.

The mesonephric duct is incorporated into the tunica muscularis and lies between the inner circular layer and the outer longitudinal layer (Figure 70). It is composed of stratified cuboidal epithelium, a layer of subepithelial connective tissue and a tunica muscularis of intermingled smooth muscle fibers.

Nerve ganglia, arteries and veins are seen outside the muscular wall of the cervix in the tunica adventitia.

Three months postnatal (pig nos. Y650, 708 and 716)

No significant morphological difference in the cervix was seen as compared to the previous age groups. Most of the cervical canal is lined with simple columnar epithelium which appears partly ciliated. Plasma cells and mononuclear lymphocyte-like cells are seen in the epithelium as well as the subepithelial tissue. A few clear cells are also seen.

The embryonic mesonephric ducts persist in the muscular coat of the cervix.

Four months postnatal (pig nos. 2250 and 9713)

At this age, the epithelium is stratified cuboidal with a few ciliated cells (Figure 72), with a tendency to become stratified squamous type in the portis vaginalis. The epithelium is infiltrated with lymphocyte-like cells some of which appear to be undergoing pyknosis. A few mitotic figures are quite apparent in the epithelium. Eosophilic cells are present in the mucosa. The tunica muscularis is well developed and the smooth muscle fibers are divided into small bundles by the connective tissue. The arteries appear to have attained considerable growth then the previous age groups studied.

The mesonephric ducts persist in the tunica muscularis of the cervix.

Six months postnatal (pig nos. 1292 and 9310)

There is no significant difference in the morphology of the cervix as compared to the previous age--the cranial part of the cervix is lined with simple columnar epithelium (Figure 73). Larger lymph sinuses are observed in the lamina propria. The embryonic mesonephric ducts still persist. The blood vessels are well developed at this age.

One year two months (pig nos. 5930, 3430-S and 2944-S)

Generally the epithelium of the part of the cervix closer to the uterus is simple columnar type as shown in Figure 73. Long mucosal folds are seen in this region and the epithelial border appears serrated. In the tunica mucosa-submucosa mucous glands are present as shown also in Figure 76. These glands are absent in the tissue sections closer to the vagina. The epithelium of the cervix towards the vagina becomes stratified cuboidal. The mucosa-submucosa is composed of fibrous connective tissue. The cellular elements in the mucosa consist of fibrocytes, plasma cells and lymphocytes. Small arterioles and venous sinuses can also be seen.

The inner circular layer of the tunica muscularis has irregularly running smooth muscle bundles. More connective tissue is present in this layer than the smooth muscle.

The outer longitudinal layer of tunica muscularis has large smooth muscle bundles with dense connective tissue separating it. The arteries and veins appeared normal (Figure 75). Large nerves and collateral ganglia were seen in the tunica adventitia of the cervix.

Epithelial invaginations were seen in the mucosa of the cervix close to the vagina. Specimen No. 3430-S was pregnant. In this specimen more connective tissue was observed in the inner circular layer of the tunica muscularis (Figure 74).

One year five months to one year eight months (pig nos. 2021-S and 3521)

The epithelium is simple columnar beginning from the os uterus and becomes stratified towards the vagina. A subepithelial layer of lymphocytes and plasma cells was observed. These cells were also seen within the epithelium and thus appeared to be migrating through the surface epithelium. At certain areas these cells segregated to form subepithelial nodules in the mucosa.

In this age a slight thickening of the tunica intima was observed in the arterioles of the cervix.

The veins had elastic fibers of 4 to 6 layers arranged concentrically in the tunica media. It appears from the morphology of these veins that they are elastic veins.

Two years and two months of age (pig nos. 6154, 6153, 2442 and 2443)

The mucous membrane was the same as described for the younger age groups. The venous sinuses were conspicuous in the mucosa. Mucosal folds near the os uterus appeared comparatively thicker than in the younger age groups.

A stray artery showed a thick intimal cushion. Arterioles in the tunica muscularis were affected with intimal thickening along with the larger arteries.

Two years six months of age (pig nos. 2153, ISU 95, 632 and 3202)

A change observed in this age group was the invagination of the surface epithelium into the mucosa. Groups of epithelial cell nests were seen along with a few cysts formed by these epithelial invaginations (Figure 78).

Most of the vessels in this age group were normal except a few arterioles in the tunica muscularis which had a slight thickening of the tunica intima.

Two years ten months to three years one month (pig nos. 1040 and 3196)

The sections of these specimens were taken closer to the vagina. The epithelium was stratified cuboidal and invaginated into the mucosa forming epithelial nests and cyst-like structures. No mucous glands are present in the endometrium close to the vagina. The mucosa-submucosa is rich in vessels.

From the studies of the vaginal sections of specimens no. 1040, it was observed that the animal was in the diestrus stage of the cycle. It appears that the inner circular layer of the smooth muscle of tunica muscularis accumulates more collagenous connective tissue during the diestrus phase of the cycle.

Arteries in the tunica muscularis exhibited thickening of the tunica intima. In a cross section of these arteries the cells of the intima were arranged in a longitudinal pattern whereas the cells of the tunica media were in a circular pattern.

A different type of artery, which does not resemble a normal artery in a section of the cervix, was observed (Figures 80, 81). The inner half of this arterial wall was composed of smooth muscle arranged in a longitudinal pattern and was of a uniform thickness. Four to 5 layers of elastic fibers ran parallel in this part of the arterial wall.

The outer half of the arterial wall was composed of circularly arranged fibers of smooth muscle mixed with elastic and collagenous fibers. The inner part of the arterial wall was not morphologically characteristic of the tunica intima. In the normal arteries of the cervix, elastic fibers are rarely seen in the tunica media.

The veins of the cervix have valves (Figure 82). The walls of the veins are composed of smooth muscle, elastic and collagenous tissue. In fact the venous walls can be described as composed of fibro-muscular tissue.

Three years seven months of age (pig nos. ISU 31, 4878 and 4876)

In this age group no conspicuous change was observed as compared to the younger age groups.

Four years of age (pig nos. 4919, 4966 and 4915)

Specimen no. 4919 was in the diestrus stage of the cycle. In the sections studied, epithelial invaginations and cysts formed by these invaginations were observed (Figure 83). The epithelial lining of these cystic forms were stratified with cell debris in their cavities, so these could be easily differentiated from the glands which are lined by mucous secreting simple glandular epithelium. It appears that in pregnancy connective tissue increases in the inner circular layer of the tunica muscularis as also seen in the inner circular layer of the uterus (Figure 74).

Arterioles in this age group were seen to be affected with intimal thickening.

Four years six months of age (pig nos. BB1, BB2, BB3 and 392)

Some of the sections taken in this age group were closer to the vagina. In the subepithelial mucosa, basophilic fibrocytes and lymphocytes were observed.

The arteries in the tunica muscularis had thick tunica intima. Enhanced elastic tissue and collagen were seen in the tunica intima. Intimal plaques were seen in a larger vessel. In other large arteries seen in the tunica adventitia of the cervix the thickened intima was one fourth of the cross sectional diameter of the entire arterial wall. The adventitia of the cervix was composed of dense fibrous tissue.

Four years eleven months of age (pig nos. 6015 and 5898)

There was no conspicuous morphological change in the cervix of this age as compared to the younger age groups.

Six years of age (pig nos. 6043, 4583, 5895 and 5813)

In this age group epithelial invaginations forming cell nests and cysts were seen in the mucosa (Figures 85, 86). Venous sinuses were present in the mucosa. In the cysts, cell debris was seen formed by desquamated superficial cells.

In the specimens which were in the diestrus stage of the cycle increased amounts of connective tissue were seen in the inner circular layer of the tunica muscularis.

Some arterioles in the tunica muscularis were affected with thickened tunica intima.

Six years five months to six years nine months (pig nos. 221 and 312)

In this age group also the epithelial invaginations were seen similar to the younger age groups (Figure 89). In specimen no. 312 a subepithelial elastic lamina was observed. The embryonic mesonephric duct persists as the canal of Gartner (Figure 87).

The arteries in the tunica muscularis and the tunica adventitia of the cervix exhibited thickened tunica intima (Figure 88). In the severely affected arteries the intima formed half of the wall thickness.

Seven years nine months to eight years three months (pig nos. 13, 1 LJFl and 1175)

The salient morphological changes were as follows:

1) Epithelial invaginations forming cystic structures in the mucosa.
2) Clear cells were observed in the surface epithelium.
3) Cell debris and neutrophils were observed in the mucous glands.
4) Extensive intimal thickening extending about one half of the wall thickness of the arteries was seen (Figure 91). Excessive collagen was seen in the tunica intima. Arteries from the mucosa to the adventitia were affected with intimal thickening and in a few arteries fibrosis of the tunica media was evident (Figure 90).

Vagina

One day postnatal (pig nos. 2, 3 and 4)

Mucous membrane The whole mucous membrane appears in folds with deep crypts. The epithelial lining consists of stratified squamous epithelium. The epithelium is infiltrated with mononuclear cells with a clear halo of cytoplasm around them (clear cells). These cells have been called clear cells and their presence in the epithelium has been observed also in the uterus, uterine tubes and the cervix.

The lamina propria is mostly constituted of areolar connective tissue and is drained by many venous and lymphatic sinuses which impart a spongy appearance to this layer (Figure 92).

The tunica muscularis consists of a circular layer of some differentiated smooth muscle fibers and young skeletal muscle whose cross striations are evident. The adventitial tissue around the muscular layer has vessels and nerves. Small ganglia are also seen in this adventitia. The mesonephric duct is seen in close approximation to the muscular wall. These have been called the canals of Gartner.

<u>One week postnatal (pig nos. 1243, 1260 and 1263)</u>

As compared to the one day old pig the vaginal epithelium appears slightly cornified and desquamating cells are seen. The nuclei of the stratum germination stain deep basophilic with H and E stain as compared to superficial cells.

In the tunica muscularis fine elastic fibrils are present intermingled with the smooth muscle fibers. The arteries of the vagina appear bigger in size and more developed than the arteries of the uterus.

<u>Two weeks postnatal (pig nos. 1320, 1322 and 1329)</u>

The vagina continues to grow in all its dimensions. The epithelium towards the cervix tends to become less stratified. The epithelial cells are three to four layers thick. In the spongy or erectile mucosal connective tissue darkly staining mononuclear cells are present. These cells appear to be infiltrating the epithelium. In the muscular wall, the mesonephric duct persists. Adventitia is mostly adipose tissue.

<u>One month postnatal (pig nos. 1652, 1653 and 1662)</u>

The difference in structure of the vagina as compared to the previous age group is the presence of large veins in the tunica mucosa in close approximation to the tunica muscularis. The smaller veins, which can be termed cavernous venous spaces, gave the tunica mucosa the appearance of an erectile tissue. The rest of the anatomical features were the same as in the vaginae of the previous age group.

<u>Two to two and a half months postnatal (pig nos. 68, 69, 92, 627, 1651, 1680 and 1682)</u>

The following salient anatomical differences were observed.

1. In this age group, the vaginal epithelium consists of five to ten layers of cells.
2. In the subepithelial connective tissue aggregates of lymph nodules appear consisting of mononuclear cells.
3. Canals of Gartner are present.

<u>Three months postnatal (pig nos. Y656, 708 and 716)</u>

The epithelium was 2 to 3 cell layers. It was observed in this age group that the cavernous spaces in the tunica mucosa of the vagina tend to be collapsed. Stray eosinophilic cells are seen in the mucosa. Clear cells in the epithelium are also present. Epithelial invaginations in the form of microcysts are seen in the mucosa (Figure 93). The canals of Gartner are seen in the wall of the vagina. Vessels and well developed nerves with ganglia lie in the tunica adventitia. Inner longitudinal layer and outer fiber muscular layer are present and become distinct.

Four months postnatal (pig nos. 2250 and 9713)

In specimen no. 9713 the epithelium lining of the vagina consisted of 10 to 20 layers of cells (Figure 95). This gilt was probably in estrus at the time of its sacrifice.

In other specimens degenerating leucocytes are seen in the epithelium. Connective tissue papillae extend into the surface epithelium. As this age is considered to be the minimum age at which the gilt reaches puberty, it can be expected that the reproductive cycle can affect the morphology of the vaginal mucosa.

Six months of age (pig nos. 1292 and 9310)

There is an overall growth in the size of the vagina. The cell layers of the vaginal epithelium vary with the individual gilt. In addition to the cavernous spaces in the mucosa small arterioles are also seen in this age group. Eosinophils in the mucosa and intraepithelial leucocytes appear as normal features. Canals of Gartner persist at this age.

One year two months of age (pig nos. 3430-S, 5930 and 2944-S)

The epithelium of the vagina varies according to the stage of the estrus cycle. A common finding in these sections of the specimen was the presence of an elastic lamina under the epithelium (Figure 96). The mucosa-submucosa is rich in vessels, i.e. arterioles, venous sinuses and lymph spaces. The mucosa-submucosa is composed of collagenous and elastic tissue. Three patterns of smooth muscle are seen in the tunica muscularis of the vagina. A thin layer of longitudinally arranged smooth muscle fibers form the innermost layer. Surrounding this layer is the fibromuscular layer composed of circularly arranged smooth muscle fibers intermingled with an equal amount of collagenous and elastic tissue. This middle layer is in fact a fibromuscular layer.

This fibromuscular layer is surrounded by a third outer longitudinal layer of smooth muscle.

In the tunica muscularis are seen the canals of Gartner which are the remnants of the embryonic mesonephric duct.

The epithelium forms invaginations which appear as epithelial nests or cysts as also shown in Figure 97.

<u>One year five months to one year eight months (pig nos. 3521 and 2021)</u>

Epithelium was 10 to 21 cell layers thick and the animals appeared to be in the estrus stage of the cycle. Clear cells and leucocytes were seen in the epithelium. Deep epithelial pegs extended into the mucosal layer. A subepithelial elastic lamina was present.

Canals of Gartner were seen in the tunica muscularis of the vagina. The vessels appeared normal.

<u>Two years and two years two months of age (pig nos. 2442, 2443, 2156, ISU-951, 632, 3202, 6153, 6154 and 2443)</u>

The epithelial cell layers varied in these specimens according to the stage of the estrus cycle. The vaginal mucósa appeared in folds. The subepithelial elastic lamina appeared to be a regular and persistent morphological feature. Plasma cells, lymphocytes and basophilic fibrocytes were seen in the subepithelial mucosa.

Epithelial invaginations, similar to the ones seen in the cervix, were present in all the tissue sections of the vagina. Cavitated cystic structures containing cell debris were observed (Figure 97).

The mucosa-submucosa was rich in venous and lymphatic sinuses. A few arterioles in the muscular layer had slightly thickened tunica intima.

The tunica adventitia of the vagina was rich in nerves. A few autonomic terminal ganglia were seen in the tunica adventitia.

Two years ten months to three years one month of age (pig nos. 1040, 3191, 1361 and 3196)

Cystic epithelial invaginations as described for the younger age groups were seen. Fibrous tissue composed the walls of the veins. Stray arterioles in the tunica mucosa and tunica muscularis had slightly thickened tunica intimas. Subepithelial elastic lamina was present.

Three years seven months of age (pig nos. ISU 31, 4878, 4876 and 5335)

No change from the previous age groups was seen. Elastosis of the arterial wall was seen in a few arterioles. Stray arterioles in the tunica muscularis showed intimal thickening.

The canals of Gartner were present in the tunica muscularis. Epithelium of the canals of Gartner invaginated around the canal to form glandular structures. In a few specimens excessive proliferation of these glandular outgrowths from the Gartner canals were seen.

Four years of age (pig no. 4919)

Invagination of surface epithelium forming cysts was a salient morphological feature. These invaginations of epithelium were deeper than the younger age groups. Arterioles in the fibromuscular layer had thickening of the tunica intima. Increased fibrosis of the tunica media was observed in a few arteries.

Four years six months of age (pig nos. BB2, BB3, and 392)

Specimens nos. BB2 and 392 were in the proestrus stage of the estrus cycle as the epithelial layers of the vagina reach 15 cells thick. Pig no. BB3 was in metestrus. There were 4 to 8 layers of epithelial cells, lymphocytes, and plasma cells were seen in the subepithelium and also within the surface epithelium. The subepithelial elastic lamina was present but appeared interrupted.

The canals of Gartner were present in the tunica muscularis.

A few arterioles were affected with intimal thickening.

Four years eleven months of age (pig nos. 5898 and 6015)

Pig no. 6015 appeared to be in the early stages of the proestrus cycle as the epithelium appeared to be proliferating. Pig no. 5898 was in the diestrus or luteal phase of the cycle as also shown in Figure 101. There were 2 layers of vaginal epithelium. Epithelial invaginations appeared deeper. The canals of Gartner were present. Not much change was observed in the arteries.

Six years of age (pig nos. 4589, 5815, 5895 and 6043)

The animals in this age group appeared to be in the post-estrus and diestrus phase of the cycle. Subepithelial elastic laminae and epithelial cysts were present in large numbers and appeared to be invading the mucosal tissue. Intimal thickening, to some extent, was seen in some arterioles of the tunica mucosa and the tunica muscularis.

Six years five months to six years nine months (pig nos. 221 and 312)

No salient changes as compared to the previous age group were observed.

Seven years nine months to eight years (pig nos. 3, LJF1 and 1175)

The salient morphological features of this age group were:

1. Deep cystic epithelial invaginations.
2. Increased numbers of arterioles in the tunica mucosa-submucosa, and the tunica muscularis were affected with intimal thickening (Figure 99).
3. Hyperplasia in the form of epithelial outgrowths from the canals of Gartner.

Table 2. Mean growth in thickness of the endometrium, inner circular layer and outer longitudinal layer of the myometrium

| Pig no. | Age days | Mean thickness endometrium(μ) | State of development | Mean thickness (microns) | | | | Total mean thickness of uterine wall(μ) | Mean wt. of the ovaries (grams) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Inner circular layer | State of develop. | Outer longitudinal layer | State of develop. | | |
| 2 | 1 | 100 | N.D.[a] | 164 | N.D. | Not dev.[b] | - | 264 | 0.02 |
| 3 | 1 | 70 | N.D. | 100 | N.D. | Not dev. | - | 170 | 0.02 |
| 1243 | 7 | 83 | N.D. | 115 | N.D. | Not dev. | - | 198 | 0.04 |
| 1263 | 7 | 109 | N.D. | 119 | N.D. | Not dev. | - | 228 | 0.03 |
| 1322 | 14 | 208 | N.D. | 118 | N.D. | Not dev. | - | 326 | 0.04 |
| 1329 | 14 | 249 | N.D. | 117 | N.D. | Not dev. | - | 366 | 0.07 |
| 1662 | 37 | 465 | Dev.[c] | 100 | Dev. | 66 | Dev. | 631 | not recorded |
| 69 | 60 | 612 | Dev. | 195 | Dev. | 64 | Dev. | 871 | 0.06 |
| 627 | 60 | 877 | Dev. | 215 | Dev. | 80 | Dev. | 1172 | 0.08 |
| 1651 | 75 | 901 | Dev. | 255 | Dev. | 114 | Dev. | 1270 | 0.16 |
| 1682 | 75 | 1030 | Dev. | 402 | Dev. | 182 | Dev. | 1614 | 0.85 |
| 708 | 90 | 710 | Dev. | 146 | Dev. | 98 | Dev. | 954 | 0.12 |
| 716 | 90 | 878 | Dev. | 347 | Dev. | 216 | Dev. | 1441 | 0.52 |
| 9753 | 120 | 1528 | Dev. | 318 | Dev. | 256 | Dev. | 2102 | 1.78 |
| 2250 | 120 | 1746 | Dev. | 398 | Dev. | 253 | Dev. | 2397 | 2.21 |
| 9713 | 120 | 1685 | Dev. | 651 | Dev. | 374 | Dev. | 2710 | 2.08 |
| 9310 | 180 | 1916 | Dev. | 825 | Dev. | 257 | Dev. | 2998 | 2.67 |
| 1292 | 180 | 2322 | Dev. | 917 | Dev. | 404 | Dev. | 3641 | 3.24 |

[a]N.D. = non differentiated.

[b]Not dev. = not developed.

[c]Dev. = developed.

Table 3. Mean thickness in growth of the tunica muscularis of the uterine tubes measured in microns at the ampulla from birth to 4 months

| Pig no. | Age in days | State of development | Mean thickness in microns |
|---|---|---|---|
| 2 | 1 | N.D.[a] | 50 |
| 3 | 1 | N.D. | 55 |
| 1260 | 7 | N.D. | 45 |
| 1243 | 7 | N.D. | 53 |
| 1263 | 7 | N.D. | 45 |
| 1322 | 14 | N.D. | 48 |
| 1320 | 14 | N.D. | 39 |
| 1329 | 14 | N.D. | 35 |
| 1652 | 37 | N.D. | 55 |
| 1653 | 37 | N.D. | 70 |
| 627 | 60 | Dif.[b] | 119 |
| 1651 | 75 | Dif. | 155 |
| 1682 | 75 | Dif. | 110 |
| Y650 | 90 | Dif. | 115 |
| 716 | 90 | Dif. | 116 |
| 2250 | 120 | Dif. | 160 |
| 9713 | 120 | Dif. | 160 |

[a]N.D. = non differentiated.

[b]Dif. = differentiated.

# DISCUSSION

## Uterine Tubes

Frommel's observations (1886) on the tubal epithelium of women have undoubtedly established the presence of two types of cells. Another type of compressed cell - "peg cell" or stiftchezellen was also reported by him. Voinot (1900) also observed ciliated and non-ciliated cells in the epithelium of the human tube. He was of the opinion that the peg cell or "stiftchezellen" is a transitory cell which could either differentiate into a ciliated or a non-ciliated secretory cell. Novak and Everett (1928) were in agreement with Voinot. Tröscher (1917) considered the peg cell to be a degenerative form of the tubal epithelial cell. Hadek (1955) reported the presence of the peg cells in the tubal epithelium of the sheep during the metestrus stage of the cycle. He has observed that the secretory cell, after shedding its nucleus, assumes the form of a peg cell. Snyder (1923) describes these cells as small round cells with a deep staining nucleus. In the present investigation peg cells were observed in the epithelium of the uterine tubes as well as the uterine epithelium (Figure 38). Their real significance in the Fallopian tubes is hard to determine. Further opinion about these cells will be expressed in the following paragraphs of this chapter.

No glands such as simple tubular or tubuloalveolar type were seen in the uterine tubes of any age group. The non-ciliated epithelial cells are thought to be the secretory cells.

Novak and Everett (1928) described a bulbous herniation of the epithelial secretory cells of women into the lumen of the Fallopian tubes carrying their nuclei with them during the luteal phase. Casida and McKenzie (1932), McKenzie _et al_. (1933), and McKenzie and Terril (1937) have been of the opinion that the presence of nuclei in the projections of secretory cells represents a holocrine secretion in the ewe. In the cow Weeth and Herman (1952) also observed nuclear extrusions from the surface epithelium of the fallopian tubes. Hadek (1955) has stated that the nucleus of the secretory cell in the sheep epithelium first moves closer to the cell surface and then wanders out. After being extruded from the cell the rounded nuclei measure 2 to 5 microns. The cells from which they originate appear thin, rod-like and without a nucleus. In his studies of the tubal epithelium of women and rabbits, Fredricsson (1959) has reported that the secretory cells are tall during the follicular phase of estrus and short during the luteal phase.

In the present work it was observed that nuclear and cytoplasmic protrusions were seen on the surface of the tubal epithelium (Figures 5, 9). Free nuclear fragments without any cytoplasmic material around them and also free cytoplasmic globules were seen in the lumen of the tube. According to the definition of holocrine secretion, the whole cell detaches from the basement membrane and is shed as a secretory product such as the cells of the sebaceous gland. In order to accept the views of Casida and McKenzie (1932), McKenzie _et al_. (1933) and McKenzie and Terril (1937) it would be expected that the entire epithelium involved in secretion should be shed. These workers have reported that mitosis was not observed in the tubal epithelium of the ewe. The present investigation is inclined to interpret the observations as a manifestation of apocrine secretion. The secretory cells appear to perform a cytotrophic function to nourish the zygote, morula or gastrula, during its passage through the Fallopian tubes. The whole cell is not lost as a secretory product, but only part of the cell - part of the nucleus or cytoplasm - appears to be shed to justify the interpretation of apocrine secretion. The present investigation is therefore more inclined towards the opinion of Hadek (1955) relating to the status of the "peg cell".

In addition to the secretory cell, ciliated cell and peg cell, some clear cells were also present in the tubal epithelium. The mucosal folds, commonly known as the fimbria, grew to the age of one month when they formed anastomotic links with the opposing or adjoining fimbria. The fimbria are more numerous and long in the infundibulium and gradually decrease in numbers and length towards the isthmus. In the isthmus they appear short and plump. There is an increase of connective tissue in the fimbria and the mucosa from the age of 4 months (Figure 10). Epithelial invaginations into the tunica propria are seen to form cystic structures at 3 years 7 months of age. These cysts are seen deeper in the tunica muscularis above the age of seven years. Further opinions on the cysts will be expressed with the cysts of the uterus.

At birth the tunica muscularis is not constituted of differentiated smooth muscle cells (Figure 4). The differentiation of smooth muscle becomes evident above the age of one month. The tunica muscularis of the Fallopian tube of the hog consists of an inner circular layer and an outer longitudinal layer. An inner longitudinal layer of smooth muscle as described by Kipfer (1950) and Toni and Maccaferri (1951) in woman is not seen in the hog.

## Uterus

A stratified layer of epithelium was observed in the uterus of the day-old pig. No distinct endometrial folds were seen and the endometrium was devoid of uterine glands (Figure 33). Hadek and Getty (1959b) also reported that there were no uterine glands in the uterus of the new-born pig. The epithelial cells which are differentiating into glandular epithelium can be recognized as they are more visiculated and show invaginated growth into the endometrium. At the age of one month the glands are well developed and the earliest differentiating epithelial cells have formed tubular glandular epithelium reaching the inner circular layer of the myometrium (Figure 40). In a day old pig the endometrium and the myometrium are not differentiated. At the age of two weeks the undifferentiated cells of the outer part of the uterine wall arrange in a concentric pattern - to form the inner circular layer. Post natal development or differentiation of the inner circular layer in the pig is identical with the myometrium of the rabbit as investigated by Yamamoto (1961). The outer longitudinal layer is also differentiated at about the age of one month from the subserous cells around the inner circular layer. It is developed more conspicuously towards the body of the uterus than in the horns (Figure 39). The first part of the myometrium to differentiate is the inner circular layer. In the rabbit uterus the outer longitudinal layer does not differentiate up to the age of 4 to 5 weeks (Yamamoto, 1961).

The growth pattern of the endometrium and myometrium is shown in Table 2 and has also been plotted (Figures 1, 2 and 3). It is interesting to observe that the mean thickness of the uterine wall is slightly reduced about the age of 3 months. The fall in mean thickness of the entire uterine wall corresponds also to a fall in the mean ovarian weights during the same period. After the age of 3 months there is a steady growth of the uterine wall and also of the ovaries.

Hadek and Getty (1959b) studied the growth pattern of the uterine wall from birth to 6 months. Their measurements of the endometrium and the entire uterine wall differ from the present results because of the fact that the uterine horns are not uniform in thickness - they are thicker caudally than in the cranial part - and measurements taken from different parts of the uterine horn are bound to vary. However the ratio of percentage of endometrial thickness to total wall thickness of the uterus as observed by Hadek and Getty (1959b) corresponds to the percentage of endometrium in Table 2.

Corner (1921), Snyder and Corner (1922), and McKenzie (1926) have reported the absence of ciliated cells in the uterine epithelium of the sow. In the present investigation ciliated cells were observed in the uterine epithelium of the sows studied. Lovell and Getty (1968) have recently confirmed the presence of cilia in the uterine epithelium of the sow with light as well as the electron microscopes. A subepithelial layer of mononucleic leucocytes was present in almost all the tissue specimens studied and could represent a second line of defense. The changing morphology of the uterine endometrium of the sow has been confirmed and reported by Corner (1921), McKenzie (1926), and more recently by Fabian (1961), to be under the influence of the estrus cycle.

The mesonephric duct has been observed to persist in close approximation to the external layer of the myometrium to the age of 4 months. According to the traditional concept, the embryonic remnant should have degenerated. In the male this duct of the urinary system is appropriated by the genital system and forms the ductus deferens. In the day old uterus clear nerve fibers were seen reaching the uterus through the mesometrial border. It is therefore evident that the uterus of the pig at birth is very well innervated.

One of the tissue samples used in these studies was that of a pregnant uterus (Pig no. 3430). It was observed that in pregnancy increased amounts of collagenous connective tissue accumulated in the inner circular layer of the myometrium. This layer in pregnancy can be called a fibromuscular layer (Figures 55 and 56). The outer longitudinal layer of the myometrium remains unaffected during pregnancy. This increase of collagen in the pregnant uterus of the sow can be compared to a degree with the increase of collagen in the pregnant woman according to the findings of Woessner (1963). This excess of collagen is completely resorbed within a few weeks in the post partum period in women (Woessner and Brewer, 1963). In the present investigation no excessive amount of collagen was seen in the myometrium of the uterus of non-pregnant sows. Based on these observations one is inclined to think that the fibrosed inner circular layer of the myometrium should not be able to contract during parturition and that the muscle of the outer longitudinal layer plays the main role in the expulsion of the fetus. Another important function of the fibrosed inner circular layer would be to strengthen or reinforce the uterine wall with increased amounts of collagen and elastin to withstand the increased growth of the fetus which causes a great expansion of the uterine wall.

## Cervix

The cervix of the sow, according to the epithelial covering, can be divided into the cranial part which is part of the endocervical canal and has been called the uterine part, and the supravaginal portion. This part in the sow is lined with simple columnar epithelium. Tubular mucus glands have been seen in the mucosa of the cervix in this area.

The epithelium of the portio vaginalis is similar to the changing epithelium of the vagina in accordance with the respective stage of the estrus cycle. Above two and a half years of age, invagination of epithelium into the mucosa was seen forming small cysts in the portio vaginalis. A variable amount of cell debris is seen in these microcysts (Figure 78). Similar cysts were also observed in the vaginal mucosa of the sow. According to Fluhmann (1961) clefts and grooves of the cervical mucosa in women run in oblique, transverse, and longitudinal directions. These clefts may extend downward and branch. Occasionally they become occluded so that an area becomes obliterated as a tunnel or blind tube. It is in these tunnels that nabothian cysts are presumed to be formed. The cystic structures observed in the mucosa of the portio vaginalis of the cervix are more superficial. If these cysts are formed due only to obliteration of the mucosal clefts, there is no reason why they should not form below the age of two and a half years. As these cysts have been found to exist in all the normal tissue specimens above the age of two and a half years, I am inclined to believe that their presence is normal and not pathological.

The embryonic mesonephric duct has been observed to persist above the age of 6 years in the wall of the cervix (Figure 87). Its significa ce is not clear in the pig as no complete record on the incidence of meson·phric tumors is available to correlate their presence. The lack of record: is due to the following reasons.

All pigs slaughtered for meat are below or about the age of six months. Sows are not usually retained for breeding above the age of approximately 3 years. Therefore the incidence of mesonephric tumors above the age of 3 years needs investigation in the pig. Embryonic nephromas below the age of one year are seen to develop in the pig (Moulton, 1961).

The presence of autonomic ganglia has not been reported along the wall of the cervix. In the present investigation autonomic ganglia were observed along the wall of the cervix (Figure 68). Therefore parasympathetic innervation of the cervix of the pig needs further investigation.

## Vagina

The cyclic changes in the vaginal epithelium have been reviewed Cystic invagination of the vaginal epithelium was normally observed beginning about the age of 3 months (Figure 93). Cell debris of desquamated epithelial cells is seen in these cystic forms (Figure 97). The significance of these cystic forms is not known and neither have they been reported in the literature.

A thin sheet of elastic fibers below the epithelium was consistently observed and has been referred to in this investigation as the subepithelial elastic lamina (Figure 96). Whether this elastic lamina is part of the basement membrane or a separate sheet of tissue below the basement cannot be stated. However, it appears that these fibers are much thicker and conspicuous when stained with elastic tissue stains as opposed to the elastic fibrils which may be the constituents of the basement membrane.

So far the tunica muscularis of the vagina of the pig has been considered to be entirely composed of muscular tissue. Three patterns of smooth muscle tissue are seen in the developed vagina - an inner longitudinal layer, a middle circular layer and an outer longitudinal layer. In the opinion of the present investigator, the middle layer should be called a fibromuscular layer because the smooth muscle bundles are intermingled with equal amounts of collagenous and elastic tissue (Figure 100). In some specimens studied more fibrous than muscular tissue was seen in this layer.

The mesonephric ducts - the canals of Gartner (Figure 94), were present in all specimens to the age of 8 years. Slight hyperplasia in the form of epithelial evaginations is seen in the older age groups above 6 years.

Clear cells have been observed in the epithelium of the entire tubular genitalia of the sow (Figure 41). The views of Glowinski (1958) that these clear cells have a hormonal function with a more or less broad spectrum have been reviewed. According to Marinov (1966), who studied the uterine epithelium of the bovine, clear cells increased during estrus, declined in number in mid-cycle and increased again in proestrus. He is of the opinion that these cells are either epithelial cells undergoing mitosis or the white blood cells penetrating into the epithelium. In the present studies it was observed that the morphology of these cells approximates those of the lymphocytes. As these lymphocytes were seen to form the subepithelial granular layer in the uterus, they could also penetrate the epithelium and appear as clear cells (Figure 41).

## Blood Vessels

### Arteries

At birth the arteries of the tubular genitalia of the pig are small and their walls are constituted of one to three layers of smooth muscle fibers (Figures 34, 67). The walls of the arteries grow steadily beyond the age of two months (Figures 6, 42, and 68). The arteries grew to normal adult size by the age of 4 to 6 months (Figures 11 and 43). The method of smooth muscle proliferation by cell division during normal growth in the arterial wall was not observed by the present investigator. Schaffer (1922) and Evans (1923) believed that the smooth muscle cells of the tunica media of arteries may develop from young endothelial cells. Malyschew (1929), in reporting his experiments with ligating the carotid arteries in rabbits, mentioned the resemblance between endothelial cells,which changed into fibroblasts, and smooth muscle cells. He quoted other authors who also suggested a metaplasia of endothelium into smooth muscle.

Slightly above the age of one year there was no apparent change in the blood vessels. From one and a half years of age onwards, the changes are discussed in the following paragraphs.

Changes in the vessels of the tubular genitalia: It is obvious from the results of the present studies that arteries and also veins exhibit a progressive thickening of the tunica intima with advancing age. In making these observations, efforts have been made to describe the general morphology of a number of sections studied. It should also be borne in mind that there are wide variations in microscopic structure along the different parts of the same vessels and that a given change is seldom uniform even throughout the microscopic section. Even the progressive changes in the tunica intima are not constant. The variation in individual vessels is so extensive that most of the published material loses significance as the individual variations cannot be considered along statistical lines.

It was Sohma (1908) who made the following remarks about the intimal thickening of the uterine arteries of women. "With each menstrual cycle and pregnancy, regeneration of the smooth muscle occurred in the subendothelium so that eventually a new vessel was formed inside the old. Whether this regeneration is due to endocrine effects or, as seems more likely, to periodic fluctuations in the functional demand has not been established." Sohma's remarks should hold also in the case of the arteries of the uterine tubes, uterus and the cervix. The only factor that Sohma did not consider while making these credible observations was the possible effect of aging on the uterine vessels.

Many reports appear in the literature about the effect of age on the arteries of man and laboratory animals. In the pig the thickening of the tunica intima of the arteries with advancing age has been reported in recent years by Skold and Getty (1961), French *et al*. (1963), Getty (1965a, 1965b), French et al. (1965), Lüginbühl and Jones (1965), Dahme (1962), Jennings *et al*. (1961) and Skold *et al*. (1966). Gottlieb and Lalich (1954) first emphasized that lesions in the aorta are common to swine and that the incidence increases with age. Lüginbühl (1965) reported his findings on different parts of the aorta in the swine and also the uterine (especially the middle uterine) arteries affected with spontaneous atherosclerosis.

In man, studies have been largely focused on the effect of age on the coronary arteries and their branches in the heart. However, all the major blood vessels of the hog and dog are now under extensive investigation as affected by normal aging process in the Department of Veterinary Anatomy at Iowa State University. In the present investigation an attempt was made to examine the blood vessels of the tubular genitalia of the sow from birth to 8 years.

As age changes in the arteries of the tubular genitalia of the sow as observed in the present studies do involve the thickening of the tunica intima (Figures 32, 63, 64, 91, 98 and 99), that part of the arterial wall which exactly constitutes the tunica intima should be defined. Bunce (1964) considers the intima to be the subendothelial layer between the endothelium and the internal elastic lamina. Thus a thick or a thin intima generally refers to the relative width of the subendothelial layer.

Gross *et al*. (1934) observed that the intima consisted at birth of a single elastic lamella (internal elastic lamina) covered with flat endothelium. With increasing age, the first change consisted of splitting of the internal elastic lamina into two membranes between which smooth muscle fibers appeared, running at times diagonally but generally in a longitudinal direction. This constituted the musculoelastic layer. The outermost of these elastic membranes continued to represent the borderline between the intima and media and accordingly retained the name lamella elastica interna (internal elastic lamina).

In the arteries of the young pig, the internal elastic lamina is present (Figure 67). Intimal thickening does become evident in some of the affected vessels about one and a half years of age. Above this age, intimal thickening can occur in any normal artery, but the earliest change is manifested with the splitting of the internal elastic lamina (Figure 52). Therefore the present observations are in accordance with the findings of Gross _et al_. (1934) and Lüginbühl and Jones (1965).

Buck (1958) considers that time is a particularly important variable in an analysis of the structure of human arteries, since an understanding of aging effects provides the basis for an intelligent approach to the study of arteriosclerosis and atherosclerosis. This concept of the effect of age induced changes of the arteries of man can be very closely compared to those of the domestic pig (Lüginbühl, 1965).

Movat _et al_. (1958) observed in their studies of the human aorta that the earliest stages of development of the subendothelial layer was represented by splitting of the internal elastic lamina and the accumulation of small pools of mucopolysaccharides. Most newborn infants showed this change, and between 6 months and one year of age additional elastic but few collagen fibers formed in this layer. Robertson (1960) reported that smooth muscle cells in the coronary arteries and aorta continued to increase throughout childhood so that by early adulthood a well developed tunica intima was present. Movat et al. (1958) also reported similar age related changes, but in the second decade the intima resembled a hyperplastic musculoelastic layer with irregularly arranged smooth muscle cells, histiocytes, occasional fibroblasts, delicate elastic fibers and ground substance. These changes were more pronounced in the abdominal aorta. Altschul (1950) considered the fibroblasts in the intima to be differentiated endothelial cells with potential differentiating properties.

With Altschul's initial observations with the light microscope and subsequent findings by other workers with the light microscope as well as with the electron microscope, it appears that the proliferation of the tunica intima is the result of the migration of both smooth muscle cells from the tunica media and also the endothelial cells migrating into the intima through the fenestrations of the internal elastic lamina and the internal limiting membrane (elastic membrane against which lies the endothelium). According to Haust et al. (1960) the migrated endothelial cells differentiate into smooth muscle cells. Rhodin (1962) observed fine intracellular fibrils in the endothelial cells. The functions of these fibrils, although not well understood, would lend some support to endothelial differentiation if the fibrils represent actin or myosin. More recently Röhlich and Olah (1967) found a myofibril-like cross striated fibril in the basal portion of endothelial cells of the arterioles of the rat myometrium.

Intimal thickening, whether it is a result of migration of smooth muscle cell or of endothelial cells or both into the intima results because the internal elastic lamina or the inner limiting membrane of elastic tissue permit the passage of these cells through their fenestra. Ham (1965) believes that elastic membranes are commonly fenestrated, probably because elastin is not very permeable and fenestra are required to permit the passage of nutrients and waste products through them. According to Ham (1965), electron microscopic studies gave no indication as to whether the elastic fibers were made up of fibrils or microfibrils, but instead they seemed homogenous. Rhodin (1962) observed that the elastic fiber was represented by an amorphous material, possibly with a mucopolysaccharide matrix, similar to basement membranes. In addition, a filamentous component has been identified, although it has not been demonstrated whether the filaments are distributed throughout the entire elastic fiber or only occur at the surface. The fenestrations in the elastic lamina have been confirmed by Pease and Paul (1960) in their studies with the electron microscope. They observed that the fenestrations often contained extrusions of smooth muscle cytoplasm. In small arteries of the heart and in major coronary arteries of man, part of the endothelial cell cytoplasm may project outward through gaps. Collagen fibrils were also seen in the fenestra by these workers.

Blumenthal et al. (1944) reported that elasticity was lost and the fibers were subsequently infiltrated with mineral salt in old age. No mineral deposit of any kind was seen in the present investigation in the arteries of the age groups studied.

Faber and Møller-Hou (1952) suggest that while total amount of collagen and elastin together increased with age, there is a drop in elastin content of the aorta from 35 per cent at age twenty to 22 percent at age 70. Harkness et al. (1957) showed that in the growing dog there is a change in the amount of elastin present in the arterial wall. Elastin content of the thoracic vessels falls from 70 to about 50 and 60 percent as the animal approaches adulthood. The changes in the peripheral vessels on the other hand are more pronounced, varying from 60 percent in the newborn to 35 percent after six weeks and finally falling to between 25 and 30 percent in the adult animal. Scarselli (1961a and 1961b) reported a marked rise from 19 to 50 percent during the first 20 years of life in the human followed by a definite decrease thereafter (26.2 percent at 63 to 88 years of age). Other workers appear to miss the initial rise and claim that there is a marked decrease in elastin content with age. Lansing (1959) stated that with the exception of the post partum uterine artery, there was little evidence that elastic fibers could be regenerated in normal adult tissue. In the present investigation it was observed that with increase in thickening of the tunica intima of the affected vessels of the tubular genitalia, the smooth muscle of the thickened intima synthesized elastin. Elastic fibers could be seen in layers in the thickened intima (Figure 32). In the cervix a somewhat different type of artery showed regularly arranged elastic fibers in both parts of its wall - an outer layer of tunica media and an inner layer (Figures 80, 81). It cannot be said definitely whether the inner layer of this vessel constituted the tunica intima. The elastic fibers were arranged somewhat parallel and in a concentric manner. In a typical intimal thickening which appears in the form of a plaque or uneven thickness the elastic fibers and collagen is quite diffusely intermingled with smooth muscle. In some of the larger mesometrial arteries (Figure 63) the elastic fibers appear in more concentric layers in the tunica intima. A number of arteries as seen in Figures 80-81 were seen along the cervix.

It seems to be an almost established fact in man that thickening of the tunica intima does take place with age in vessels such as the aorta and coronary arteries which have been studied quite extensively. In the arteries of the domestic pig intimal thickening has been reported by Gottlieb and Lalich (1954), French et al. (1963), Dahme (1962), and Lüginbühl and Jones (1965). Age changes in the middle uterine arteries of the pig were studied by Getty (1965a) and Lüginbühl (1965). According to their findings the arteries (especially the middle uterine) of the sow had extensive intimal thickenings. These consisted of smooth muscle cells arranged in a specific pattern, and of collagen, reticulum and elastic fibers. In several instances a considerable quantity of ground substance staining for mucopolysaccharides, was also present. Fat deposition and atheromatous change were not observed (Lüginbühl, 1965). In the present studies, the mesometrial and the endometrial vessels of the uterus, which are the terminal ramifying branches of the uterine arteries, exhibited intimal thickening. No lipid deposits were seen in the thickened intimae with the Oil Red O stain nor were foam cells detected. The main cell type observed in the tunica intima was the smooth muscle cell. The multipotential properties of the smooth muscle have been recognized by French (1966), Bal (1966) and Wissler (1968). Smooth muscle does synthesize collagen and elastin and can also accumulate lipids. In the tunica intima this cell has been called the myointimal cell by Buck (1962).

It cannot be said with any certainty whether this myointimal cell retains the property of contractility in the tunica intima where they are arranged in a longitudinal pattern (Figures 61, 63, 32).

As is evident from the present studies regarding the effect of age as one of the possible factors which causes thickening of the tunica intima of the arteries supplying the tubular genitalia of the sow, a number of other etiologic factors have been suggested by many workers. According to Dahme (1965) lesions of spontaneous arteriosclerosis in man and domestic animals present themselves as being of polyetiologic origin. Some of the factors rated by Dahme (1965) are hemodynamic or mechanical influences, disturbances of endothelial permeability and nutrition to the vessel wall, metabolic and enzymatic disorders of the arterial tissue and in respect to cases of true atherosclerosis, in disturbances of general lipid metabolism. Many hypotheses concerning etiology and pathogenesis have been projected. According to Waters (1965) atherosclerotic lesion is basically an inflammatory reaction of vascular tissues to injury. He has described all the progressive changes in the atherosclerotic lesion (experimental) in the arteries of the dog. The initiating factor can possibly be a hemodynamic cause as acute lesions occur predominantly at bifurcations or other locations of maximal stress. It was also observed in the present investigation that thickened intimal cushions are present at the bifurcation of the arteries (Figure 14).

Normal changes in the uterine arteries: Goodall (1910) pointed out that the uterus renews all its arteries after each pregnancy and that the changes in the walls of the blood vessels of the uterus are closely allied to those around the atrophic corpus luteum in the ovary of women. Bal (1966) described coiled or helical arteries in the ovaries of the sow. The smooth muscle in the walls of these arteries is arranged in a longitudinal pattern. Okkels and Engle (1938) described two types of arteries in the uterus of rhesus monkeys. The coiled arteries rich in elastic fiber supplying the endometrium and smaller ones devoid of elastic tissue supplying the base of the endometrium. The coiled arteries undergo a fibroelastic degeneration after spaying and also disappear after menopause. Restitution of the coiled arteries could be produced by treatment with estrone. According to Delson _et al_. (1948) estrogen has a particular predilection for spiral, arterial structures in the uterus and the ovaries. Williams (1948) has stated that estrogen is influential in producing early changes in the vascular pattern, including antimesometrial hyperaemia, edema and mitosis in the rat uterus. Progesterone alone was observed not to influence the vascular architecture. Progesterone intensifies the effect of estrogen on the blood vessels of the endometrium to produce vasodilation.

Goodall (1910), Shaw (1914) and more recently Maher (1959) have described the changes in the elastic components of post partum uterine blood vessels. Based upon their observations they suggest that the distribution of elastic tissue could aid in differentiation between the nulliparous and multiparous uteri and that there is an increased amount of elastic tissue in the vessels of the parous uteri.

Maher (1959) also observed that the elastic tissue of blood vessels loses its normal staining properties for 25 to 30 days after parturition and that the normal staining reaction is restored 30 days post partum. Albert (1967) reported that in the guinea pig, the subendothelial cells of mesometrial arteries proliferate followed by fragmentation of the internal elastic membrane during the early stages of pregnancy. Later with the further proliferation of the subendothelial cells the elastic fibers disappeared. After 22 days post partum the subendothelial cells disappeared followed by reappearance of the internal elastic membranes. Guinea pigs with unilateral pregnancies exhibited the above changes on the side of the pregnant horn.

In the cow, Mochow and Olds (1966) observed increased amounts of elastic tissue in the thickened medial layer of the endometrial arterioles. Elastosis was found in 3.9 percent of animals which had not calved and in 56.5 percent of animals which had calved. It would seem that there was an increase in elastosis with increasing numbers of pregnancies.

Nieberle and Cohrs (1967) have described the post partum involutionary change in the vessels of the uterus as physiological pregnancy sclerosis. The change, according to these authors, is so characteristic that earlier pregnancy can be diagnosed with absolute certainty. In the young pig the terminal uterine arteries are straight, and in the animal which has once become pregnant the vessels are stouter and run in spiral loop-forming courses. Histologically there is reorganization in the vessel wall in the form of hyperplasia of elastic fibers in the intima and media (elastosis), hyaline change in connective tissue and atrophy of muscle fibers. The intima is hypertrophied and the internal elastic lamina consists of two or more layers.

In the present studies the arteries of pig no. 1040,which was pregnant, did not show distinct elastic fibers in the thickened intima or media (Figure 57).

The opinions of many investigators on the arteries of man, laboratory animals and a few domestic animals including the pig have been reviewed. In the domestic pig, the arteries of the tubular genitalia appear to have increased thickening of the tunica intima in older age groups accompanied by an increasing amount of elastosis and fibrosis. The effects of pregnancy and ovarian hormones on these vessels cannot be ignored although the study of this phenomenon was beyond the scope of the present investigation. As the tissues collected for this research project were from the normal animals, pathological causes leading to the intimal thickening of the vessels remain doubtful.

So far the study of arteriosclerosis on the major arteries (aorta, coronary arteries, femoral arteries) has been carried out in a collapsed condition and not in the natural state while the vessels are distended with blood. Bunce (1964) devised a means of obtaining the arteries distended with blood by means of a double hemostat. He arrived at the conclusion that intimal thickening is not as pronounced in arteries studied with his device as compared to the relaxed vessels. He concludes that the descriptions in the literature of intimal thickening as observed by workers in relaxed vessels could be slightly exaggerated.

Veins

It was observed in the present studies that most of the venous drainage of the mucosa of the tubular genitalia was by peculiar large venous sinuses lined by a continuous endothelium and devoid of a muscular wall (Figures 12, 44, 47 and 77). Their morphology can be compared with the capillaries except for their very large size. Tissue fluid which nourishes the tissues permeates at the capillary ends and is also resorbed by the venous capillaries. The significance of the venous sinuses could be to facilitate increased permeability during estrus and also during pregnancy to transfer nutrients or metabolites to and from the developing embryos. Burr and Davies (1951) reported that the largest veins in the rabbit ovary were composed entirely of a single layer of endothelial cells. No muscle tissue or fibrous tissue was present. The walls of the veins were thickened by a thin layer of connective tissue where they emerged from the ovary. Similar veins (venous sinuses) were observed in the ovaries of the domestic pig (Bal, 1966). Lymph appeared to be drained by large lymphatic sinuses morphologically similar to the venous sinuses.

Regular or larger veins draining the tubular genitalia of the sow had thin walls composed of fibroelastic tissue (Figure 60). If any smooth muscle is present, it is concealed mostly by elastic and collagenous fibrous components of the wall. As the smooth muscle fibers were not clearly discernible it cannot be suggested if the elastic fibers are elaborated by smooth muscle or any other cell (elastoblast). The walls of a few large veins were richly supplied by vasa vasorum (Figure 49) in the uterus. A few veins in an 8 year old sow had thick plaques in the tunica intima (Figures 28, 29). Not much information is available about intimal thickening in veins in the literature. Similar intimal plaques were observed also in the large veins of the pig ovaries (Bal, 1966).

According to Trautman and Fiebiger (1957) the veins of the uterus and cervix lack valves. In the present studies distinct valves were observed in the veins of the cervix (Figure 82).

In animals that have gone through a pregnancy, the tunica intima of the arteries and veins exhibits cushion-like intimal thickenings and in all layers of the wall shows increased amounts of elastic tissue which forms clumps in old subjects (Trautman and Fiebiger, 1957).

## SUMMARY

1. The tubular genitalia (uterine tubes, uterus, cervix and vagina) of 66 sows was studied with the light microscope for age changes from birth to 8 years.

2. The secretory pattern of the epithelial cells, the cell types, and their changing morphology as influenced by the estrus cycle (especially in the vagina) have been observed. Post natal development of the uterine glands has been investigated.

3. Growth and differentiation of the layers of the tunica muscularis in the uterine tubes, myometrium of the uterus and changes with age have been studied.

4. Growth and morphology of the veins and arteries and their changing morphology with age has been discussed.

5. Other morphological manifestations of age changes in the tunica mucosa, such as the formation of cysts in old age and the persistence of embryonic mesonephric ducts in the tubular genitalia up to a maximum age have been observed.

### Uterine Tubes

1. At birth the uterine tubes are lined by simple columnar to pseudostratified columnar epithelium with evidence of apocrine secretory activity. Ciliated and secretory cells along with a few peg cells constitute the epithelium. The fimbria are short and not well developed. The external tunic, although present, is not well developed and differentiated into the respective layers of the tunica muscularis.

2. At one week of growth, the fimbria extending from the mucus membrane show branching. Early differentiation of the tunica muscularis begins to be manifested.
3. At two months of age the fimbria of the uterine tube are well developed and branch, forming avastomotic connections with the adjoining fimbria. The fimbria are very long at the infundibulum and ampulla but gradually become short towards the isthmus. The smooth muscle fibers of the tunica muscularis are differentiated and develop into inner circular and outer longitudinal layer. Venous sinuses become distinct and are seen in the mucosa in all age groups.
4. From 4 to 6 months of age, increased connective tissue is seen in the lamina propria forming the core of the fimbria. The arterioles grow in the tunica muscularis. The vessels in the mesosalpinx attain an adult normal size. The tunica muscularis is very thin at the infundibulum and becomes gradually thicker towards the ampulla and more so at the isthmus.
5. Small arterioles in the mucosa of the uterine tubes appeared affected slightly with intimal thickening observed above the age of 2 years.
6. At the age of 3 years and 7 months the surface epithelium invaginates into the subepithelial propria forming epithelial nests, and cysts. Arteries in the tunica muscularis and mesosalpinx were affected with thickened tunica intima.
7. The intimal thickening of the arteries within the tubes and the mesosalpinx show gradual thickening of the tunica intima beyond 4 years of age.

8. From 6 to 8 years the epithelial ingrowths forming cystic structures are seen to have extended deep into the tunica muscularis. Increased fibrosis of the mucosal tissue and the muscular tunica is evident. The arteries appear severely affected with intimal thickening and some intimal plaques are seen in large veins also.

Uterus

1. At birth, the uterus is a tubular organ lined with epithelium with slightly depressed vesiculated epithelial ingrowths indicating the areas from which the glands would develop. The entire uterine wall is constituted of undifferentiated cells as the endometrium and myometrium cannot be distinguished. The embryonic mesonephric duct persists close to the wall of the uterus. Small vessels and nerves are seen at the mesometrial border. The whole of the uterine wall is invested by a serous membrane (mesometrium).
2. At 2 weeks age the glands are seen half way grown into the undifferentiated subepithelial cells. This subepithelial layer appears to be differentiating into the endometrium. Outside this cell layer cells with somewhat longer nuclei appear to be arranging in a concentric manner to form the inner circular layer of the myometrium. This layer is surrounded by a few undifferentiated cells occupying a subserous position.
3. At one month the uterine glands are well developed. The myometrium is also differentiated into inner circular and outer longitudinal layers.

The outer longitudinal layer extends into the broad ligament enclosing vessels and nerves. Mononuclear leucocyte-like cells and eosinophils are seen in the subepithelial region of the endometrium from this age onwards. Clear cells appear in the epithelium.

4. At 4 to 6 months of age the uterus is well developed. The endometrium forms 2/3 of the uterine wall thickness. Venous and lymphatic sinuses are seen draining the endometrium and myometrium. The myometrium and the arteries grow to adult normal size.
5. Arteries, veins, lymphatic and venous sinuses appear normal in the endometrium to the age of one year.
6. Intimal thickening in the arterioles of the endometrium appears about 1 year 6 months of age. The mesometrial arteries appear normal. The walls of the veins are constituted of fibroelastic tissue.
7. Above 3 years of age almost all the arterioles in the endometrium exhibit thickened tunica intima rich in elastic and collagenous tissue.
8. Over 4 years of age more of the mesometrial arteries exhibit increased thickening of the intima. Some of the veins also appear affected. In the severely affected arterioles the tunica intima formed 2/3 of the entire arterial wall in cross section.
9. Above the age of 6 years glandular cysts are seen in the endometrium.
10. In the endometrium of the 8 year old animals, the entire arterial wall of some arterioles exhibited elastic tissue and it is difficult to delineate the tunica intima from the tunica media. The mesometrial arteries were affected with more thickened tunica intima than in the younger age groups. An increased number of glandular cysts were present.

Cervix

1. At birth the cervix is a tubular organ with folds in the mucus membrane. Mucosal connective tissue and the muscular wall-to-be is not very well differentiated. The mesonephric duct is seen close to the external layer, the adventitia, in which are present vessels and nerves. The walls of the arteries have 2 to 3 layers of smooth muscle. Small ganglia are also present in the adventitia.
2. Above one month the mucosal folds appear deeper. The mucosal connective tissue and the tunica muscularis is differentiated into an inner circular and outer longitudinal layer. Paired mesonephric ducts are seen one either side of the cervix between the two muscle layers.
3. About 3 months of age, mononuclear leucocytes in the subepithelial regions and eosinophils in the mucosal layer are seen. A few clear cells are spotted in the epithelium. Further growth in the walls of blood vessels is apparent.
4. At 4 to 6 months, the cervix is well developed. The epithelium tends to become stratified at the portio vaginalis. The tunica muscularis and the blood vessels are well developed. The paired mesonephric ducts (canals of Gartner) persist. The walls of the veins are rich in elastic tissue and have valves.
5. In pregnancy it appears that the inner circular layer of the muscular wall exhibits increased amounts of fibrous tissue.
6. At 1 year 6 months age, a slight thickening of the tunica intima was observed in small arterioles.

7. About 2 years of age, epithelial cystic invaginations were seen at the portio vaginalis of the cervix.
8. At 3 years of age, increased numbers of arteries showed intimal thickening in the cervix.
9. At 4 years 6 months of age, the thickened intima of the arteries in the cervix accumulates more elastic and collagenous fibers. In the large arteries of the tunica adventitia the intima forms 1/4 of the cross sectional diameter of the entire arterial wall.
10. At 6 to 8 years of age, the epithelial cysts (mentioned also in paragraph 7) contain cell debris. Complete fibrosis of the tunica media was seen in a few arteries. In the larger arteries of the adventitia, the tunica intima forms 1/2 the cross sectional diameter of the arteries. The embryonic mesonephric duct was observed in the cervix to 6 years 5 months of age.

Vagina

1. At birth, the vaginal mucous membrane appears in folds and crypts. The subepithelial propria is rich in lymphatic and venous sinuses. Clear cells are seen in the epithelium. The outer muscular layer appears somewhat differentiated. A few nerve ganglia were observed in the adventitia.
2. At 1 to 2 weeks mononuclear leucocytes and eosinophils are seen in the tunica propria. Mesonephric ducts persist as canals of Gartner.

3. At one month some cornification of the epithelium is seen. The tunica muscularis is rich in elastic tissue.
4. Above 2 months the lamina propria appears spongy and is similar in structure to the erectile tissue. More adipose tissue is seen in the adventitia which has vessels, nerves and ganglia.
5. At 3 months of age, epithelial cystic invaginations form in the sub-epithelial mucosa. Three layers of muscle, the inner longitudinal layer, the middle circular layer rich in fibro-elastic tissue, (fibro-muscular layer) and an outer longitudinal layer become distinct.
6. Above the age of 4 months approximating puberty, the number of cell layers of the vaginal epithelium depends on the stage of the estrus cycle. In the subepithelial mucosa mononuclear leucocytes and plasma cells are generally present. A subepithelial lamina of elastic tissue was present from this age onwards. The canals of Gartner were also present in the vagina of all specimens studied. Clear cells are present in the epithelium.
7. To the age of 8 years, epithelial invaginations from the surface into the mucosa forming cell nests and cystic forms appears to be a regular normal feature.
8. Intimal thickening of small arterioles was seen in a few arterioles in the tunica muscularis. Most of the vaginal arteries do not seem to be affected with intimal thickening to any great extent as compared to the arteries of the corresponding age groups of the uterine tubes, uterus, and cervix.

9. In older age groups (6 to 8 years) epithelial hyperplasia of the canals of Gartner is seen. A few arterioles in the vagina were affected with intimal thickening.

10. No glands of any kind were observed in the vagina of the sow.

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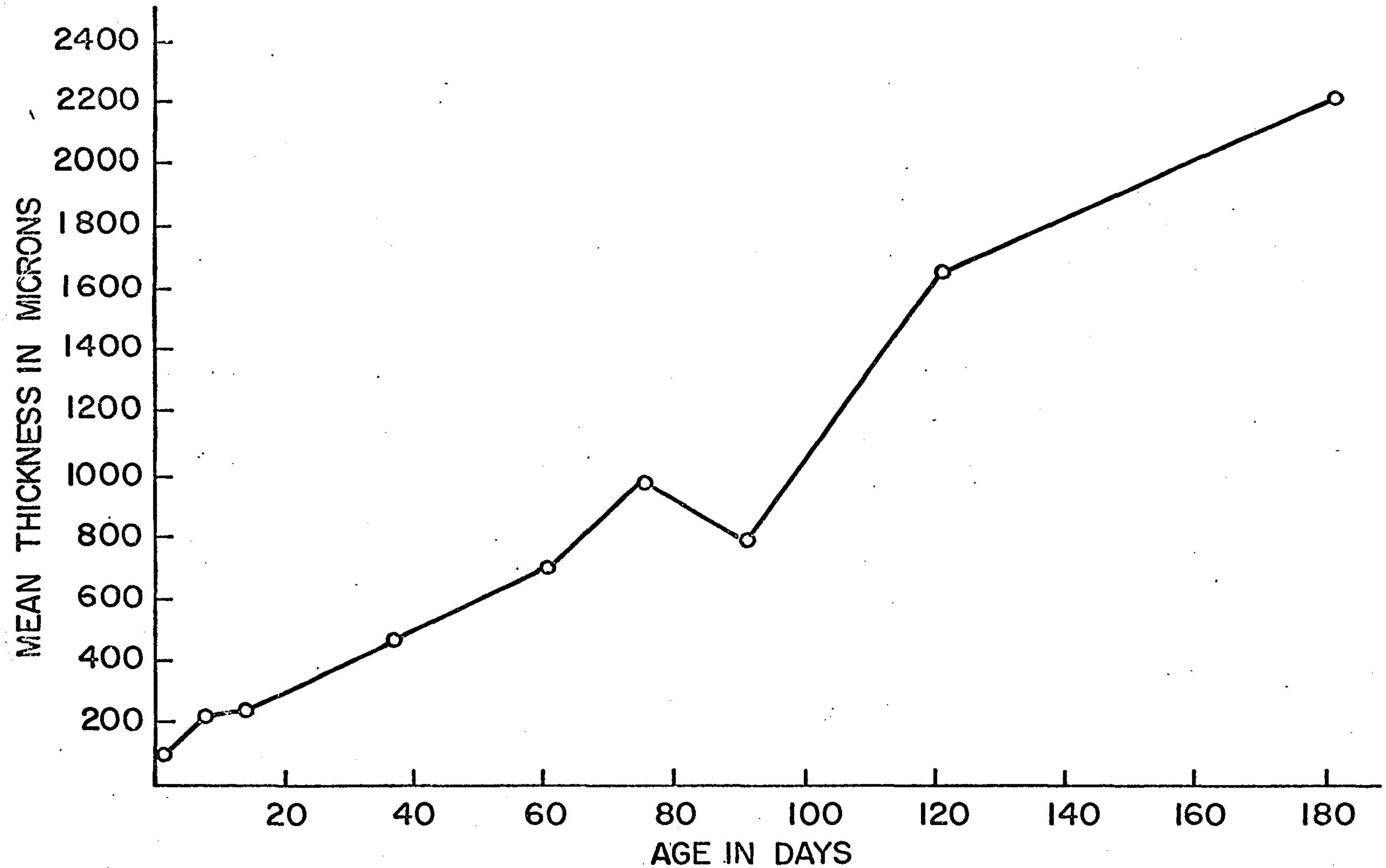
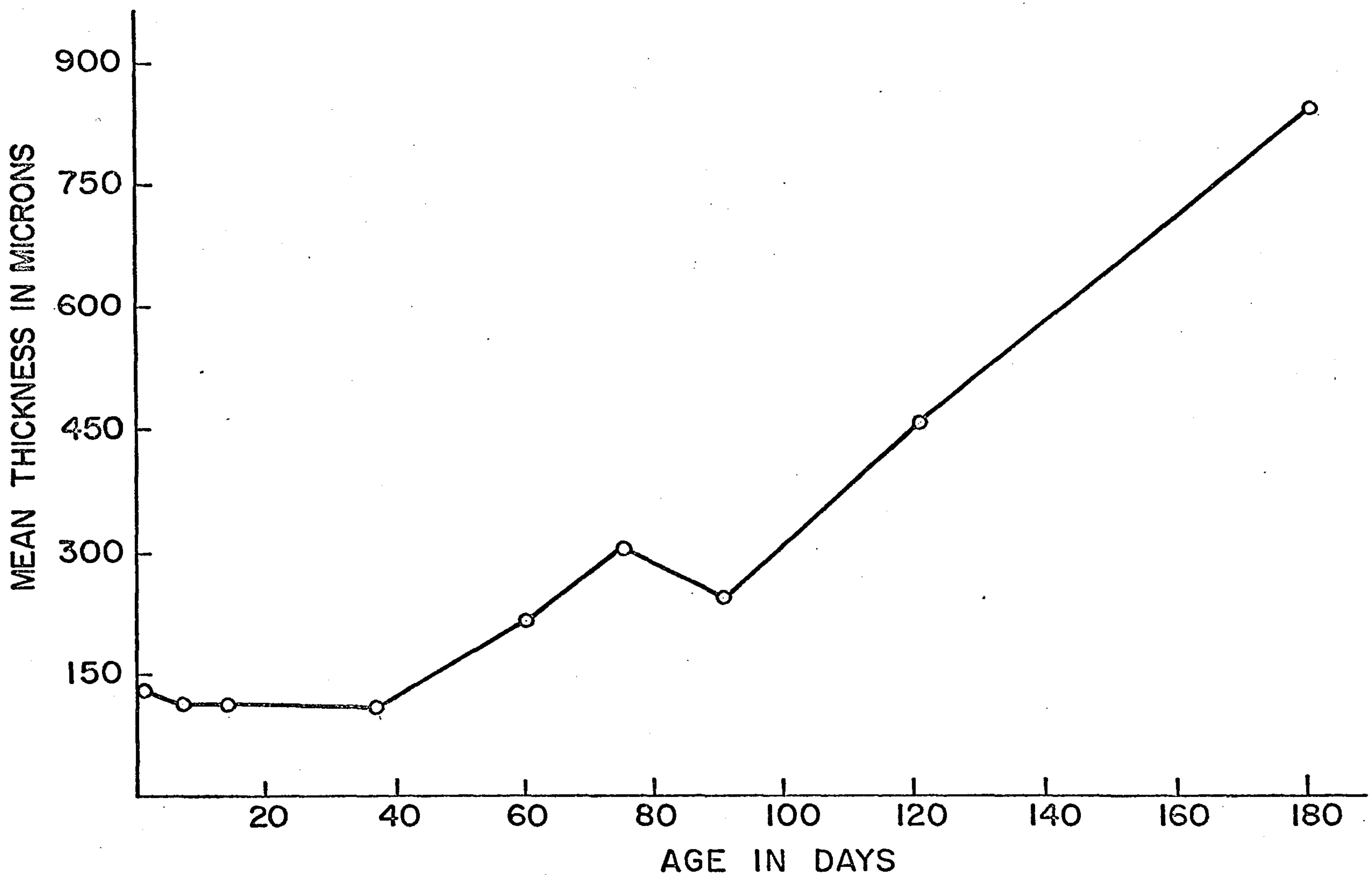
# APPENDIX

Figure 1. Mean endometrial growth in thickness of the uterus from birth to 6 months.



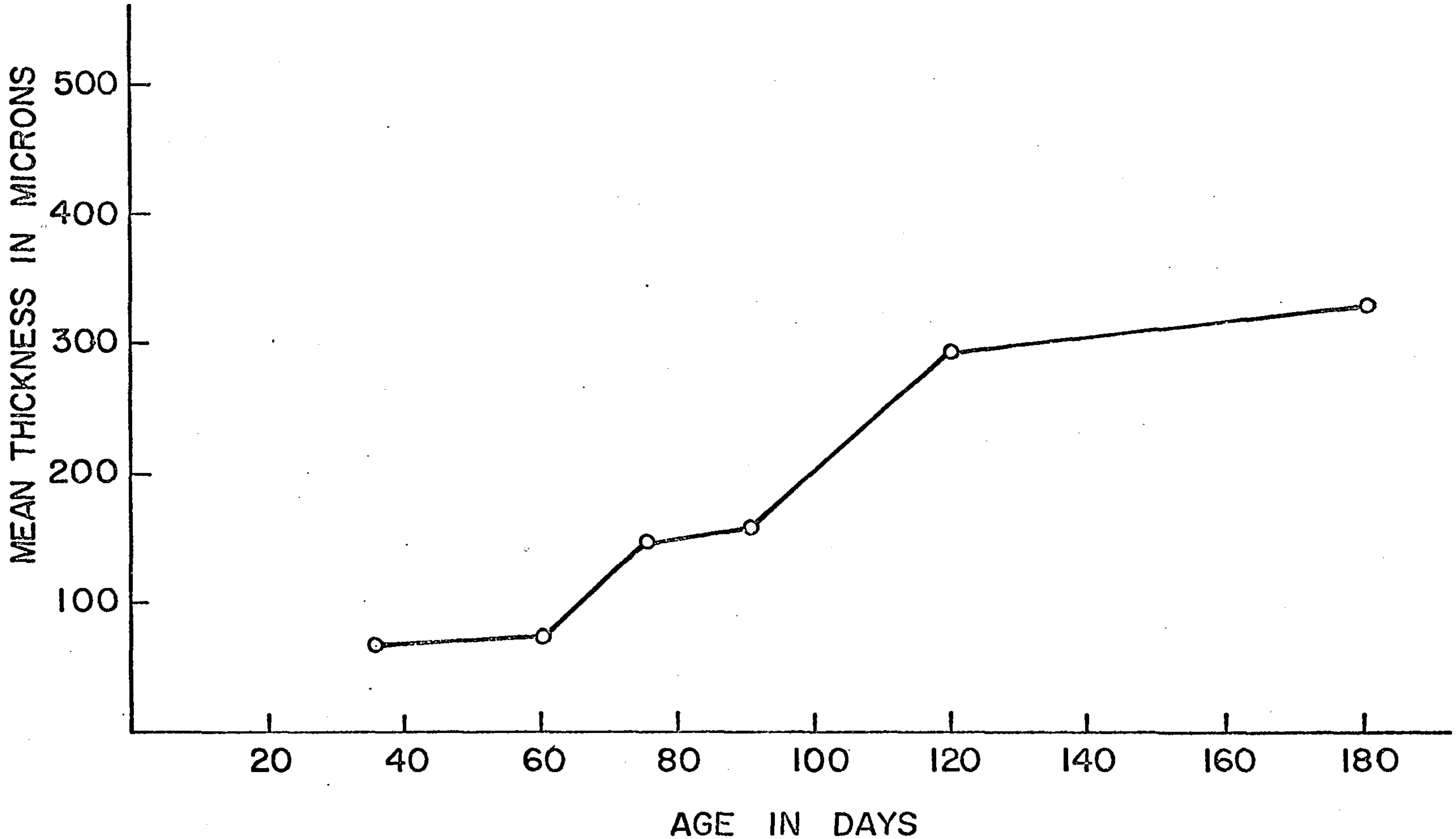

MEAN ENDOMETRIAL GROWTH OF THE SOWS BIRTH TO 6 MONTHS

Figure 2. Mean growth in thickness of the inner circular layer of the myometrium from birth to 6 months.




MEAN GROWTH OF INNER CIRCULAR LAYER OF THE SOWS BIRTH TO 6 MO.

Figure 3. Mean growth in thickness of the outer longitudinal layer of the myometrium from birth to 6 months.




MEAN GROWTH OF EXT. LONGITUDINAL LAYER OF THE SOWS, BIRTH TO 6 MO.

Figure 4. Pig no. 2: age 1 day. A section of the uterine tube at the ampulla showing the small developing fimbria and undifferentiated wall consisting of primitive mesenchymal cells, H and E stain. 100X.

Figure 5. Pig no. 3: age 1 day. A section of the epithelial surface of the uterine tube showing detached nuclear and cytoplasmic fragments indicating an apocrine type of secretion probably, H and E stain. 250X.

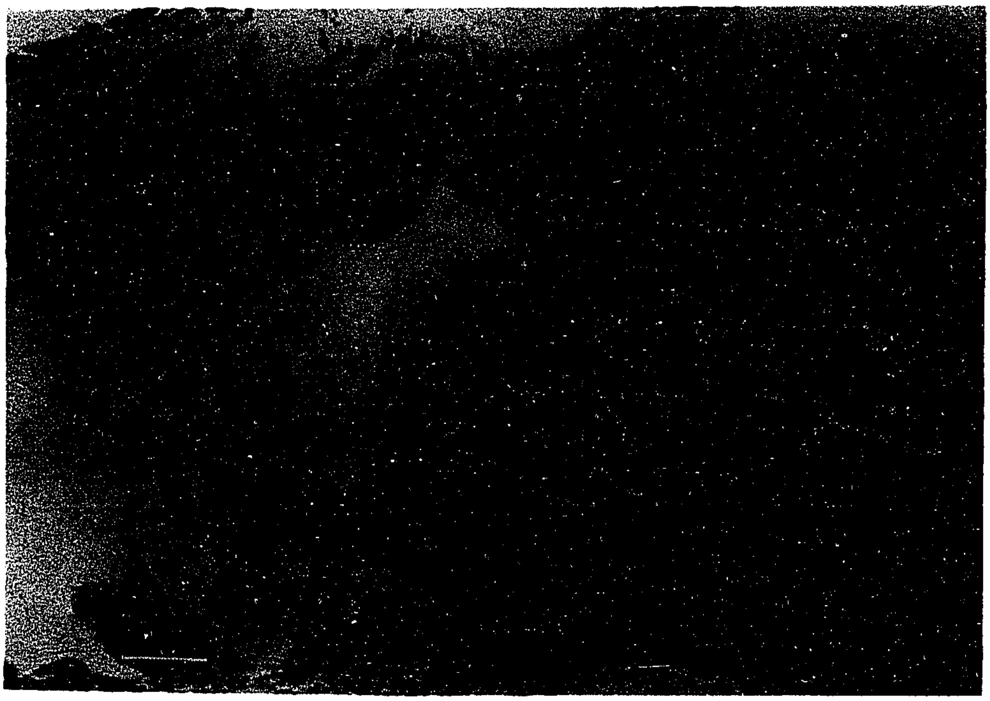


Figure 6. Pig no. 1653: age 1 month 1 week. Artery and veins in the mesosalpinx of the uterine tubes. Weigert's, van Giesen's and Heidenhain's stain. 250X.

Figure 7. Pig no. 68: age 2 months. Section of the uterine tube through the infundibulum showing the increased growth of the fimbria forming anastomotic connections with each other. H and E stain. 100X.

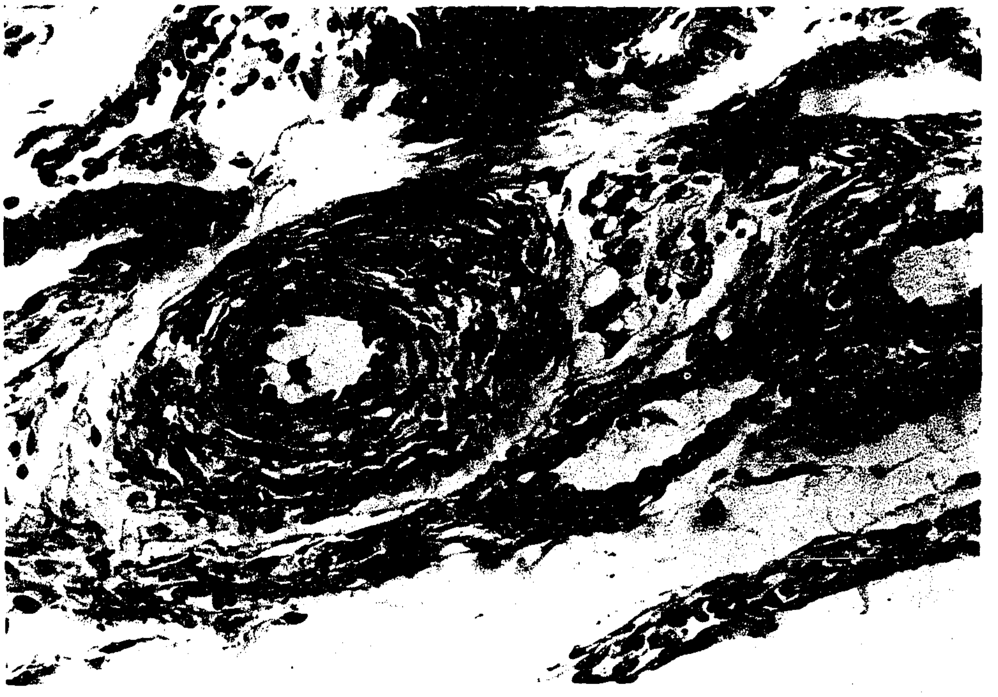
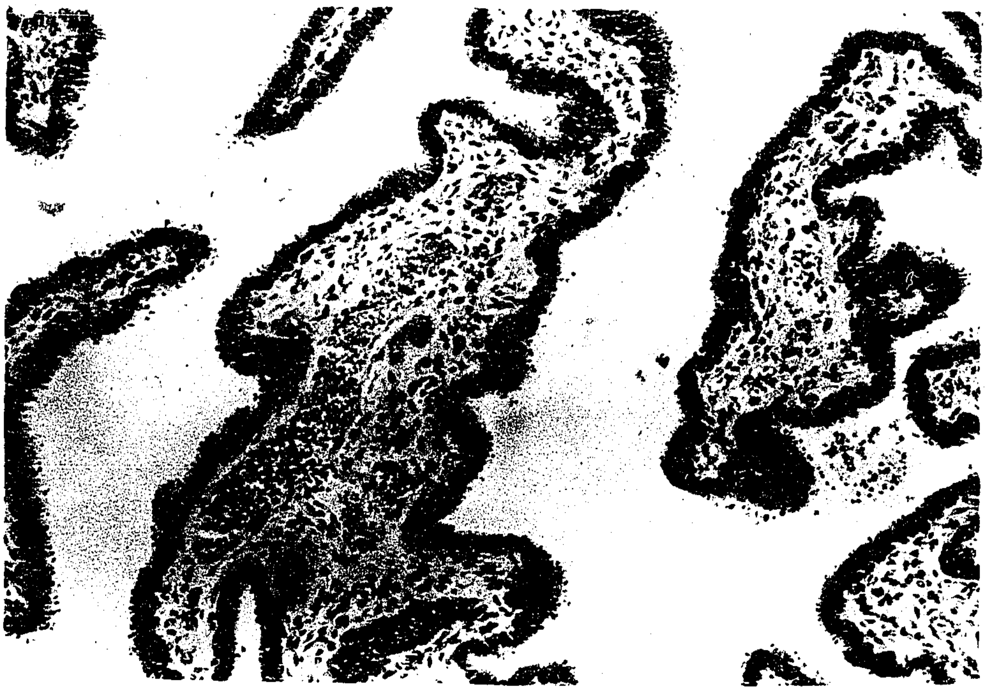


Figure 8. Pig no. 69: age 2 months. Section of the uterine tube through the isthmus showing thick tunica muscularis and short fimbria. H and E stain. 100X.

Figure 9. Pig no. 1680: age 2 months 2 weeks. Section through the infundibulum of the uterine tube showing cytoplasmic and nuclear fragments being secreted or shed by the epithelial cells of the fimbria indicating apocrine secretion. H and E stain. 400X.

Figure 10. Pig no. 2250: age 4 months. Section through the infundibulum of the uterine tube showing further growth of the fimbria with increased amount of connective tissue. H and E stain. 100X.

Figure 11. Pig no. 9713: age 4 months. Section of the uterine tube showing a well grown normal artery. Weigert's, van Gieson's and Heidenhain's stain. 250X.



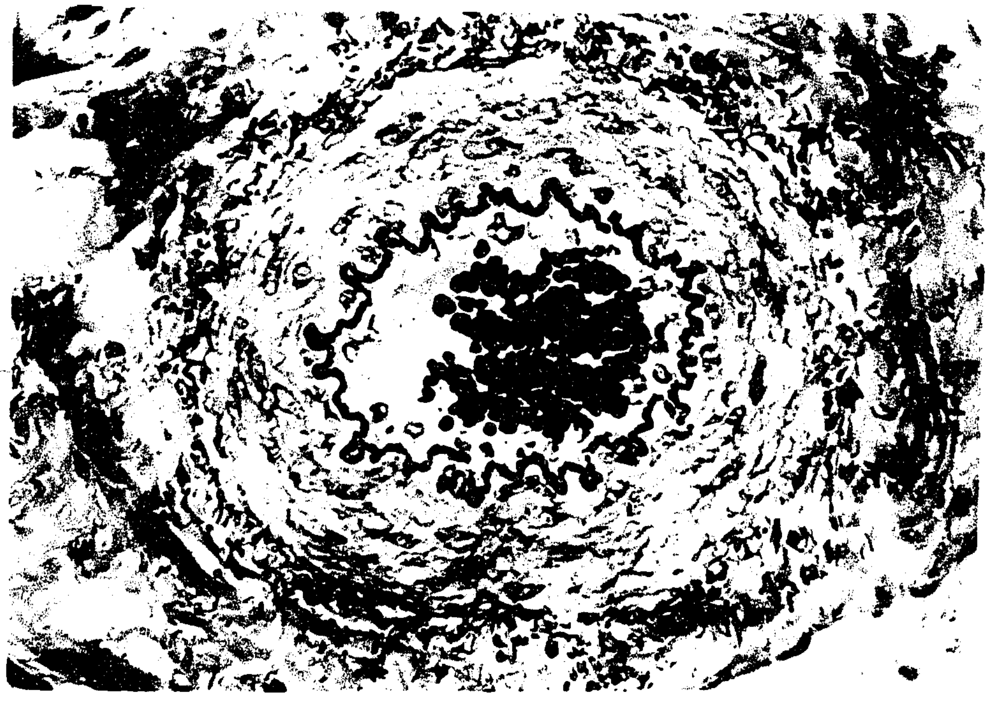
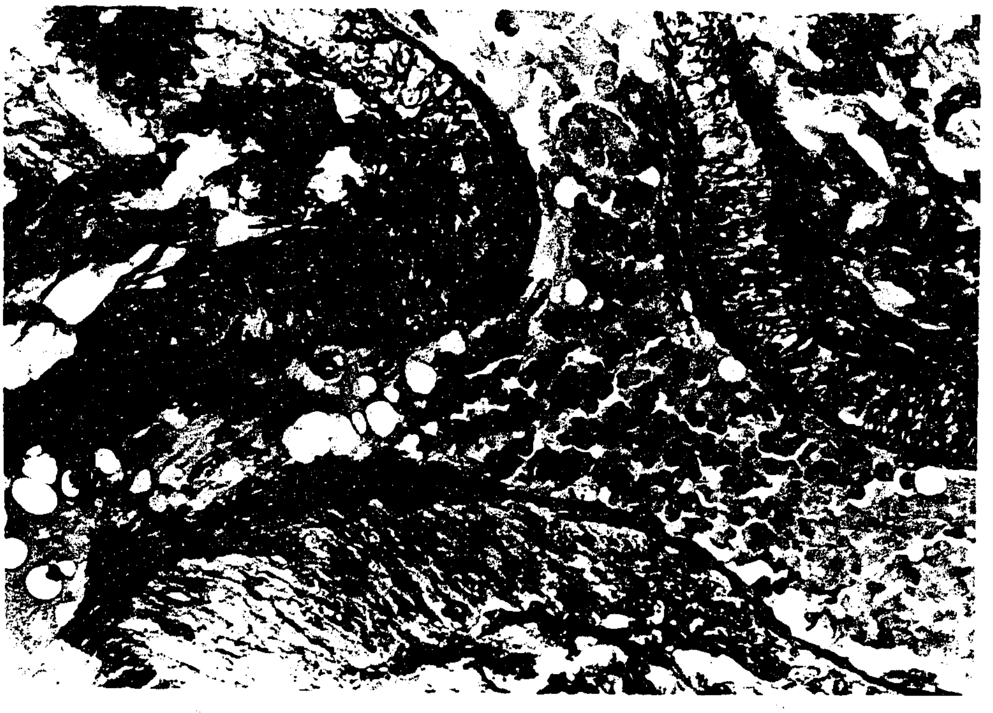


Figure 12. Pig no. 2944: age 1 year 3 months. A venous sinus in the uterine tube lined by the endothelium and surrounded by the connective tissue of the mucosa. Verhoeff's elastic stain. 250X.

Figure 13. Pig no. 3251: age 1 year 8 months. Normal arteries of the uterine tube at the border of the mesosalpinx. Verhoeff's elastic stain. 40X.

Figure 14. Pig no. 2442: age 2 years 2 months. A branching artery in the uterine tube showing endothelial cushions at its bifurcation. Weigert's van Gieson's and Heidenhain's stain. 250X.

Figure 15. Pig no. 1040: age 2 years 10 months. Artery in the uterine tube showing thickened tunica intima. Verhoeff's elastic stain. 100X.



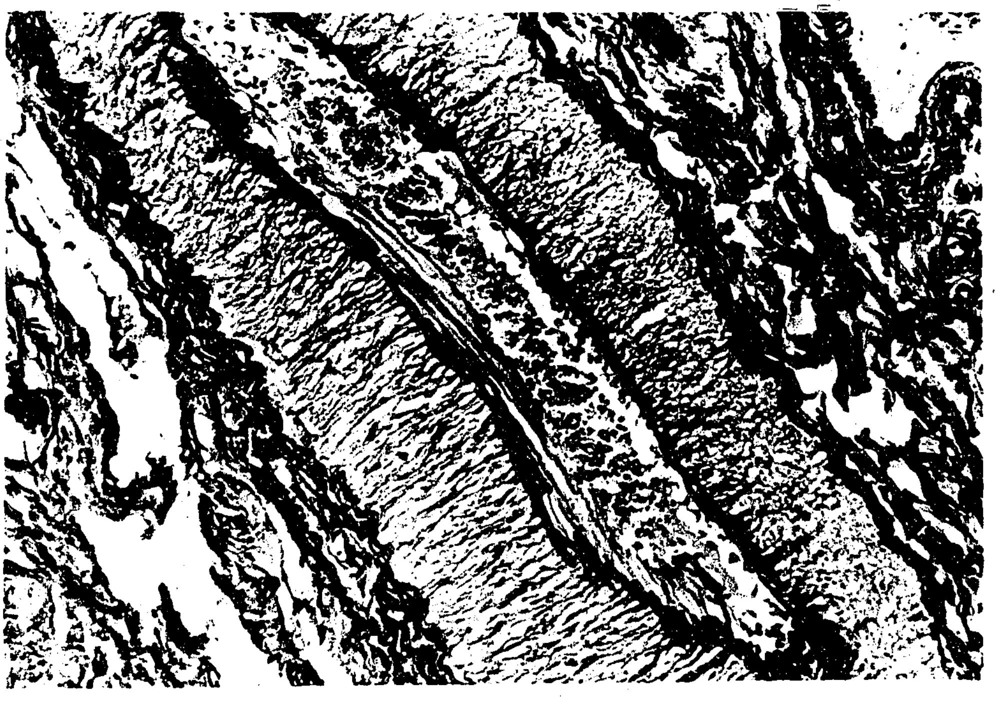
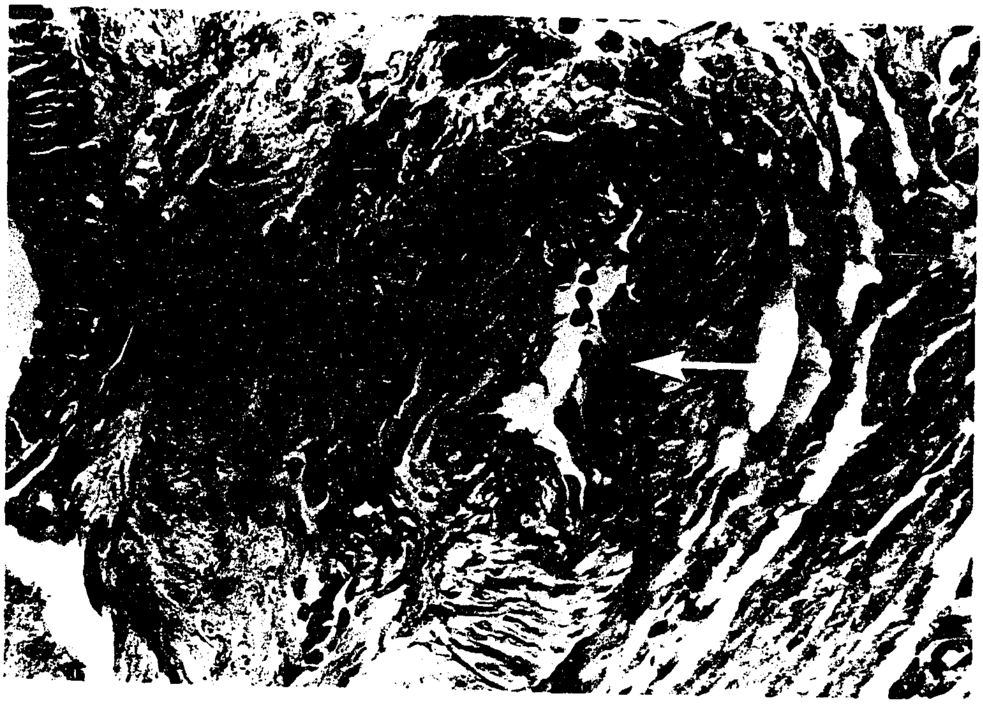


Figure 16. Pig no. 1361: age 3 years. Artery in the tunica muscularis of the uterine tube showing thickened tunica intima. Weigert's, van Gieson's and Heidenhain's stain. 250X.

Figure 17. Pig no. 4915: age 4 years. Invaginated epithelium forming enclosed cystic structures deep in the mucosa of the uterine tubes. Verhoeff's elastic stain. 100X.

Figure 18. Pig no. 4915: age 4 years. Artery in the tunica muscularis of the uterine tube showing thickened tunica intima. Weigert's van Geison's and Heidenhain's stain. 250X.

Figure 19. Pig no. 4966: age 4 years. Arteries with thickened tunica intima towards the salpingial border of the uterine tubes. Verhoeff's elastic stain. 100X.



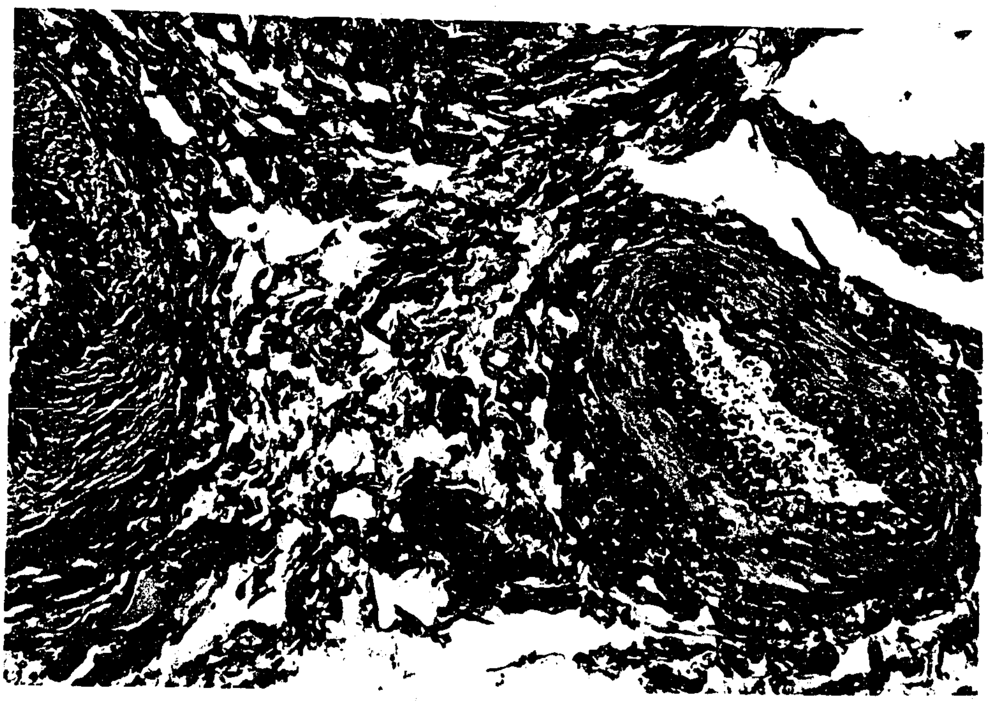


Figure 20. Pig no. 5898: age 5 years. A longitudinal section of a tortuous vein in the uterine tube. Weigert's, van Gieson's and Heidenhain's stain. 100X.

Figure 21. Pig no. 5895: age 6 years. Epithelium invaginating into the mucosa of the uterine tube. H and E stain. 100X.

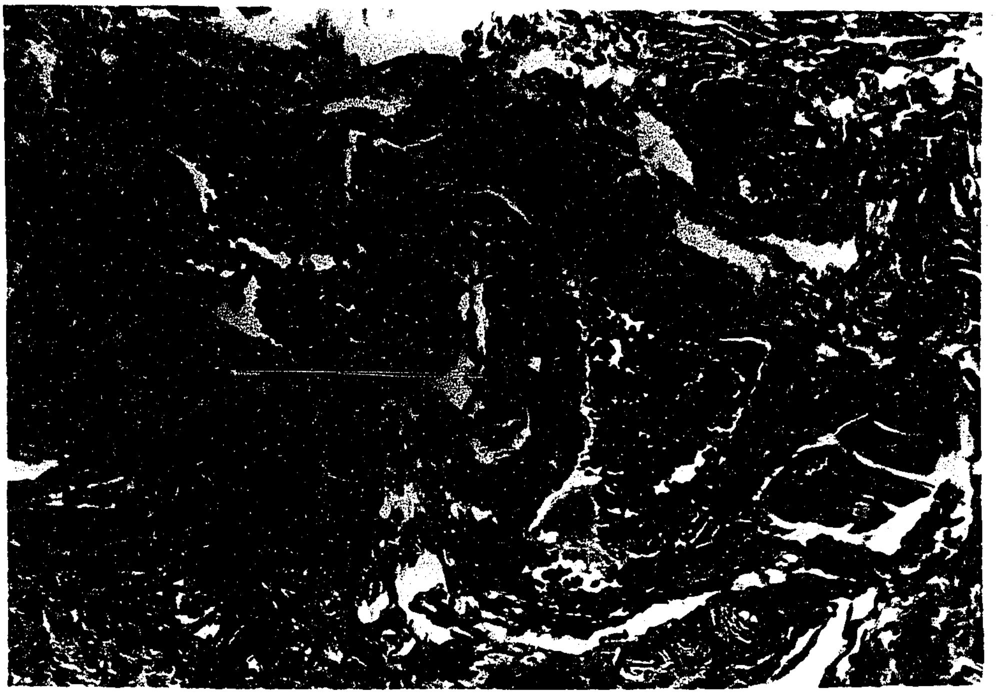


Figure 22. Pig no. 5895: age 6 years. A thickened tunica intima in the salpingial artery of the uterine tube. Verhoeff's elastic stain. 100X.

Figure 23. Pig no. 4583: age 6 years. Epithelial invagination in the mucosa of the uterine tubes. H and E stain. 100X.

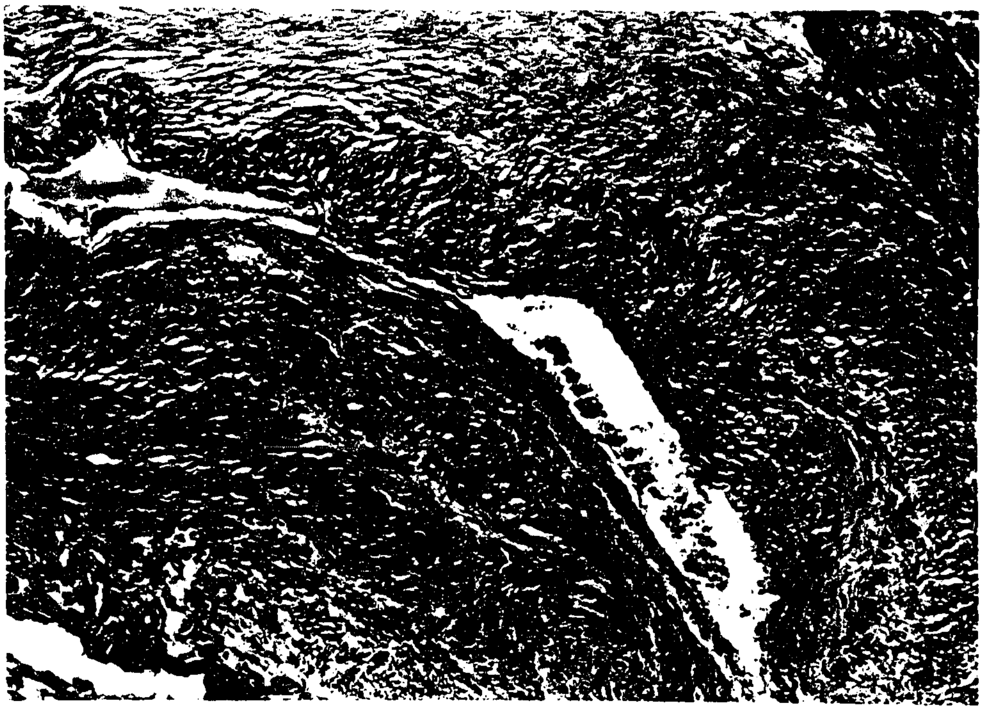


Figure 24. Pig no. 4583: age 6 years. Epithelial invagination in the fimbria of the uterine tube. H and E stain. 100X.

Figure 25. Pig no. 312: age 6 years 9 months. Cystic forms of epithelial invaginations in the layer of the tunica muscularis of the uterine tube. H and E stain. 100X.

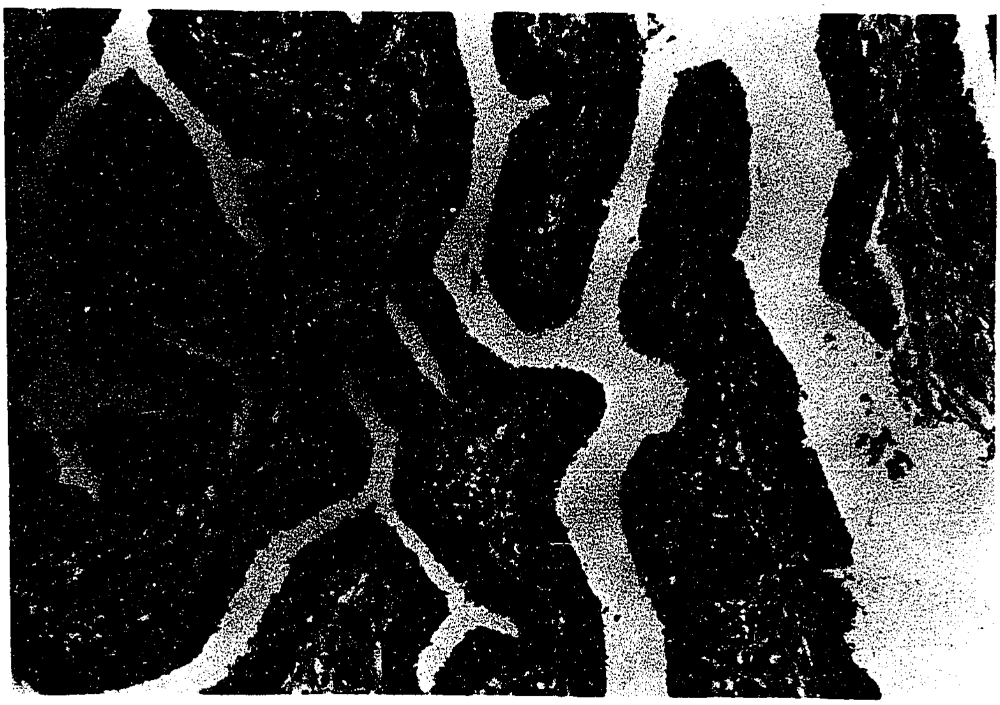


Figure 26. Pig no. LJF 1: age 8 years. Arteries of the mesosalpinx thickened tunica intima. Verhoeff's elastic stain. 40X.

Figure 27. Pig no.ljf 1: age 8 years. Intimal thickening in the salpingial artery of the uterine tube. Verhoeff's elastic stain. 100X.

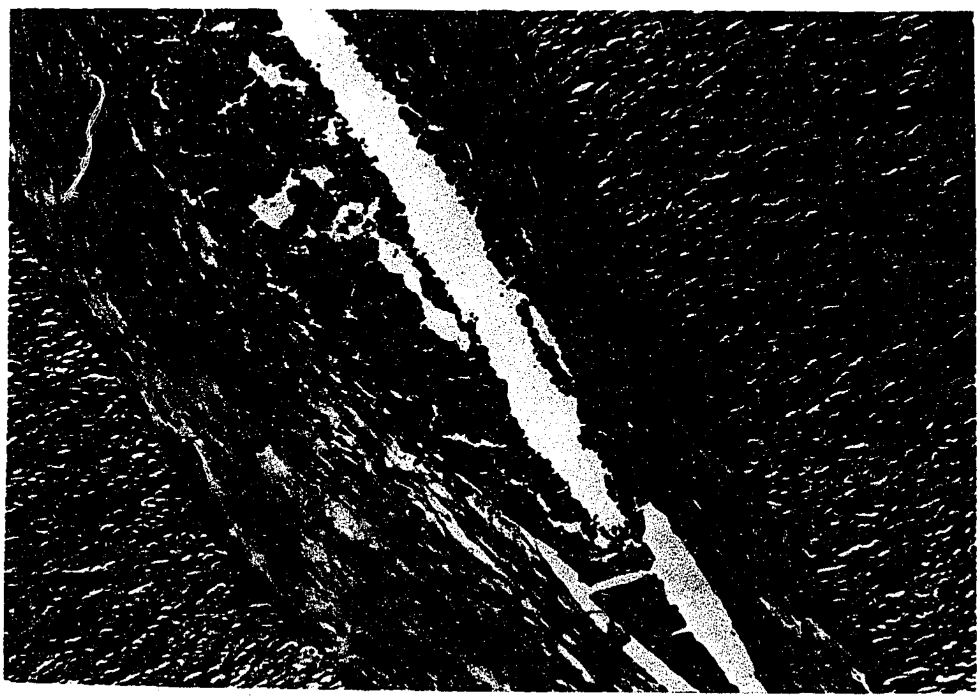


Figure 28. Pig no. ljf 1: A section of the vein in the uterine tube showing plaques in the tunica intima. Verhoeff's elastic stain. 100X.

Figure 29. Pig no. Ljf 1. Age 8 years. Same as Figure 17. 40X.

Figure 30. Pig no. 1175: age 8 years. A section of the uterine tubes showing cysts in the fimbria of the mucous membrane. H and E stain. 40X.

Figure 31. Pig no. 1175: age 8 years. Epithelial cysts deep in the tunica muscularis of a section of the uterine tube. H and E stain. 40X.

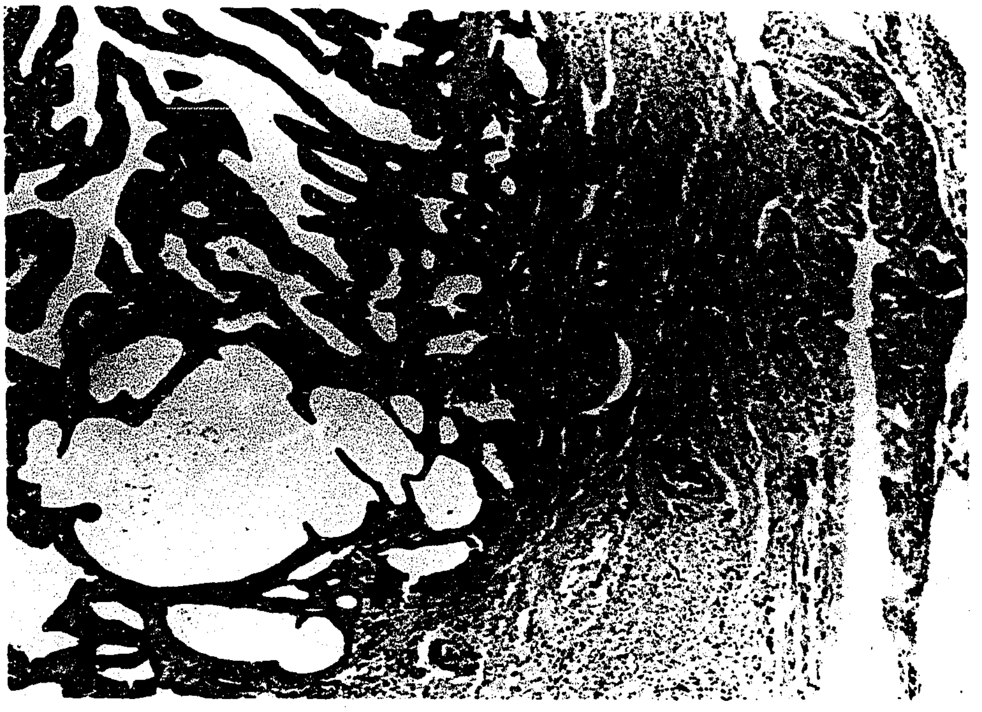


Figure 32. Pig no. 1175: age 8 years. A section of an artery in the mesosalpinx of the uterine tube showing a thickened tunica intima. Verhoeff's elastic stain. 100X.

Figure 33. Pig no. 2: age 1 day. Section through the horn of the uterus. Endometrium and myometrium is undifferentiated and the uterine glands are not developed. Trough-like invaginated areas (arrow) on the surface epithelium are the points from where the uterine glands would invaginate. H and E stain. 100X.

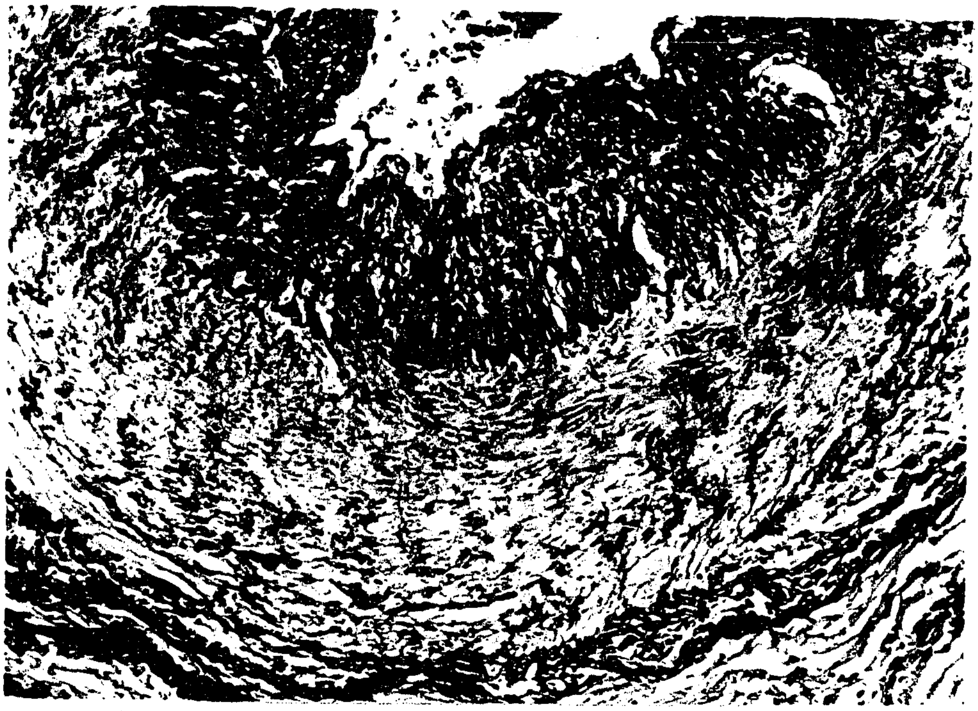


Figure 34. Pig no. 1263: age 1 week. Section through the uterine horn showing the young vessels in the mesometrial border of the broad ligament. Cells of the nondifferentiated endometrium and the circular layer of the myometrium can be recognized. Outer longitudinal layer of the myometrium is not developed at this age. H and E stain. 100X.

Figure 35. Pig no. 1260: age 1 week. Section through the uterine wall showing the persistent mesonephric duct close to the uterine wall (arrow), at the mesometrial border. Nerves and young vessels are seen in this area. Mallory's triple stain. 100X.

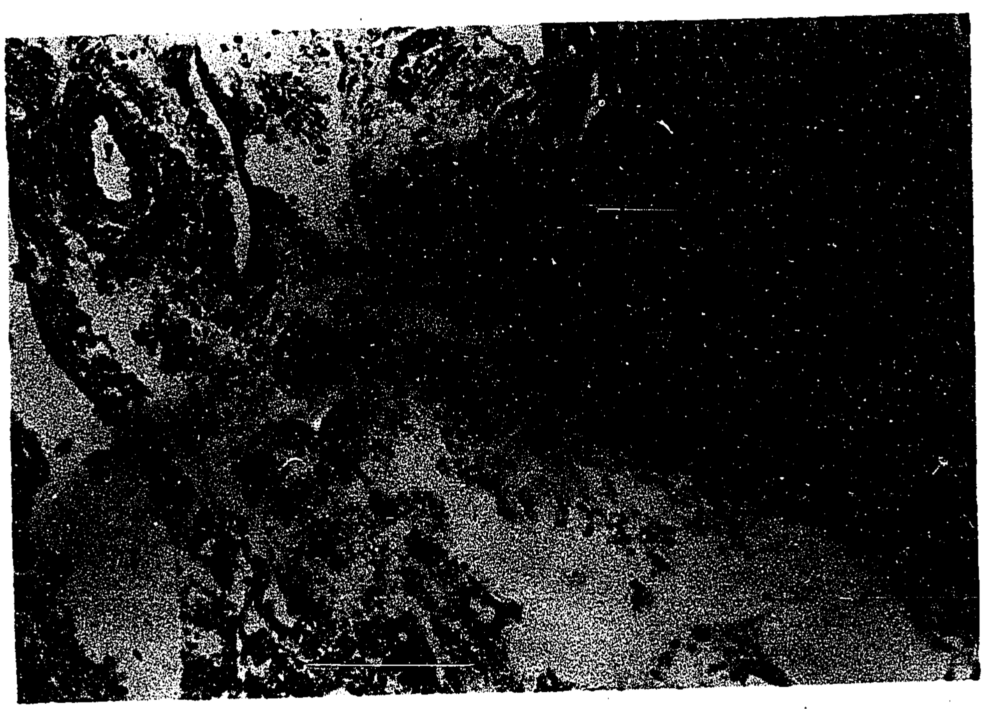


Figure 36. Pig no. 1322: age 2 weeks. Section through the endometrium of the uterus showing the developing uterine gland. The glandular epithelial cell appears vesiculated. H and E stain. 250X.

Figure 37. Pig no. 1329: age 2 weeks. Section showing the early development of the outer longitudinal layer of the myometrium from the cells of the subserous layer. The undifferentiated cells of the subserous layer continue into the broad ligament. H and E stain. 100X.

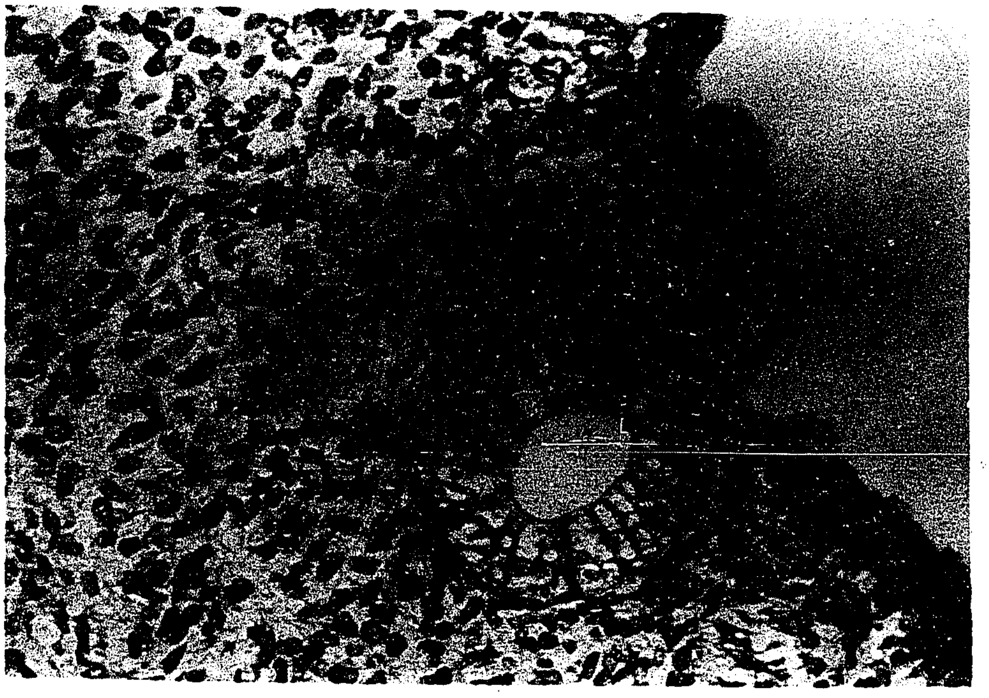


Figure 38. Pig no. 92: age 2 months. Section through the uterine endometrium showing the dark staining nuclei of the "peg" cells also called "stiftchezellen" in the epithelium. Mallory's triple stain. 400X.

Figure 39. Pig no. 1653: age 1 month 1 week. Section of the uterine horn near the body of the uterus showing the partly developed external longitudinal layer of the myometrium. Weigert's, van Gieson's, and Heidenhain's stain. 100X.

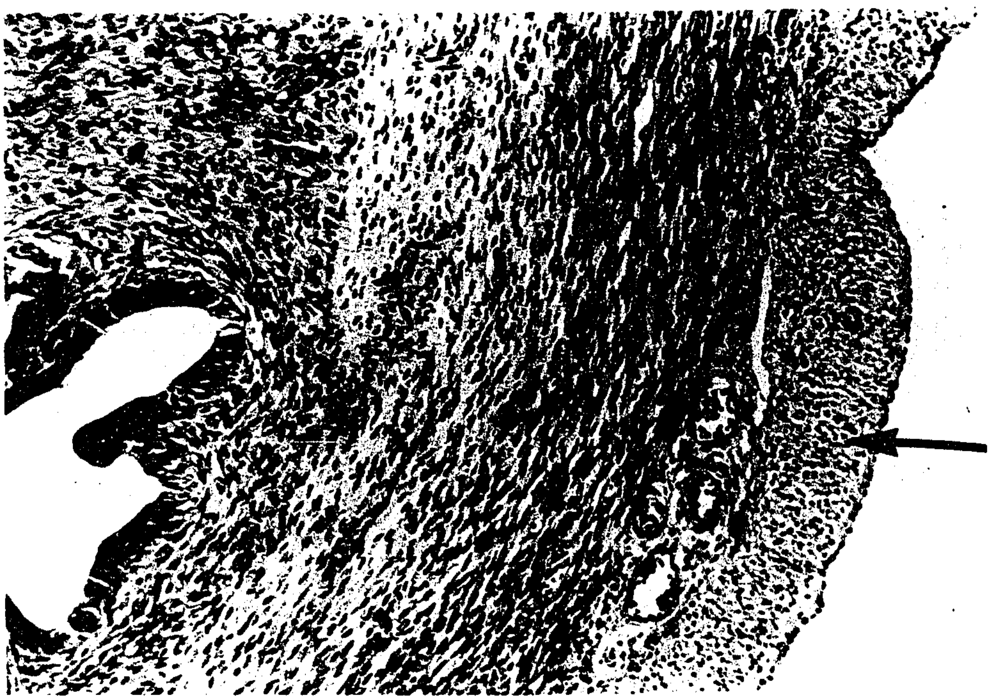
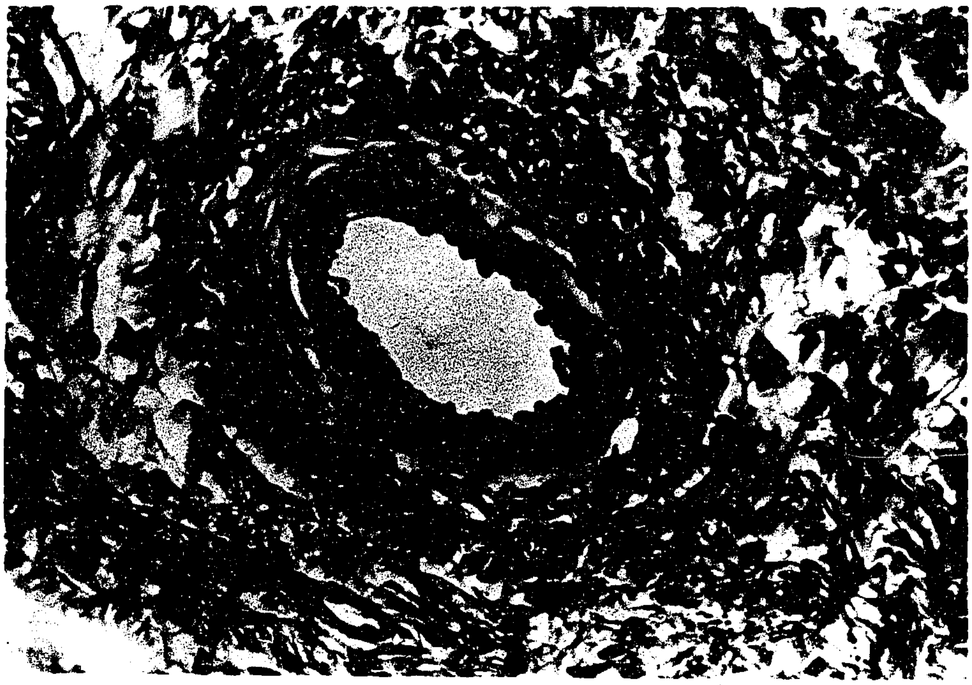


Figure 40. Pig no. 1662: age 1 month 1 week. Section through the uterine horn showing the invagination of glands reaching the myometrium. The inner circular layer and the outer longitudinal layer though apparent are not yet completely differentiated. H and E stain. 100X.

Figure 41. Pig no. 69: age 2 months. Section of the uterine endometrium showing clear cells in the epithelium and lymphocytes in the sub-epithelium. H and E stain. 400X.

Figure 42. Pig no. 92: age 2 months. Section through the mesometrial border showing a developing artery. Verhoeff's elastic stain. 250X.

Figure 43. Pig no. 9713: age 4 months. Section of the endometrium of the uterus showing an artery and a few glands. Weigert's, van Geison's, and Heidenhain's stain. 400X.



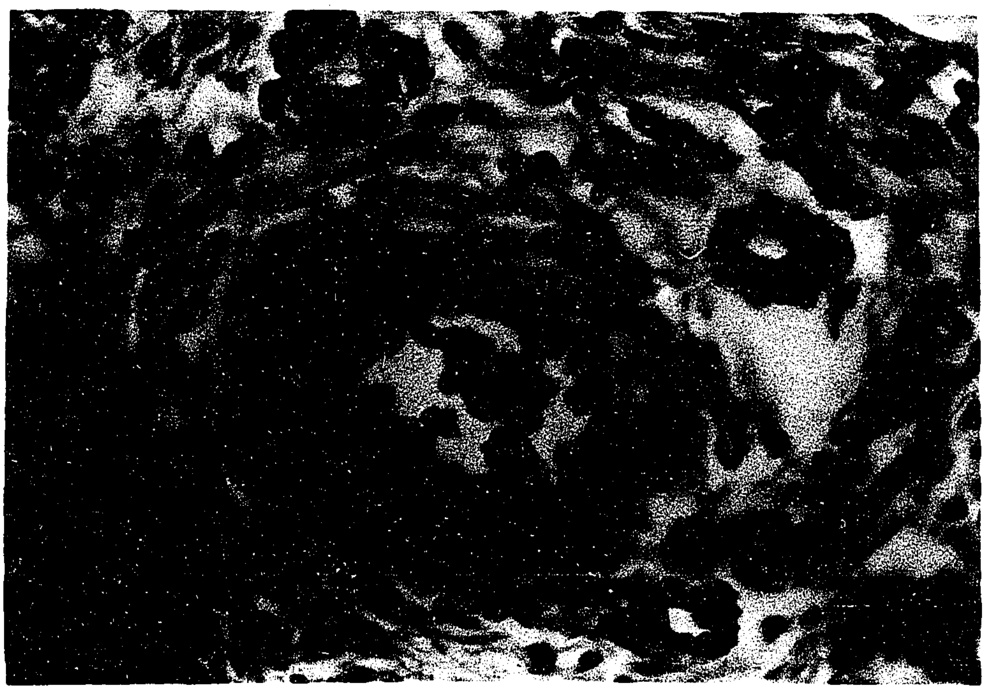
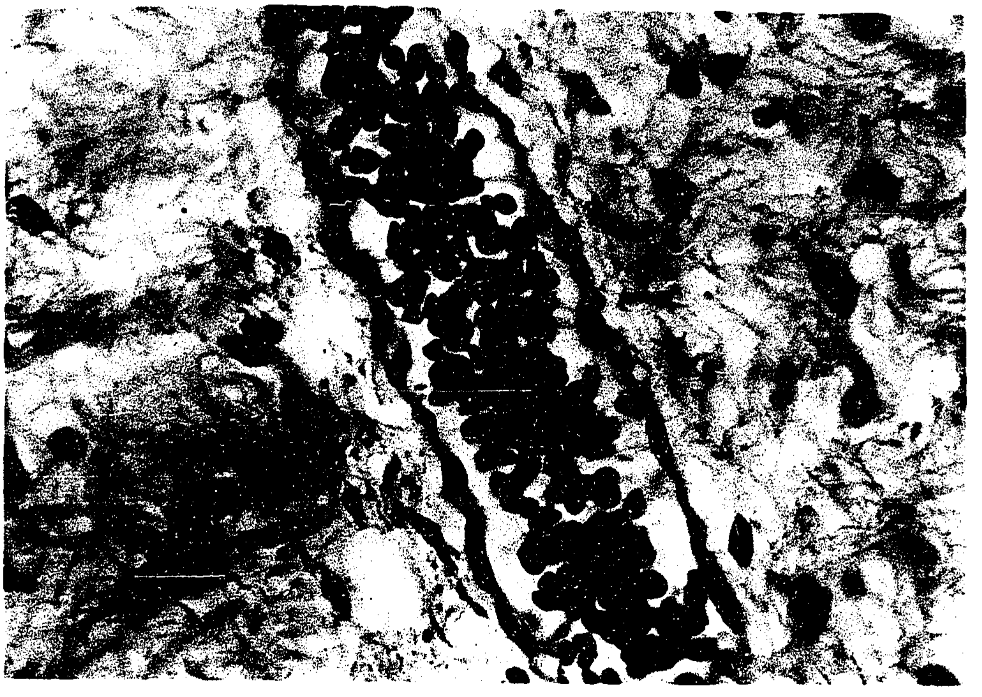


Figure 44. Pig no. 1292: age 6 months. Section of the endometrium showing longitudinal section of a venous sinus lined with endothelial cells and surrounded by areolar connective tissue of the endometrium. Weigert's, van Gieson's, and Heidenhain's stain. 400X.

Figure 45. Pig no. 1292: age 6 months. Section of a normal artery in the mesometrium. Weigert's, van Gieson's, and Heidenhain's stain. 100X.



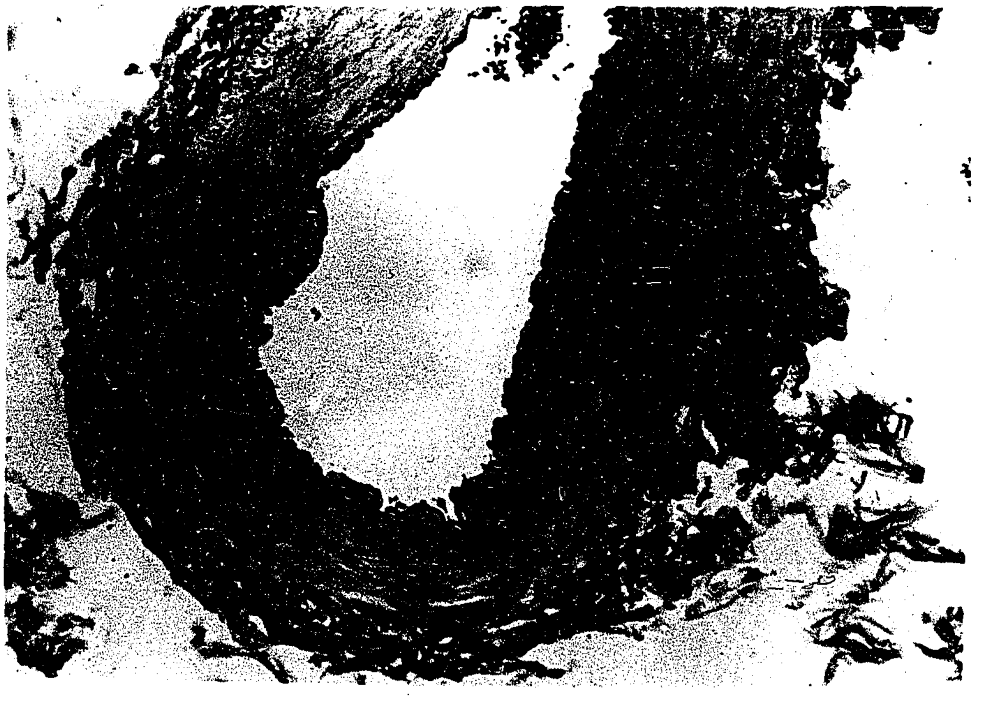


Figure 46. Pig no. 2944: age 1 year 3 months. Section of a normal arteriole in the endometrium of the uterus. Verhoeff's elastic stain. 250X.

Figure 47. Pig no. 2944: age 1 year 3 months. A section of the uterus showing venous sinuses in the endometrium. Verhoeff's elastic stain. 250X.

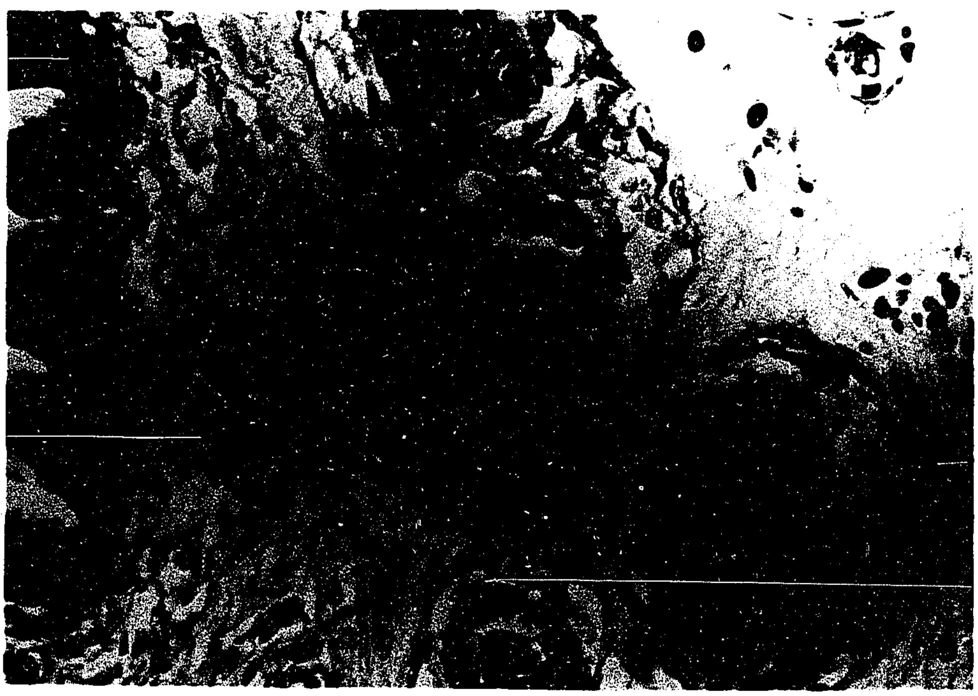


Figure 48. Pig no. 2021-s: age 1 year 5 months. An arteriole in the endometrium of the uterus showing thickened tunica intima. Verhoeff's elastic stain. 100X.

Figure 49. Pig no. 2021-s. Age 1 year 5 months. A section of the wall of a vein in the uterus showing vasa vasorum. Verhoeff's elastic stain. 250X.

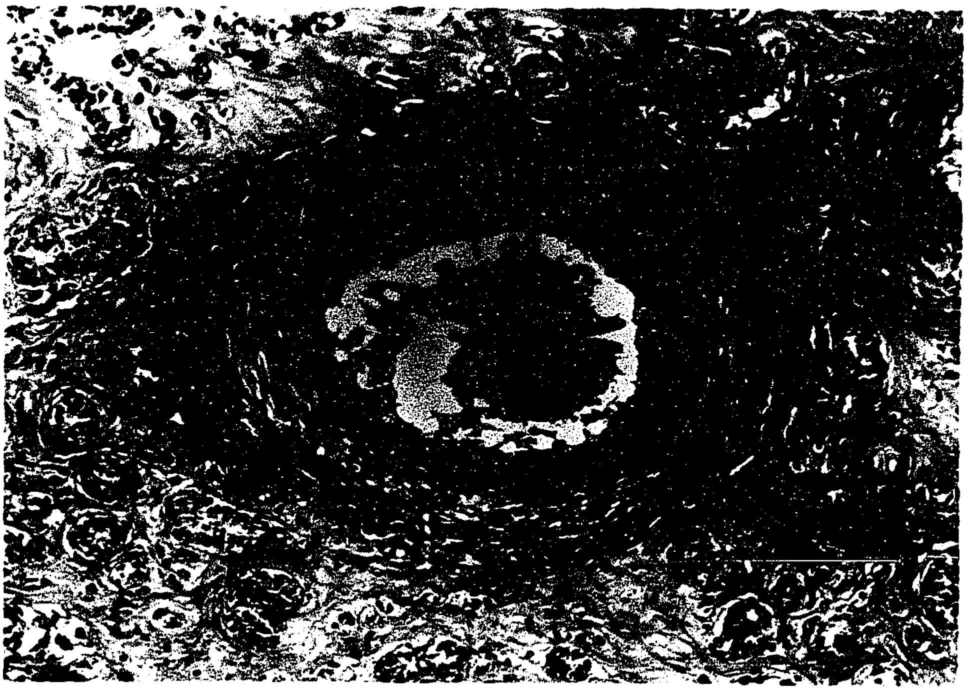


Figure 50. Pig no. 6154: age 2 years. A mesometrial artery in a section of the uterus showing thickened tunica intima. Verhoeff's elastic stain. 100X.

Figure 51. Pig no. 6154: age 2 years. A section of the uterus showing an arteriole with an intimal thickening in the endometrium. Verhoeff's elastic stain. 40X.

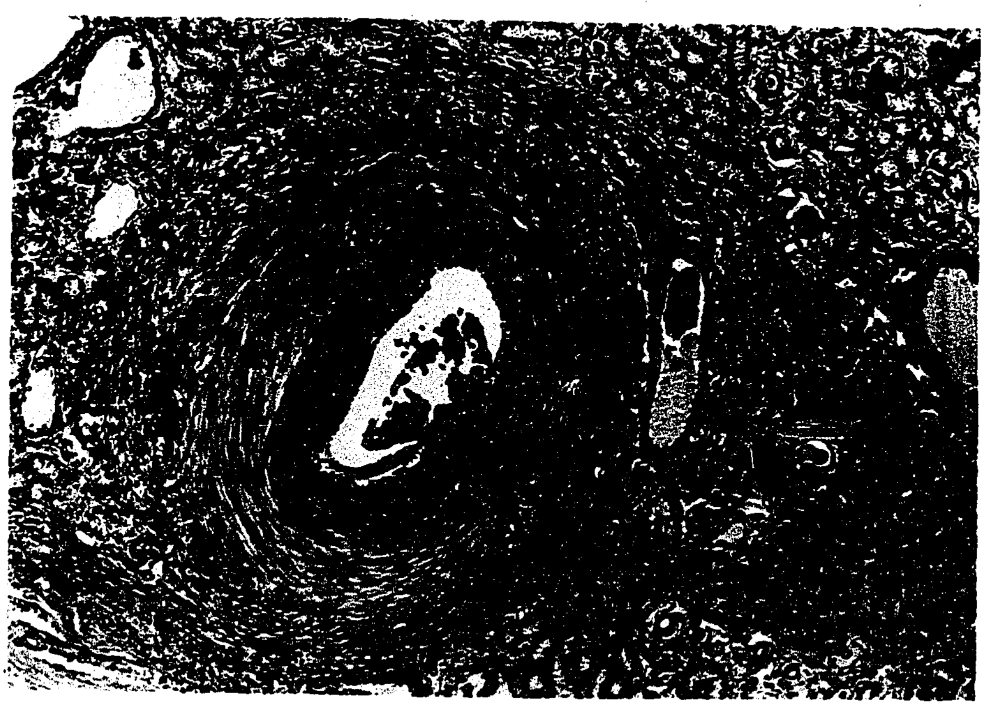
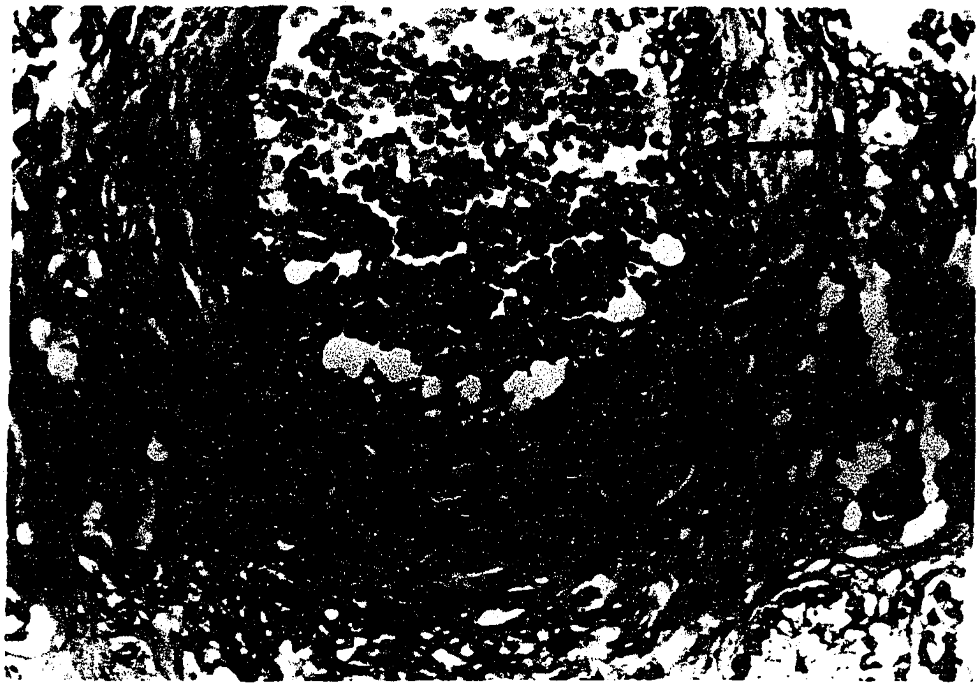


Figure 52. Pig no. 2442: age 2 years 2 months. An endometrial arteriole in a section of the uterus showing splitting of the internal elastic lamina. Verhoeff's elastic stain. 250X.

Figure 53. Pig no. 632: age 2 years 6 months. An endometrial arteriole in a section of the uterus showing intimal thickening. Verhoeff's elastic stain. 100X.



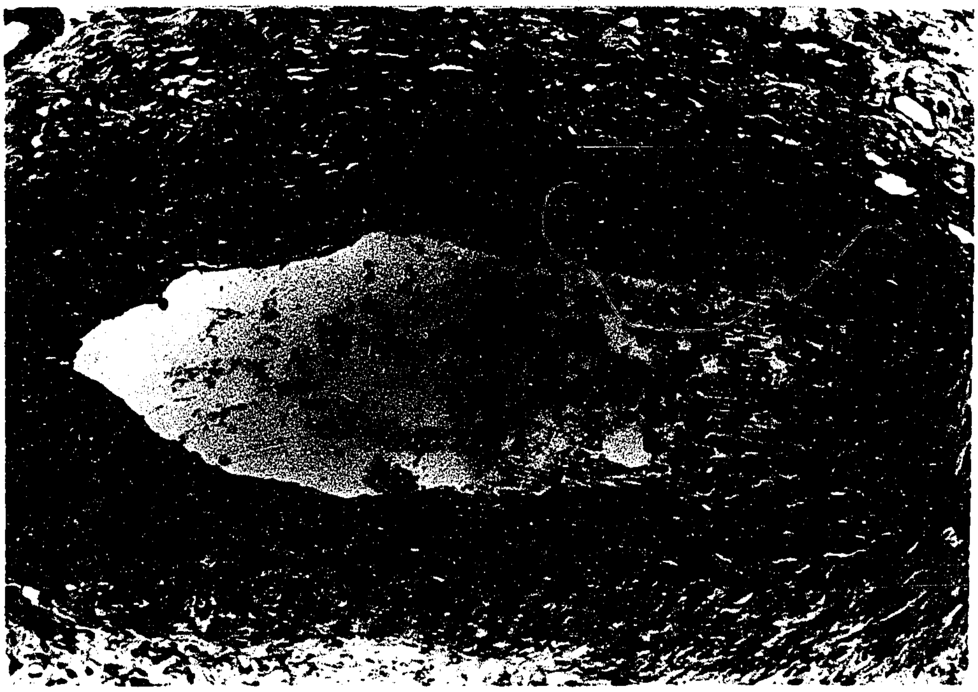


Figure 54. Pig no. 3201: age 2 years 6 months. Tunica intima forming almost half arterial wall of an endometrial arteriole in a section of the uterus. Verhoeff's elastic stain. 250X.

Figure 55. Pig no. 3430-s: age 1 year 2 months. A section of a pregnant uterus showing formed fetal membranes and epethelio-chorial relation. H and E stain. 100X.

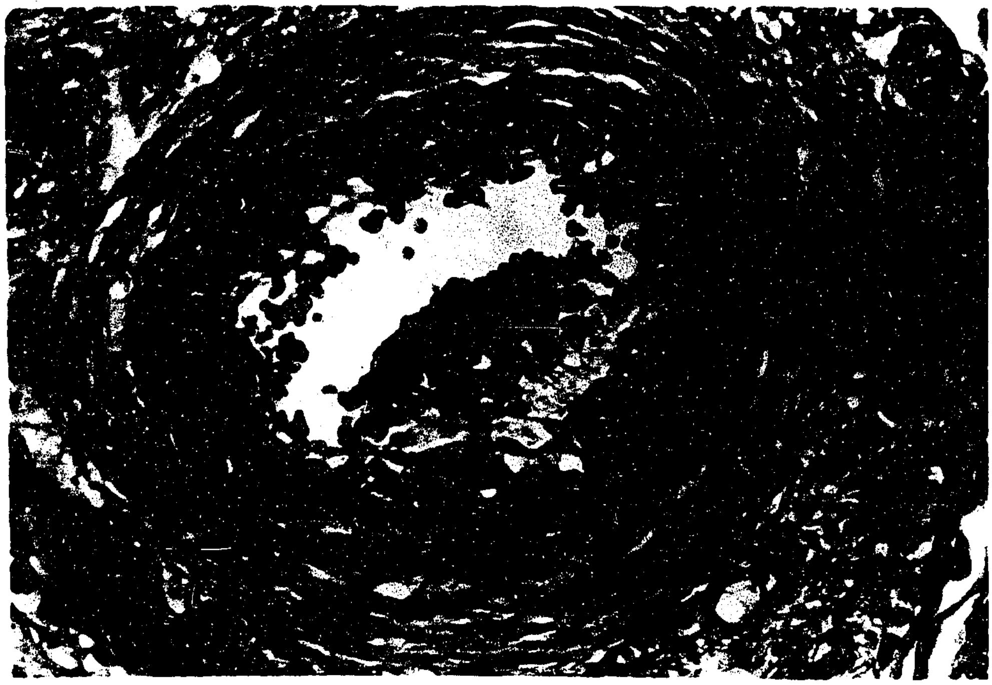


Figure 56. Pig no. 3430-s: age 1 year 2 months. A section through the myometrium of the uterus showing increased amount of connective tissue in pregnancy. Verhoeff's elastic stain. 100X.

Figure 57. Pig no. 1040: age 2 years 10 months. A section of an arteriole devoid of elastic tissue in the thickened tunica intima in pregnancy. Internal elastic lamina is indistinct. Verhoeff's elastic stain. 250X.

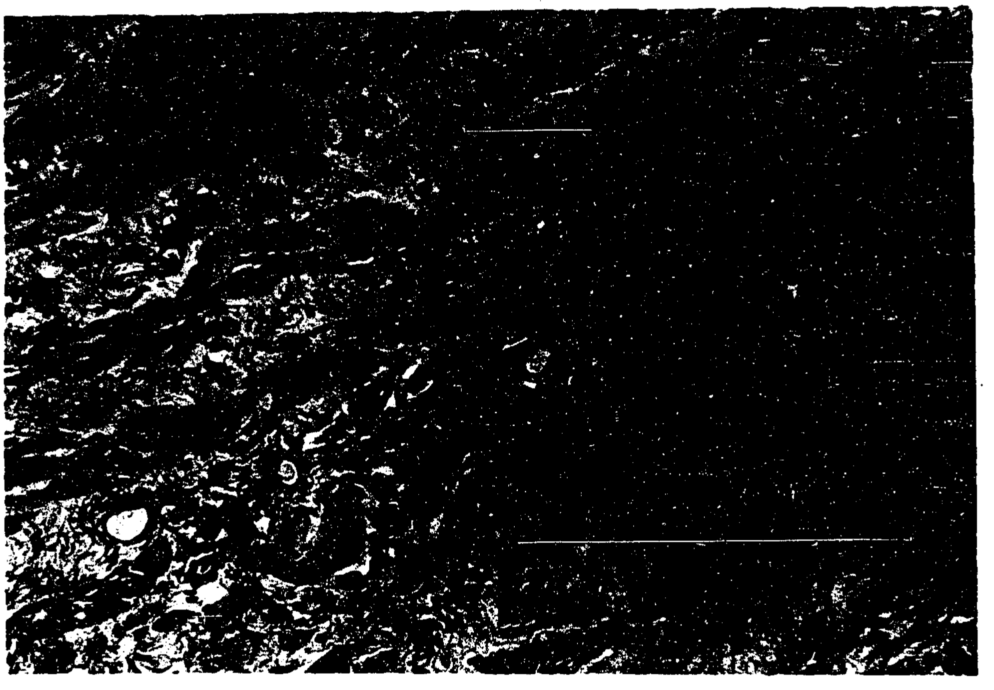


Figure 58. Pig no. 6015: age 4 years 11 months. A section of the uterus showing thickened tunica intima in endometrial arteries. Weigert's, van Geison's and Heidenhain's stain. 100X.

Figure 59. Pig no. 5895: age 6 years. A section of the uterus showing intimal thickening in a mesometrial artery. Verhoeff's elastic stain. 100X.

Figure 60. Pig no. 5895: age 6 years. A section of a vein of the uterus showing more fibro-elastic tissue in the wall than cellular elements. Verhoeff's elastic stain. 250X.

Figure 61. Pig no. 1: age 7 years 9 months. A section of an endometrial artery in which the tunica intima forms about 2/3 of the wall. Verhoeff's elastic stain. 250X.

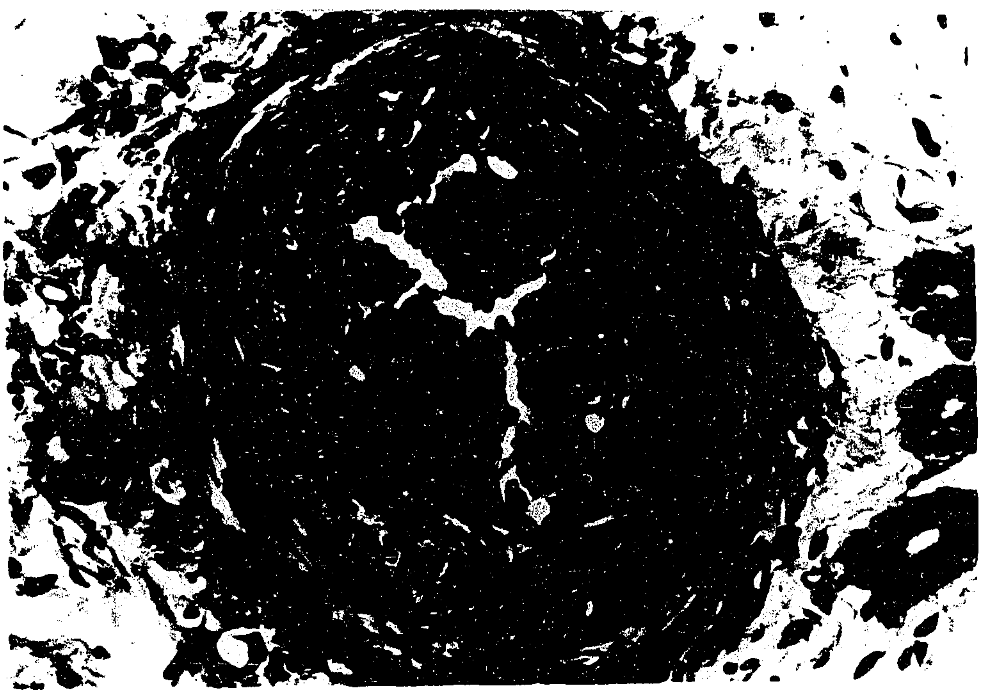


Figure 62. Pig no. 1175: age 8 years. A section of the uterus showing the formation of glandular cysts in the deeper part of the endometrium. H and E stain. 40X.

Figure 63. Pig no. 1175: age 8 years. A section of the uterus showing thickened tunica intima in the mesometrial arteries. Verhoeff's elastic stain. 40X.

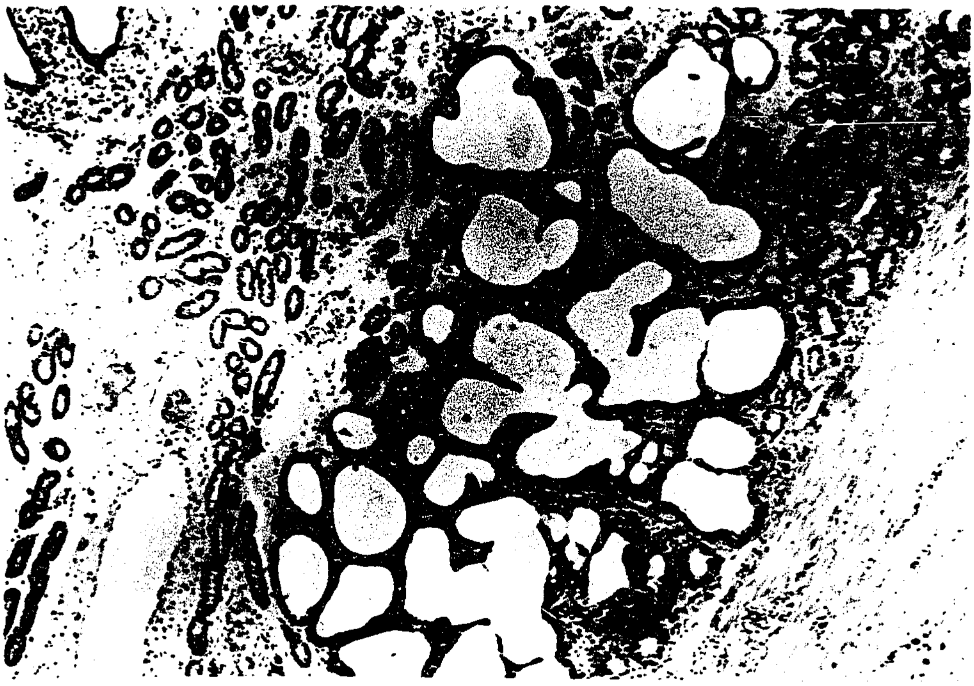
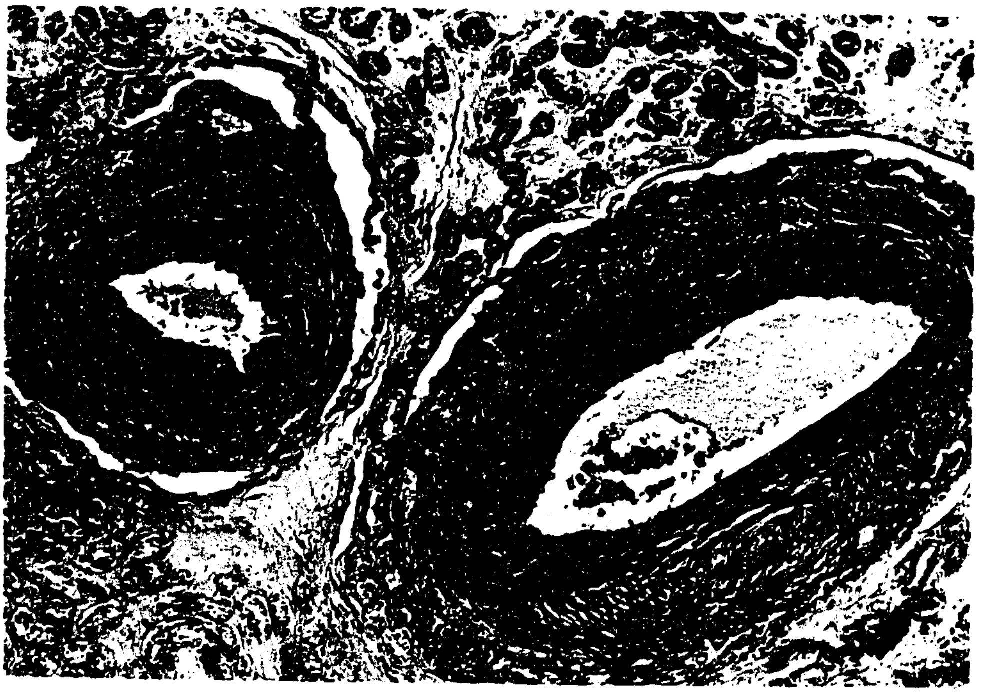


Figure 64. Pig no. 1175: age 8 years. A section of the uterus showing thickened tunica intima in the endometrial arteries. Verhoeff's elastic stain. 40X.

Figure 65. Pig no. 2944: age 1 year 3 months. A section of the uterus showing the epithelium, capillaries and glands of the endometrium. H and E stain. 100X.



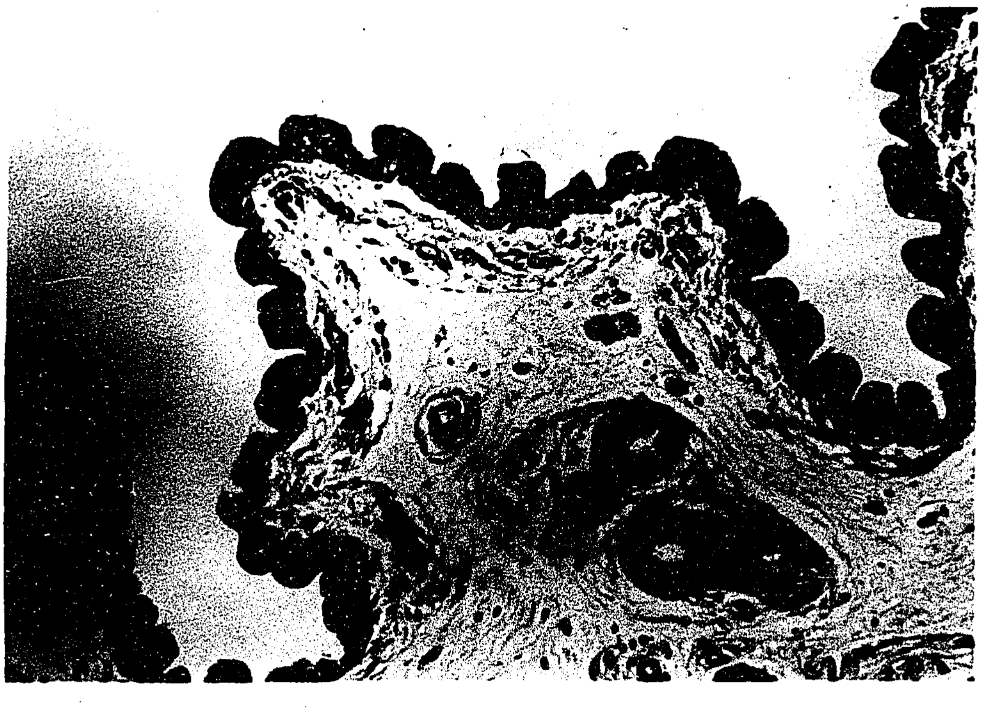


Figure 66. Pig no. 2: age 1 day. Section through the cervix showing the epithelium lining and the mesonephric duct in the wall. H and E stain. 40X.

Figure 67. Pig no. 2: age 1 day. Young artery and a vein in the tunica adventitia of the cervix. Weigert's, van Gieson's, and Heidenhain's stain. 250X.

Figure 68. Pig no. 1263: age 1 week. Section through the tunica adventitia of the cervix showing an autonomic ganglia and blood vessels. Weigert's, van Gieson's, and Heidenhain's stain. 250X.

Figure 69. Pig no. 1320: age 2 weeks. Section of arteries in the cervix of tunica adventitia showing increased growth of the arterial wall. Weigert's, van Gieson's, and Heidenhain's stain. 250X.

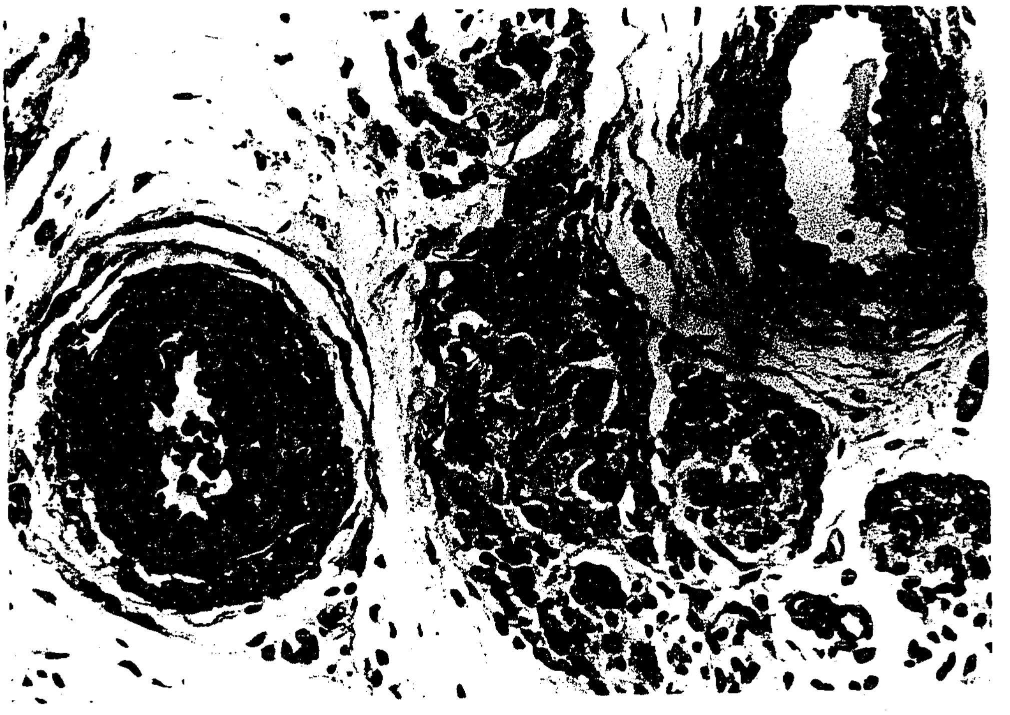
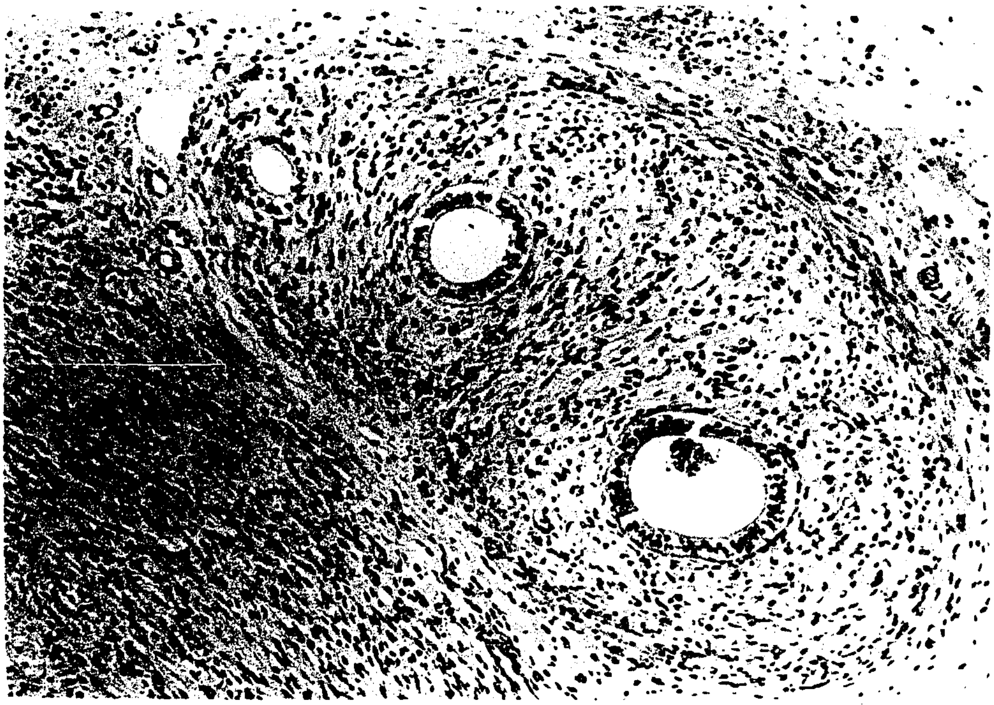


Figure 70. Pig no. 1653: age 1 month 1 week. Section through the wall of the cervix showing the persistent mesonephric ducts. H and E stain. 100X.

Figure 71. Pig no. 627: age 2 months. Section of the cervix indicating the differentiated layers of the tunica muscularis. Mallory's triple stain. 40X.



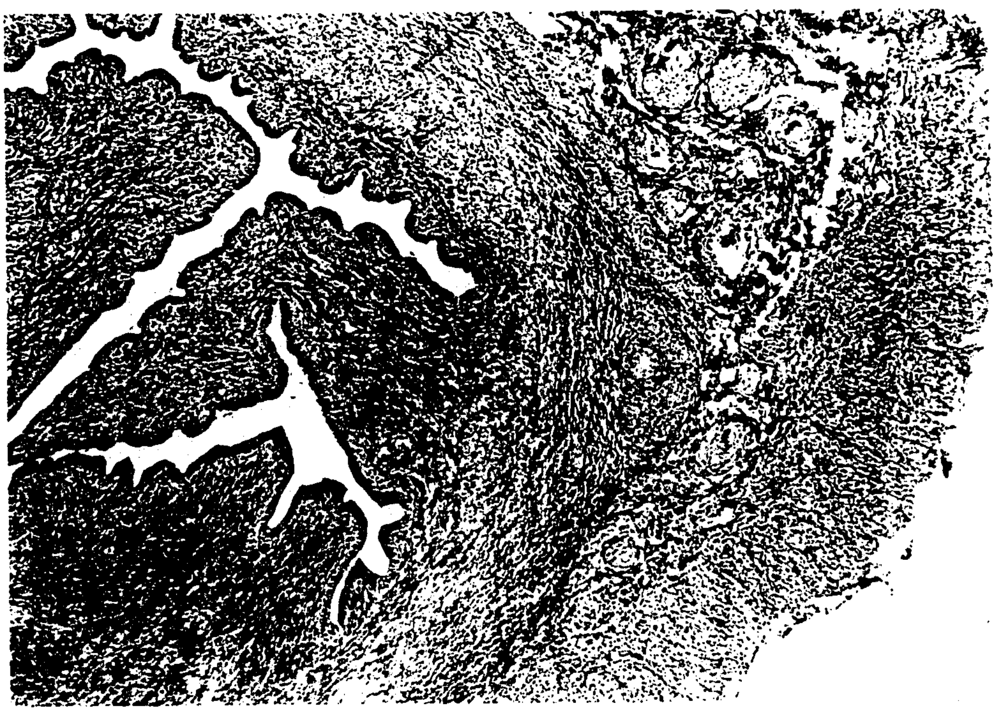


Figure 72. Pig no. 2250: age 4 months. Section of the mucosa of the cervix at the portio vaginalis showing some ciliated epithelium (black arrow) and clear cells (white arrow). H and E stain. 400X.

Figure 73. Pig no. 9310: age 6 months. Section of the portio supravaginalis or cranial part of the cervix showing the mucosa lined with simple columnar epithelium. H and E stain. 100X.

Figure 74. Pig no. 3430-s: age 1 year 2 months. A section of the tunica muscularis of the cervix showing increased amount of connective tissue in pregnancy. Verhoeff's elastic stain. 100X.

Figure 75. Pig no. 5930-s: age 1 year 2 months. A section of the cervix showing normal arteries in the tunica muscularis. Verhoeff's elastic stain. 100X.

Figure 76. Pig no. 6154: age 2 years. A section of the cranial part of the cervix showing mucous glands in the mucosa. H and E stain. 100X.

Figure 77. Pig no. 2442: age 2 years 2 months. A section of the cervix showing a venous sinus and an arteriole in the mucosa. Verhoeff's elastic stain. 250X.

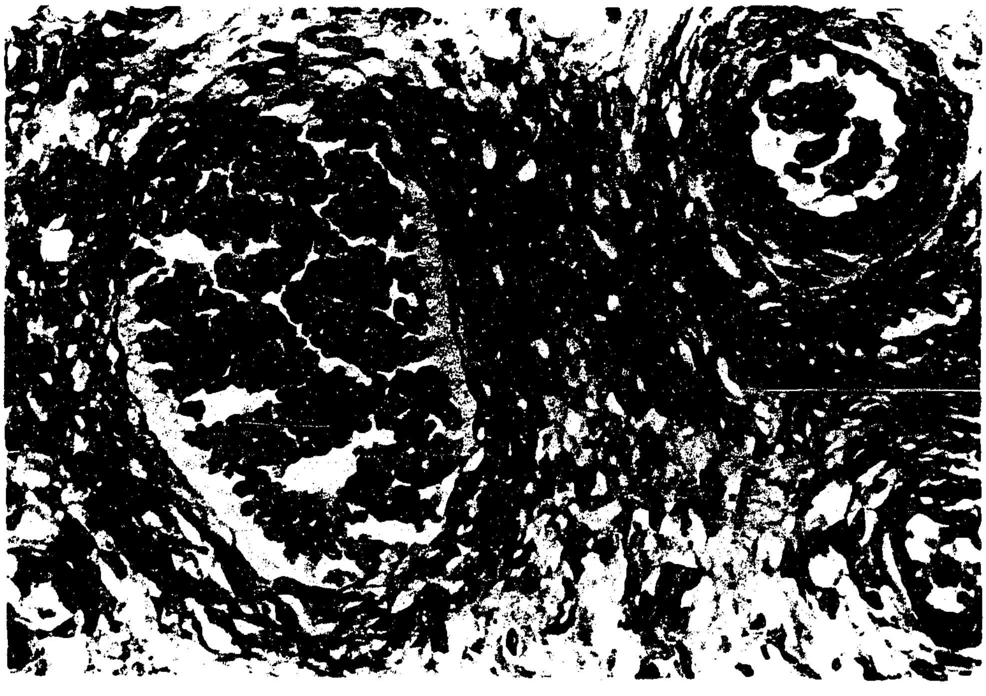


Figure 78. Pig no. ISU 95: age 2 years 6 months. A section of the cervix showing epithelial nests and cystic forms in the mucosa. H and E stain. 100X.

Figure 79. Pig no. 1040: age 2 years 10 months. An artery of the cervix showing thickened tunica intima. Verhoeff's elastic stain. 250X.

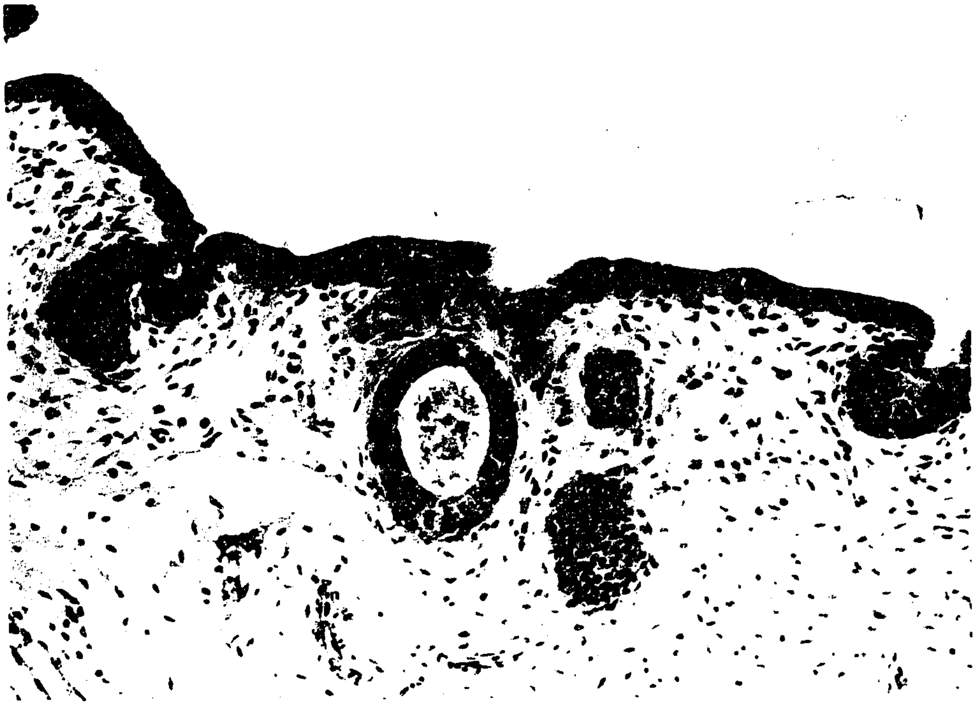


Figure 80. Pig no. *1040*: age *2 years 10 months*. *A vessel of the cervix* in a cross section showing two layers of smooth muscle forming the wall. Both the inner longitudinal layer and the outer circular layer have elastic tissue. Verhoeff's elastic stain. 40X.

Figure 81. Same vessel as Figure 80. 100X.

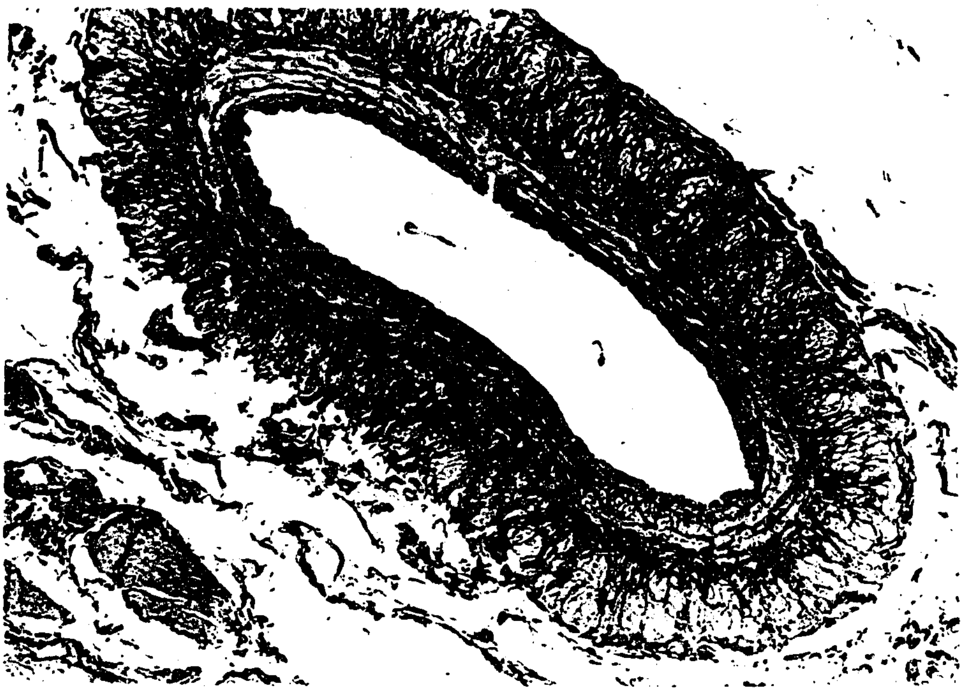
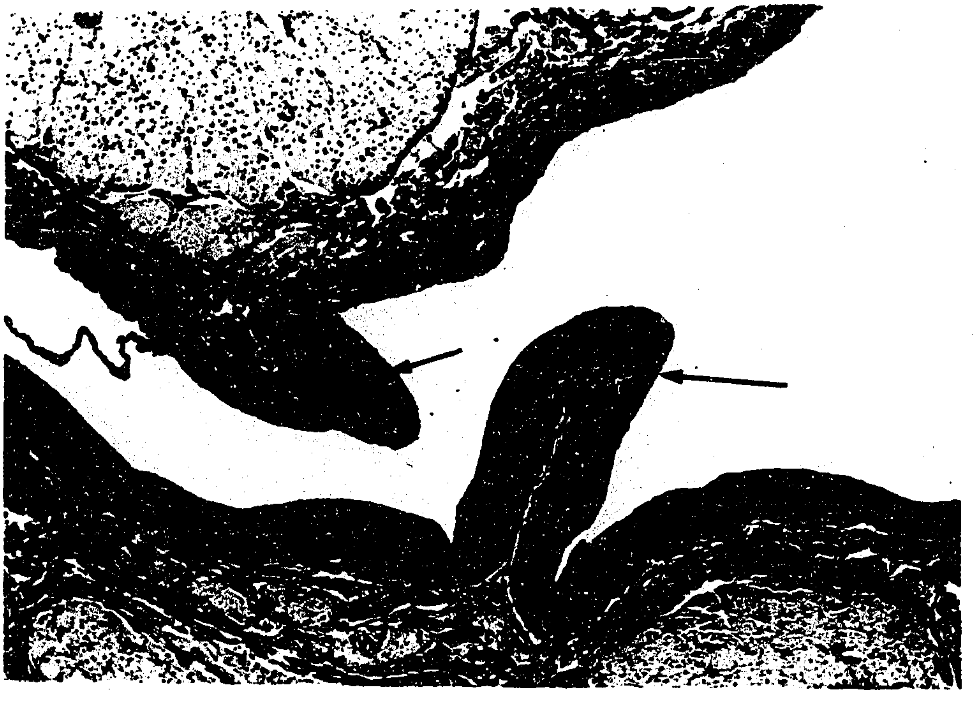


Figure 82. Pig no. 3196: age 3 years 1 month. A vein of the cervix cut in a longitudinal section showing valves in the lumen. Verhoeff's elastic stain. 40X.

Figure 83. Pig no. 4919: age 4 years. A section of the cervix showing epithelial invaginations forming micro-cysts in the mucosa. H and E stain. 100X.



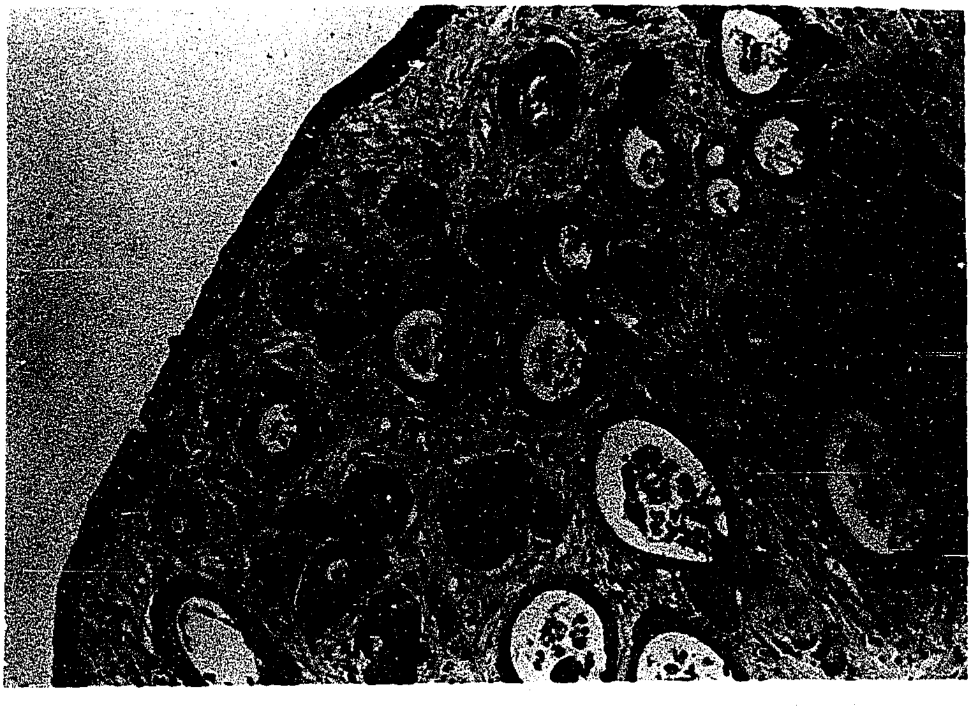


Figure 84. Pig no. BB 1: age 4 years. A section of the cervix showing an artery with thickened tunica intima. Verhoeff's elastic stain. 100X.

Figure 85. Pig no. 6043: age 6 years. A section of the cervix showing epithelial cysts containing necrotic cells in the mucosa. H and E stain. 100X.

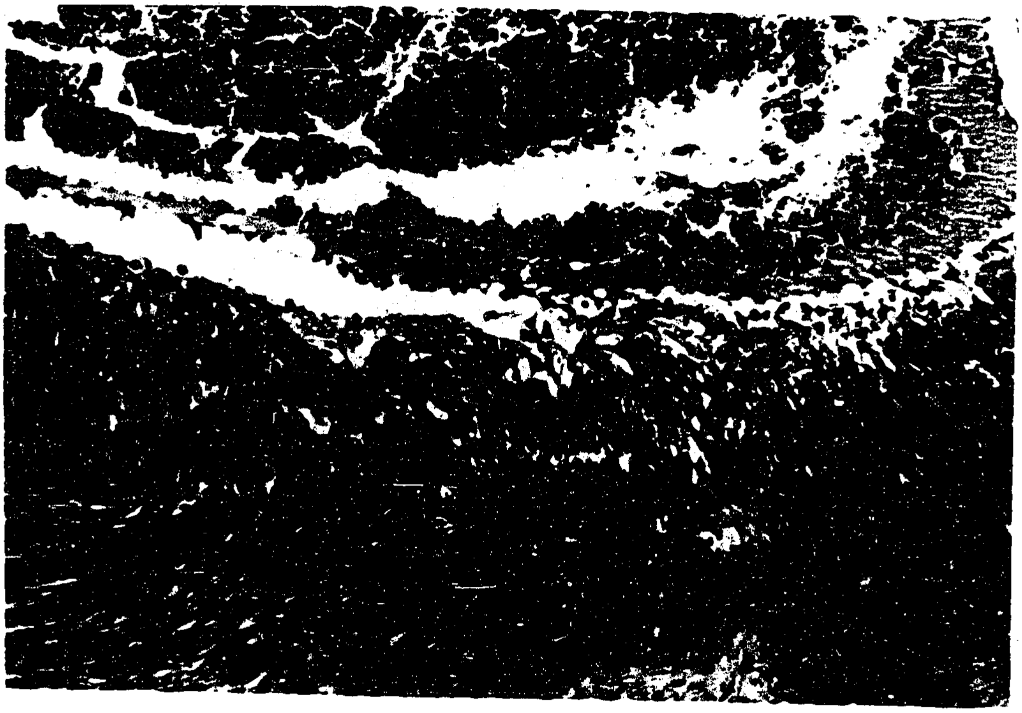


Figure 86. Pig no. 6043: age 6 years. Necrotic cells in the epithelial cysts in the mucosa of the cervix. H and E stain. 250X.

Figure 87. Pig no. 221: age 6 years 5 months. A dilated canal of Gartner (embryonic mesonephric duct) in the tunica muscularis of the cervix. H and E stain. 100X.

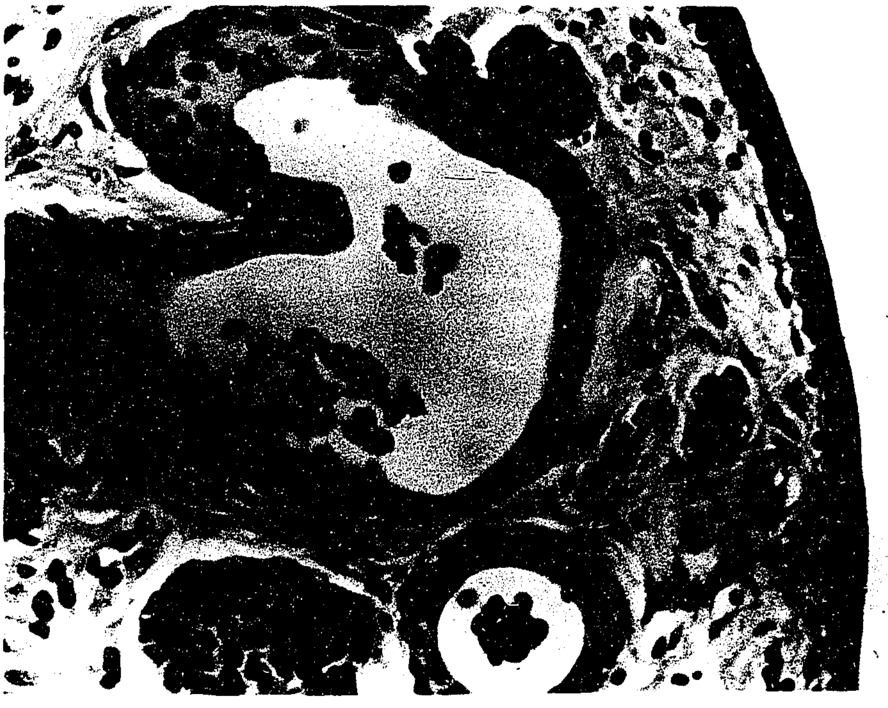


Figure 88. Pig no. 221: age 6 years 5 months. An arteriole in the tunica muscularis of the cervix showing thickened tunica intima. Verhoeff's elastic stain. 250X.

Figure 89. Pig no. 312: age 6 years 9 months. A section of the cervix showing stratified cuboidal epithelium. A subepithelial elastic lamina (arrow) and a cyst. Verhoeff's elastic stain. 100C.

Figure 90. Pig no. 13: age 7 years 9 months. An artery in the cervix showing fibrosis of the arterial wall. Verhoeff's elastic stain. 250X.

Figure 91. Pig no. 1175: age 8 years. Arteriole in the mucosa of the cervix showing the intima forming about half of the arterial wall. Verhoeff's elastic stain. 250X.

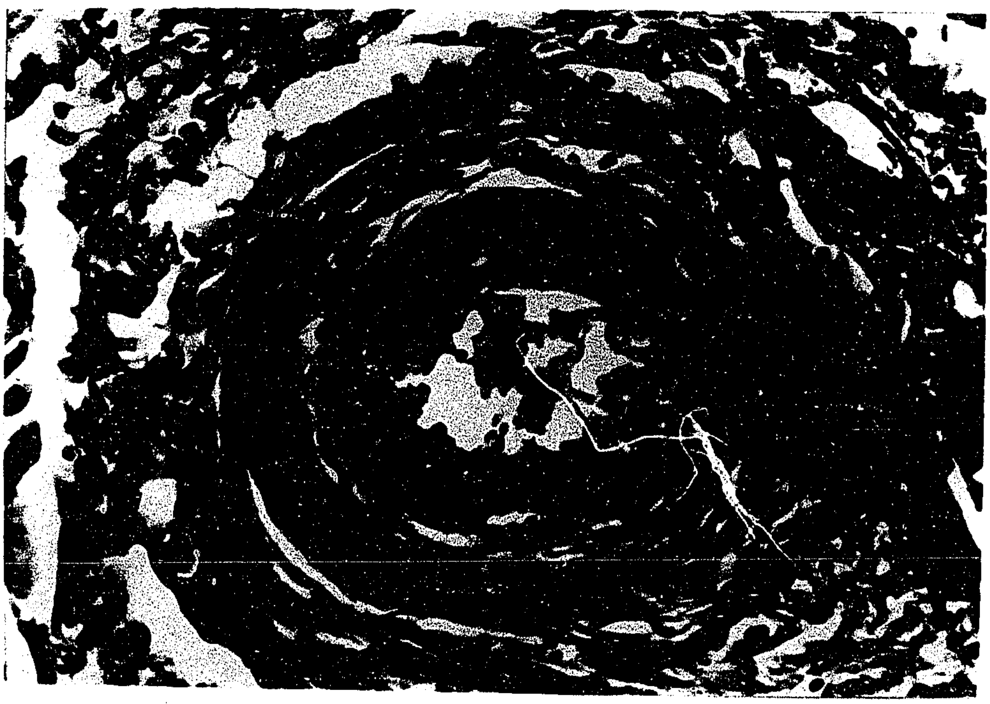
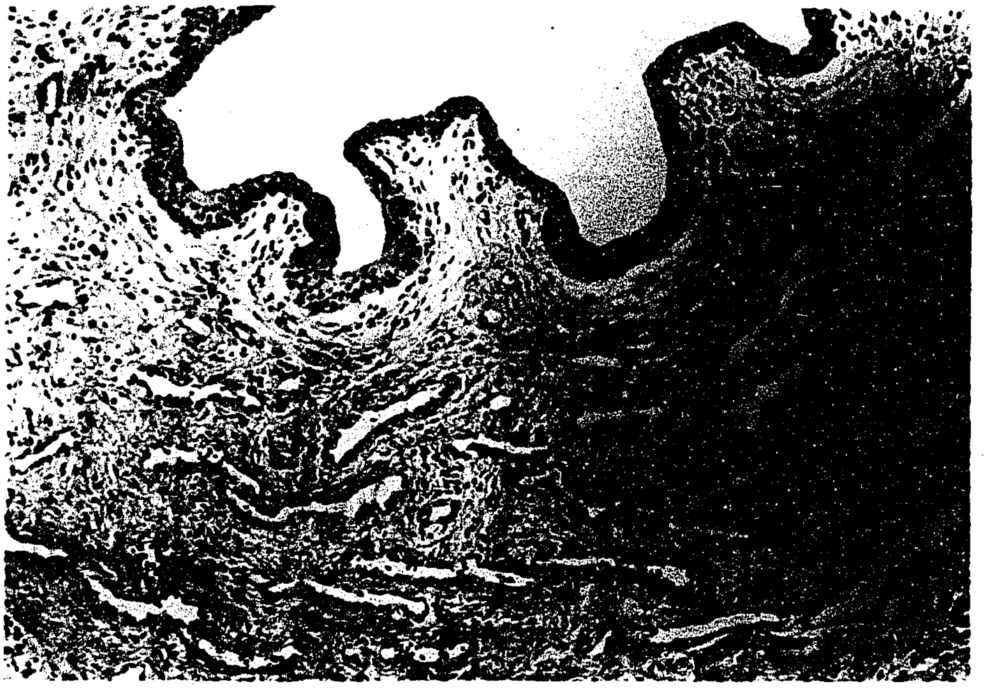


Figure 92. Pig no. 2: age 1 day. Section of the vagina showing a stratified epithelium and a spongy mucosa with many vascular channels simulating cavernous spaces of the erectile tissue. H and E stain. 100X.

Figure 93. Pig no. 708: age 3 months. Section of the vagina showing the epithelial invaginations into the underlying mucosa forming cystic structures. H and E stain. 100X.



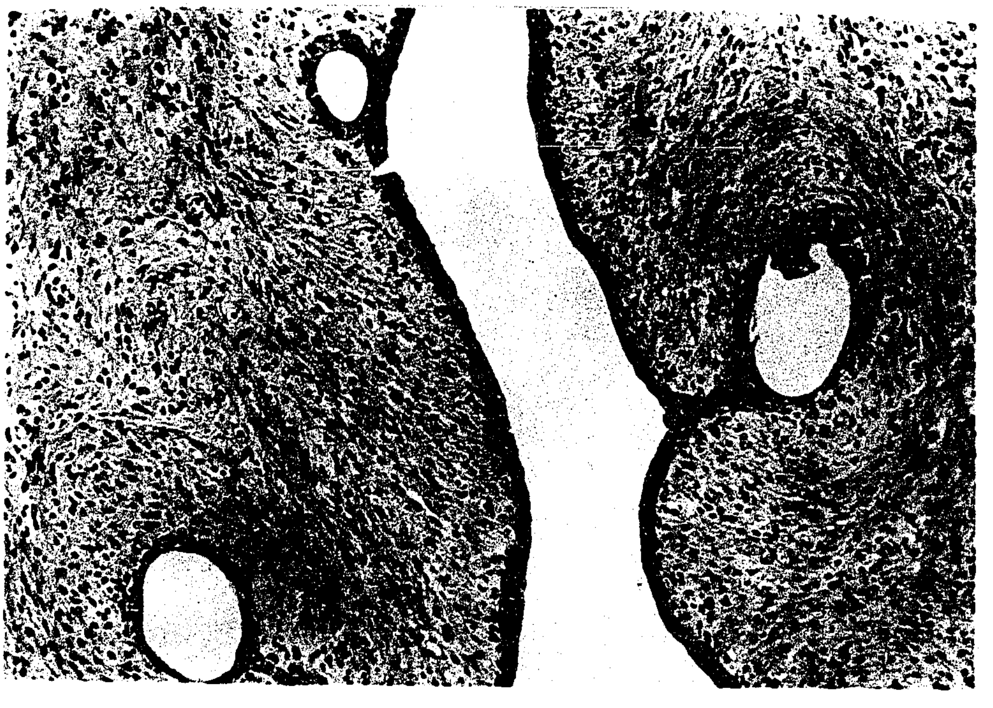
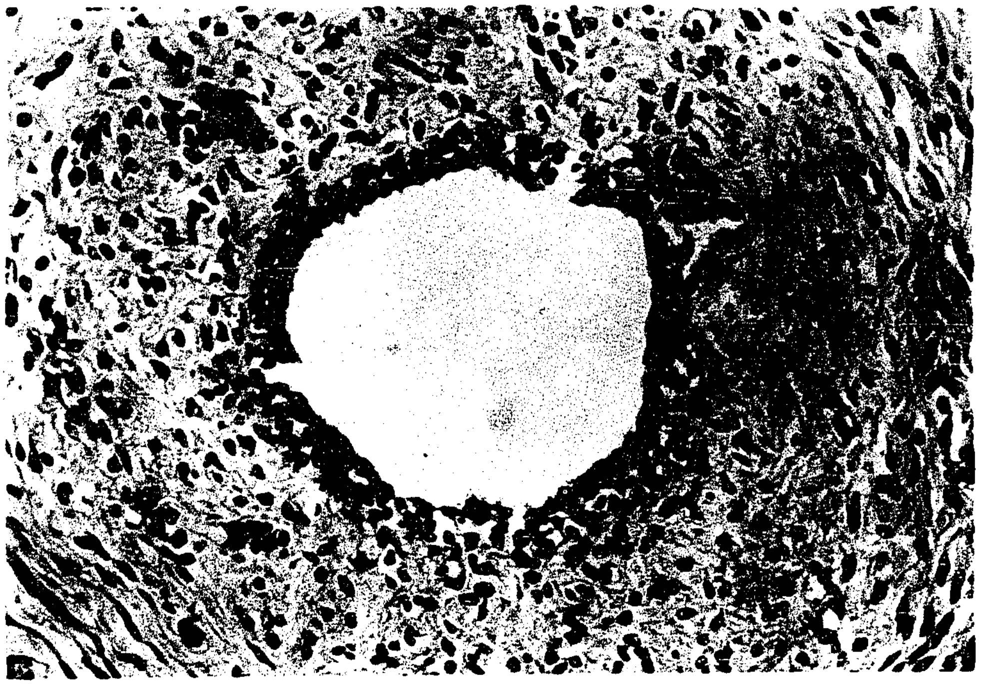


Figure 94. Pig no. 708: age 3 months. Section of the vagina showing an embryonic mesonephric duct or the canal of Gartner in the wall. H and E stain. 250X.

Figure 95. Pig no. 9713: age 4 months. Section of the vaginal mucosa showing over 15 layers of stratified epithelium characteristic of the estrus. H and E stain. 250X.



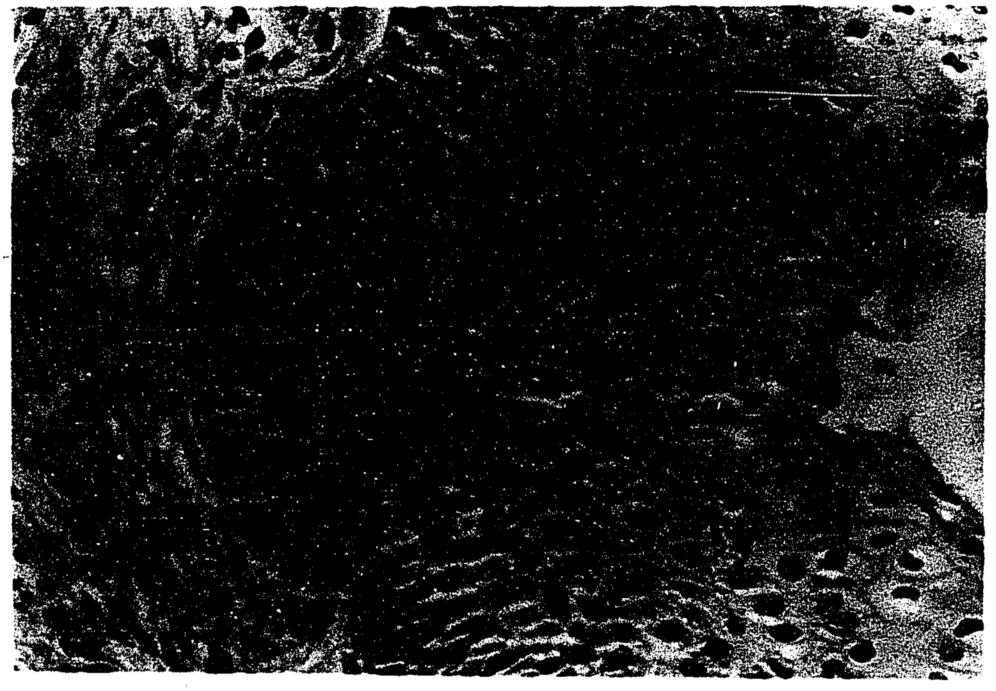
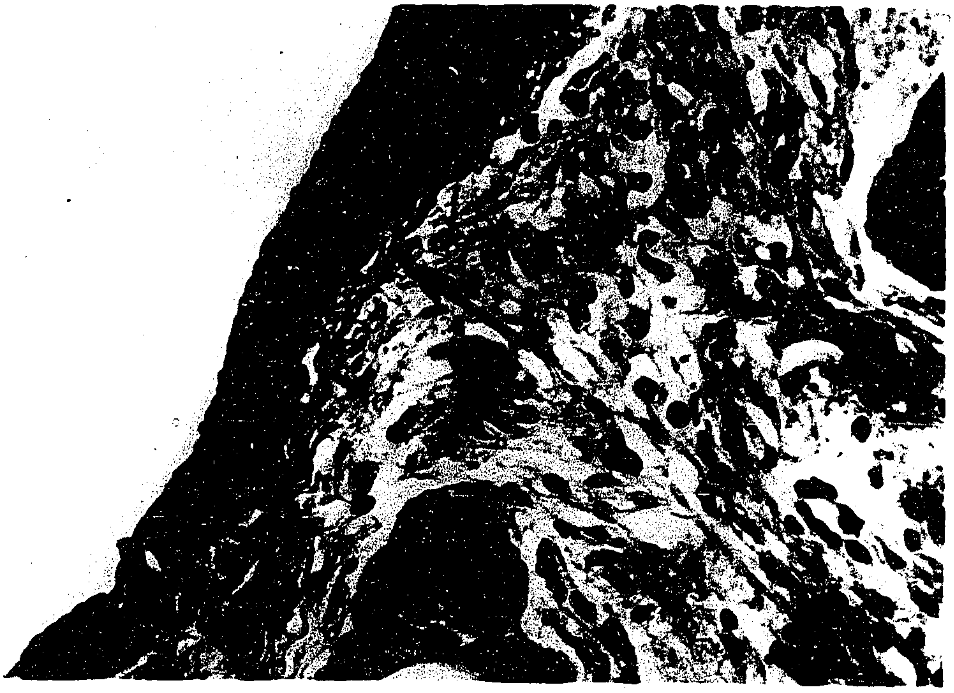


Figure 96. Pig no. 3202: age 2 years 6 months. A section through the vagina showing the subepithelial elastic lamina (arrow). Weigert's, van Gieson's, and Heidenhain's stain. 250X.

Figure 97. Pig no. 3202: age 2 years 6 months. A section of the vagina showing epithelial invaginations into the mucosa. Weighert's, van Gieson's and Heidenhain's stain. 100X.



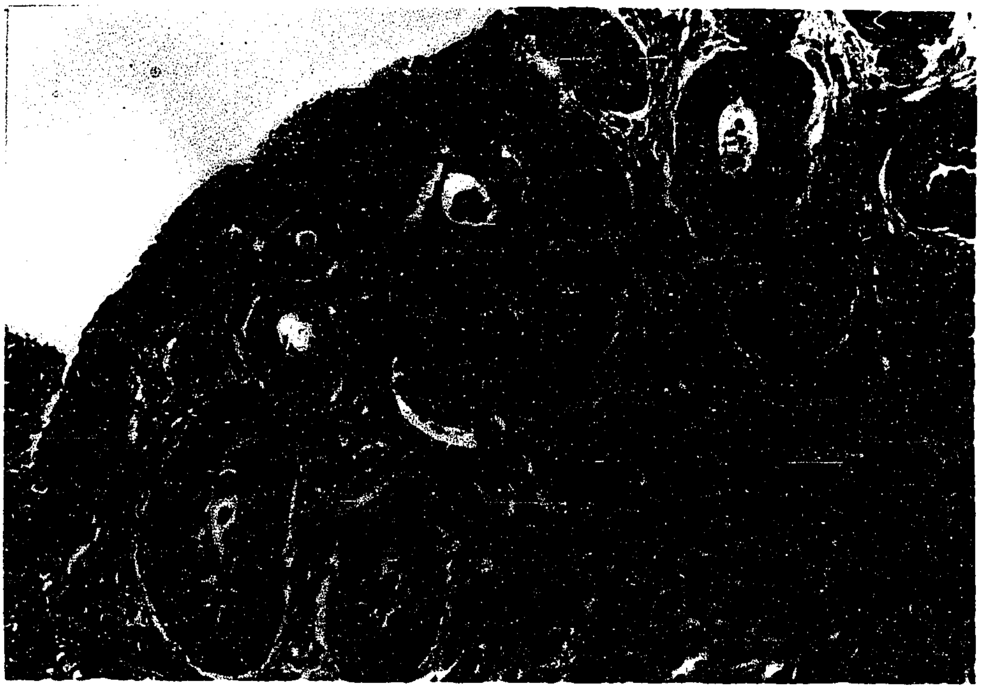


Figure 98. Pig no. 1175: age 8 years. A section of an arteriole in the tunica muscularis of the vagina showing thickened tunica intima. Weigert's, van Gieson's and Heidenhain's stain. 250X.

Figure 99. Pig no. LJF 1: age 8 years. A section of an arteriole in the tunica mucosa of the vagina showing thickened tunica intima. Weigert's, van Gieson's and Heidenhain's stain. 250X.

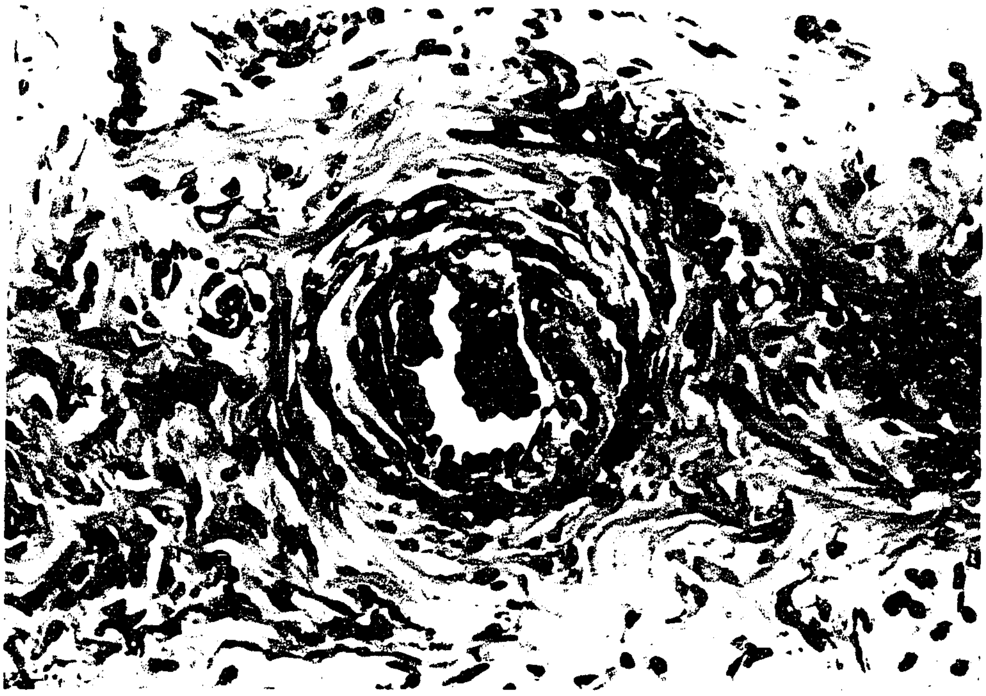


Figure 100. Pig no. LJF 1: age 8 years. A section through the fibro-muscular layer of the vagina. Weigert's, van Gieson's and Heidenhain's stain. 100X.

Figure 101. Pig no. LJF1: age 8 years. A section of the mucosa of the vagina showing two layers of epithelium characteristic of the diestrus or luteal phase of the estrus cycle. H and E stain. 250X.