MORPHOLOGICAL CHANGES IN THE OVARIES OF THE SOW (SUS SCROFA DOMESTICUS) AS INFLUENCED BY AGE FROM BIRTH TO EIGHT YEARS

by

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INTRODUCTION

The subject of Veterinary Anatomy encompasses the study of the "normal structure" of the tissues and organs of the domesticated animals. What tissue appears normal or physiological and what is pathological cannot be differentiated specifically until studies of such tissues in chronological order from birth to senility are made scientifically. In addition to the accepted properties of protoplasm, which is the organized basis of life, senescence should also be recognized as one of its fundamental properties. The object of gerontological research, therefore, is to identify the changes in structure and function of the tissues and organs influenced by the passage of time. It was Dr. E. V. Cowdry who brought to the attention of the Josiah Macy, Jr. Foundation the need for a comprehensive survey of the field of aging in the year 1937. Scarcity of factual information regarding what is normal in the domestic animals was recognized also by Dr. Robert Getty, Professor and Head, Department of Veterinary Anatomy, at the Iowa State University over 10 years ago. As a result of this realization a comprehensive gerontologic program was initiated.

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

With these thoughts in mind, the present studies of morphological changes in the ovaries of the sow, as influenced by age, from birth to 8 years were undertaken as one of the gerontological projects in this department. The breeding of the domestic pig (Sus scrofa domesticus) is a profitable and a rewarding enterprise for a farmer. It is also an excellent laboratory animal.

First reports on age changes in the ovaries of the domestic pig from birth to 33 months appear in the publication by Hadek and Getty (1959). In the present studies, it was decided to probe the age changes in the ovary of the sow beyond 33 months and carry them to the 8 year age limit.

The literature relating to the ovarian tissue is mostly about the follicular development and growth, and development of the corpus luteum, and the pattern of vascularization. Endocrine properties and functions of the ovary have been studies to a great extent in man and animals.

The ovary is a dynamic organ. Its constituent parts such as the graafian follicle, the corpus luteum and its germinal epithelium are changing simultaneously in a cyclic form induced mostly by hormones of the anterior pituitary. These structures are either developing or in stages of transformation, regression and involution. Blood vessels - especially arteries - are the only constituents of these organs which do not change much. There is, however, proliferation of arterioles and capillaries which supply the growing follicles as well as the corpus luteum. Therefore, more attention is focused on the structural changes taking place in the arteries of the ovary.

Efforts have been made to describe the morphology of the whole organ

in a particular age group along with the probable stage of the cycle through which it was passing when sacrificed for tissue collection. Many other peculiarities that have been observed in the tissue samples are reported.

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

The Ovary

Development

Gonads in the young embryos are exhibited during the indifferent stage of development. At this stage there is no evidence of whether they are going to develop into testes or ovaries. Along with the gonads the undeveloped stage of the male and female duct systems is present. If the developing embryo is a prospective female, one of these duct systems forms the uterine tubes, uterus, and vagina and the others remain rudimentary. If the embryo is destined to develop into a male, the female potential ducts remain rudimentary and the other set of duct system, the transitory function of which is excretory during that stage of development, gives rise to the duct system of the testes.

In dealing with the development of the reproductive organs, the indifferent stage is the starting point for the consideration of the later stages of the development of the reproductive organs in either sex (Patten, 1958). Gonads are closely associated with the nephric system and arise as ridge-like thickenings (gonadal ridges) on its ventro-mesial face. The ridge has a mesenchymal core and is enveloped by mesothelium. This stretch of mesothelium is continuous with the mesothelium covering the mesonephros. The mesothelium grows thicker and its cells round out and grow comparatively larger than their neighbors. This modified layer of mesothelium, with some large rounded cells, is now termed germinal epithelium. The large cells in or beneath the germinal epithelium are the

primordial germ cells. These primordial germ cells do not differentiate from the mesothelial cells. It is maintained that they are found in the yolk sac entoderm from where they migrate to the germinal epithelium of the gonads to settle down and raise their families. In case the gonad develops into an ovary, the primordial germ cells grow down into the mesenchymal core to differentiate into ovarian follicles containing ova.

Opinions differ regarding the modes of migration of the germ cells to the gonadal ridges:

(1) Mammalian germ cells are claimed to migrate largely by their own active ameboid movements aided by histiolytic action (Mintz, 1959).

(2) In amphibians, the primordial germ cells are drawn into the gonadal ridge passively from an extra embryonic to an intraembryonic position. This may be due to differential growth.

(3) In birds, at least, the vascular path is responsible for the transportation of primordial germ cells that first become segregated in the area pellucida. From here they migrate into the developing mesodermal blood islands of the area vasculosa, and enter the peripheral circulation which then carries them to the genital ridge area (Pasteels, 1953). Simon (1957) dissected the chick embryo at the level of the 20th somite and found that primordial germ cells situated at the germinal crescent reached the severed caudal region of the embryo given that the vascular path remained intact. In a second experiment Simon (1957) grafted the caudal half of another embryo on to the blastoderm and found that if the graft became vascularized, primordial germ cells originating from the cranial region of the host embryo were also able to reach the graft.

(4) Chemotaxis: Experimental evidence supports the view that induction substances responsible for the movement of mammalian primordial germ cells in the right and left ovaries of birds may well be mediated by chemotactic substances. An alternative explanation for the uneven distribution of the primordial germ cells between the two avian ovaries is that the right ovary which is destined to become nonfunctional is poorly vascularized at very early stages of development and gets a smaller quota of germ cells than the left ovary.

During migration it is believed that no multiplication of germ cells occurs. Mintz (1957), with histochemical methods has observed that on the 8th day of gestation mouse embryos contain about 100 primordial germ cells, whereas four days later the numbers are about 5000. The concensus of opinion, however, is that the primordial germ cells undergo a period of mitotic proliferation once they have reached the genital ridges and those that fail to reach their destination degenerate.

As already mentioned above a connection is established between the gonads and the mesonephros. The connection remains rudimentary in the female and does not become functional to transport germ cells as it does in the male. Before sex differentiation sets in, solid cellular cords called rete cords connect the medulla with the more cranially situated tubules of mesonephros. In the female, the rete cords do not develop after the onset of sexual differentiation. The rete ovarii remains capable of becoming functional for a certain time during ovarian differentiation, and may form a functional urogenital connection, if sex reversal occurs (Witschi, 1931; Foot and Witschi, 1939).

Regarding the inducing effects of the primordial germ cells on the further development of the gonadal ridges various experiments have been conducted with inconclusive results. Burns (1955) however concludes that (1) the local appearance of genital ridges is conditioned by regional influences, (2) primordial germ cells alone cannot induce a genital ridge and are not essential for its origin, and (3) the formation of the ridge is an activity of the structural elements.

Goldsmith (1935) and Bounoure (1937) observed a marked reduction in the size of the developing gonads if the primordial germ cells were prevented from reaching the genital ridges; and that primordial germ cells which, as a result of grafting, aggregate in ectopic positions are capable of inducing local thickenings of the coelomic epithelium which do not, however, differentiate to form a typical gonad (Humphrey, 1928).

Macroscopic and microscopic structure

Ovaries in the female species, like the testes in the male, are the essential organs of reproduction. The shape of the mammalian ovaries simulate the testes, although they are smaller in size.

The ovaries of the sow are situated in the abdominal cavity on the lateral wall of the pelvic inlet. Their position depends on the number of pregnancies and the young ones born by the sow. The broad ligament forms the ovarian bursa and is attached to the flank and the lateral pelvic wall. Therefore, the ovaries lie anterior to the broad ligament, sustained by the vessels that supply and drain the ovary and by the ligament of the ovary, composed of smooth muscle strands which attach it to the uterus.

This situation in the swine ovaries sometimes changes and they occupy cavities, analogous to those of the male scrotum in the perineal region.

Their shape is somewhat ovoid measuring on an average 1" by 1½" and they weigh about 3 grams to 1 pound depending on the functional state of the organ. Their surface, unlike that in the other domestic animals, is not smooth but presents rounded prominences which are the projections of graafian follicles and corpora lutea. There is no ovulation fossa although a distinct hilus is present.

In the newborn pig, the ovary has a trilobed macroscopic appearance and sections on microscopic examination showed clefts, two in number. These clefts lost their predominance at the age of 5 to 6 weeks and were no more discernible at 3 months of age. Occasionally, the clefts persisted in the ovaries of mature hogs (Hadek and Getty, 1959).

Vessels and nerves

The ovarian artery, a branch of the abdominal aorta, reaches the attached border of the ovary passing between the layers of the mesovarian.

The large numerous veins drain the ovary. The lymph drainage is towards the lumbar lymph nodes.

The nerves are derived from the sympathetic system through the renal and aortic plexus and accompany the vessels (Sisson and Grossman, 1958).

Microscopic structure

The microscopic structure of the ovary would depend on the plane of the section to be studied and the physiological state, i.e., phase of the ovarian cycle. For descriptive purposes, the ovary is divided into (1)

cortex, (2) zona parenchymatosa, and (3) zona vasculosa or medulla.

Germinal epithelium covers the ovary and the cells are usually cuboidal in shape consisting of a single layer of cells. Below this epithelial layer is the tunica albuginea made up of condensed connective tissue framework derived from the cortical stroma. The mesovarian is attached at the hilus where the medullary substance extends to the surface through the cortex and the tunica albuginea.

In their studies on the age changes in the ovary of the sow from birth to 33 months Hadek and Getty, (1959) observed alterations in the shape of germinal epithelial cells in the different parts of the ovary. At certain areas germinal epithelial cells were tall columnar measuring 30 microns and next to these appeared low cuboidal to flat squamous type measuring only 10 microns.

Cortical stroma in the sow ovary is a highly reactive tissue. This may be attributed to the undifferentiated potential properties of the cells constituting the stroma. These may be called stromal fibroblasts and are capable of differentiating into wandering macrophages, storing dyes, and accumulating and storing lipids. They assume an epithelioid character and perform nutritive and secretory functions in the follicles. They occur singly or in clusters in the stroma as the interstitial cells (of bitch and cat). Although the evidence regarding the physiological function of these cells is limited, the possible elaboration of, estrogen, progesterone and androgen by them is not ruled out. Since these cells are present from birth to old age and exhibit numerous potentialities of differentiation correlated with the reproductive age and ovarian cycles, they may prove to be the most important ovarian gland cells when their function is completely

understood (Mossman et al. 1964). In the ovaries of the mare pigmented cells are found which decrease with age. The cortical stroma appears studded with primary follicles in different progressive and regressive stages of development. In the sow these follicles are quite numerous and number about 60,000 in each ovary. They vary in shape and size and tend to become larger from the periphery inwards.

In carnivores and ruminants these follicles are found in clusters and are uniformly distributed below the tunica albuginea.

Larger Graafian follicles are found in the deeper layers. In the sow the primary oocytes are hard to distinguish because of their delicate structure. The mesenchyme proliferates continuously forming the cortical stroma and the tunica albuginea which separates the germinal epithelium from the cortex. The consolidation of the tunica albuginea, it is claimed, stops further ingrowth of germinal epithelial cells to form follicles. There is evidence that new follicles may be formed throughout life, at least in some species, e.g. the bitch (Raps, 1946) by ingrowth of epithelial cords through the tunica albuginea.

Allen (1904), from his study of the mouse ovaries, concludes that germinal epithelium is numerous enough or is at a maximum during the estrus period when the genital tract and the ovaries are hyperemic. Blood supply to the ovaries is at a minimum level when the ovaries are almost anemic during the diestrus stage and consequently mitosis in the germinal epithelium is retarded and at its lowest ebb. Therefore ovogenesis is a postpubertal phenomenon by means of which young ova are added to the cortex of the adult ovary at each normal estrus period. About

400 to 500 ova differentiate from the germinal epithelium at each normal estrus and one percent of them survive from postpubertal ovogenesis. The low percentage of survival, resulting from postpubertal ovogenesis necessitates the recognition of the extensive degeneration of ova in the primary follicles.

The establishment of ovogenesis during sexual maturity makes this process more analogous to spermatogenesis. In the human ovaries the cortical stroma is composed of spindle shaped cells and intercellular substance. The cell bundles and fibers run in various directions and appear "swirly" with heavily unrelated cells. The tunica albuginea beneath the germinal epithelium differs from the stroma in that its fiber constituents are arranged more or less parallel with the surface, and the amount of amorphous intercellular substance is greater (Ham, 1965).

The medulla, in contrast to the cortex, is more loosely arranged and contains more elastic tissue and smooth muscle cells, extensive convolutions of blood vessels, especially veins, which are large and distended appear deceptively hemorrhagic. Vascular supply is extended to the cortex from the medulla.

In the gonads of cattle, Bascom (1923) describes interstitial cells in the ovary homologous to the interstitial cells of the testes. During embryonic life, when medullary cords or cords of Pfulger are present, the position of the interstitial cells between the cords seem as good a criterion for the ovary as for the testes. Their staining properties and their morphology resemble those of testes.

At 6 to 8 weeks after birth, cells in short strands or in groups of

4 or 5 that strikingly resembled the interstitial cells of bull testes appeared in the connective tissue network of the thickened theca interna cells with Mallory preparation. These cells, according to Bascom (1923), should be called interstitial cells.

Development of the graafian follicles

Of the total number of follicles in the ovaries a few percent of them mature to ovulate after puberty, as already stated in previous pages. A primary follicle consists of a large oogonium enveloped by a single layer of cells (Ham, 1965). The size of the primary follicle in the human ovaries is 40 to 50 microns. Degeneration is the ultimate fate of most of these follicles as they are or after their spurious development. Follicles exhibiting such changes are termed atretic follicles.

The nucleus of the oogonium is large with prominent chromatin granules and a pale interior with the exception of well stained nucleolus. In further growth of the primary follicle the follicular stimulating hormone (F.S.H.) triggers off proliferation of follicular epithelial cells to be known as cells of the membrana granulosa. At first these cells are cuboidal then columnar and ultimately become stratified after division. The primary oocyte contained by the granulosa cells increases in size. When the size of the follicle has doubled, a thick membrane - the zona pellucida - developes around the oocyte. This membrane is rich in glycoproteins and opinions vary as to its formation. Some investigators think that it is formed by the oocyte itself and others think that it could also be formed by the follicular cells (Bailey, 1964). In atresia of the follicle the zona pellucida persists longer than the ovum.

The cytoplasm of the oocyte contains yolk granules and appears pale. In vertebrates, there is evidence that the liver synthesizes the yolk protein which is brought to the egg via the circulatory system (Porter and Bonneville, 1964) and, without detectable change, is incorporated into the egg cell. There is no indication that the follicular cells enclosing the ovum nourish it, although opinions differ. The zona pellucida has been observed to be penetrated by the microvilli of the egg. These mocrovilli, on the other hand, are in contact with the projecting membranes of the follicular cells and desmosome-like structures have been observed. The plasma membranes of the egg and the follicular cells are not disrupted, but are intact. Bailey (1964) in his text states that the corona radiata cells supply nutritive substances to the ovum. As seen with the phase microscope the processes of the corona radiata cells extend through the zona pellucida and are in contact with the plasma membrane of the oocyte and vice versa. A narrow perivitelline cleft between the zona pellucida and the oocyte has been observed.

After the continued growth of the follicular cells covering the ovum, fluid accumulates in the form of small pools. These islands of small pools fuse until the follicle is filled with the follicular fluid surrounding the ovum. The ovum is anchored to the small germ hill of cells known as the cumulus oophorus. The follicle at this stage is the vesicular follicle. The stroma, surrounding this follicle, differentiates into the theca interna, the vascular layer; and the theca externa, the outer fibrous layer. The interna is interwoven with a rich network containing fat granules (referred to as interstitial) seen among the theca interna cells.

Between the theca interna and membranogranulosa is interposed a glassy basement membrane.

Blood supply and lymphatic drainage: In the sows ovary Anderson (1926) observed a double wreath of capillaries after the differentiation of the theca interna which has a single layer of capillaries on its inner surface. This double wreath of capillaries constitutes the follicular blood supply. The blood vessels do not enter the granulosa of the unruptured follicle.

The lymphatics are slow in their growth towards the interna. A coarse mesh of lymphatic capillaries also lines the interna.

Many miniature vessels approach the mature follicle through the stroma of the ovary. On approaching the follicle, the vessels branch to supply twigs to the neighboring growing follicles and corpora lutea. Branches from these vessels enter the theca externa and spread through it. The arteriols are seen to branch out uniformly through the externa, rarely anastomosing. The vessels form an anastomosing network. When these vessels reach the inner surface of the externa, they form a single layer as already described.

When a follicle attains its full size, on maturing, it ruptures due to the influence of leutinizing hormone (L.H.) secreted by the hypophysis cerebri. The oocyte is released and ovulation accomplished.

During the stages of development, preceding ovulation, Porter and Bonneville (1964) observed the theca interna cells to contain lipid droplets and that their mitochondria simulated those of the interstitial tissue of the adrenal cortex. Histochemical studies have confirmed the

presence of steroids and, therefore, the theca cells probably elaborate estrogens at this phase of the ovarian cycle. Barker (1951), in his studies of lipids in sow ovaries, had arrived at a similar conclusion. The lipid droplets in the theca cells are present prior to ovulation and in the theca lutein cells 15 days after ovulation.

Development of the corpus luteum

The average period of estrus in the sow is about 3 days. Ovulation occurs during the 2nd day of estrus or 30 to 48 hours after the onset of heat. The average size of a graafian follicle in the sow ranges from 3.5 to 5 mm or larger ones may measure 6 to 7 mm in diameter. They reach about 7 to 10 mm in size 2 to 3 days before the onset of estrus. This growth is influenced by the follicular stimulating hormone elaborated by the pars distalis of the hypophysis cerebri. The theca interna proliferates receiving a rich vascular supply. The cumulus oophorus breaks its attachment from the wall of the follicle and frees the ovum. The first step in maturation division is accomplished by the ovum with the formation of the first polar body which is extruded. When rupture of the follicle takes place a second polar spindle is formed but the formation of the second polar body is delayed until the penetration of the ovum by a male germ pronuclei takes place. If fertilization does not occur, the second polar body is not formed.

Rupture of the follicle between 30 and 48 hours from the beginning of estrus has been reported (Corner, 1919b). Excessive accumulation of follicular fluid bulges the mature follicle on the surface of the sow ovary pushing the layer of the tunica albuginea above it. This part,

covering the follicle, is almost blanched, being either removed away from the blood supply or deprived of it and it becomes weak and ruptures. The follicular fluid carries the ovum or ova surrounded by the cells or corona radiata into the fimbria of the uterine tube.

The fate of the ruptured follicle, as it transforms into a corpus luteum, is well documented.

The history of the corpus luteum dates back prior to the enunciation of the cell theory by Schleiden and Schwan as referred to by Corner (1919b). In his monograph "De Ovi Mammalium Genesi", Von Baer, as quoted by Bischoff (1878) stated that the corpus luteum was derived from the theca interna cells. Bischoff (1878) opposed this view in favor of the membrana granulosa as the site of origin. Both were partially correct. During those days the first nuclear stain was not discovered. Histology was studied by pincettes and needles rather than by section. It was upon the developments of histological technics that the findings of Waldeyer (1870) and His (1919) approached the descriptions of recent years. Sobota (1895), in his studies of the corpora lutea in 200 mice, is of the opinion that the granulosa cells do not undergo any proliferative cell division but hypertrophy to become the lutein cells. Meanwhile, the cells of the theca interna divide and its spindle cells invade the lutein tissue and differentiate into the connective tissue elements of the corpus luteum. Blood capillaries grow in from the theca interna and provide the corpus luteum with a rich circulation characteristic of an endocrine gland. Van der Stricht (1901) believed that the theca cells remain at the periphery of the corpus luteum or entered a short distance into it. After a few days,

when the granulosa cells accumulate fatty droplets, the two types of cells resembled each other and could no longer be distinguished.

Stratz (1898) agreed with Sobota (1895) except for a minor difference about the fate of the theca interna. Stratz considers the cells of the theca interna of the mature follicle as a zone of blood vessels in which the theca cells are either constituents of the vascular walls or of the adventitia.

Zwicky (1844), a student of Henle, made another important contribution concerning the corpus luteum of the sow. Henle, as referred to by Corner (1919b) believed that the hemorrhage occurring in the ruptured follicle with the coagulated blood formed a scar tissue and this transformation of the follicle represented the corpus luteum. Zwicky (1844) was acute enough in his observation to correct the error of his teacher. He sided with the opinion of Von Baer (1827) and Bischoff (1878) in favor of the granulosa origin of the corpus luteum but he was, also probably, including the theca interna as part of the granulosa.

In their account on the human corpus luteum, Timofeiev (1913) and Wallart (1914) stated that the theca cells remain in groups in the periphery of the corpus luteum and atrophy later. They do not differentiate into the spindle cells of the connective tissue. They described the lipoid bodies in the granulosa lutein cells a few days after the formation of the corpus luteum and were also described by Corner (1919b) in the ovaries of the sow. After the completion of ovulation the size of the ruptured follicle is reduced from 8.5 to 10 mm, to 4 to 6 mm, due to loss of follicular fluid and contraction of the smooth muscle fibers around it. All of the contents of the follicle are retained, except the oocyte and cells of the cumulus

oophorus which were expelled at the time of rupture. The granulosa cells hypertrophy and their cytoplasm becomes laden with lipoid-like fats. They become lutein cells. No cell proliferation with mitosis takes place in the granulosa lutein cells. On the other hand, lipid laden cells of the theca interna proliferate by mitosis and pass into the developing corpus luteum to become lodged between the granulosa lutein cells. Blood capillaries grow in from the theca interna to ramify between the theca and granulosa luteal cells giving the newly formed structure a characteristic resemblance to an endocrine gland. Corner (1921) does not regard these thecal cells as forming a connective tissue reticulum or fibroblasts with spindle shape present in the corpus luteum. At about the end of a week's growth the corpus luteum attains a size of 8 to 9 mm. in diameter. This growth is further enhanced by the following pregnancy by a few more millimeters. At this stage the corpora lutea project from the surface of the ovary. In comparison the remainder of the ovarian tissue is dwarfed. The consistency of the corpora lutea at this stage is solid and a few in the ovary may form cysts. Unlike the cow and the human ovaries, the cut surface of the corpus luteum of the sow appears pink and velvety without trace of yellow pigments characteristic of the bovine ovary.

The hypertrophied granulosa cells reach a size of 30 or 40 microns and contain a considerable amount of lipoid or a mixture of lipoids and proteins. These oily substances round up when subjected to the action of water. On stained sections vacuoles previously occupied by lipoids are seen in the cytoplasm. They are dissolved in xylene and alcohol or ether in the process of staining, etc. The theca cells in the corpus luteum

are also seen as they invade the developing follicle and enter along the collapsed folds of the follicles and also with the blood capillaries. These theca cells are quite distinct in the 8 to 10 day old corpus luteum. They are smaller than the granulosa lutein cells measuring 10 to 25 microns. They have a deeply staining cytoplasm and foamy appearance due to vascuoles and the presence of fat globules. Their cell outlines are square or irregular and they occupy the interstices between the granulosa lutein cells. The ramification of capillaries in the corpus luteum is supported by the reticular framework. Every cell of the corpus luteum acquires a surface relationship with the blood capillary and their secretions are easily transferred into the general circulation. It is not possible to distinguish the corpus luteum of pregnancy from that of non-fertilized ovulation. If pregnancy does not occur, the corpus luteum degenerates rapidly on about the 14th or 15th day. The changes are so rapid they defy an exact analysis of the events of regression. The size is almost reduced from 10 to 6 mm. within 2 or 3 days. The color changes from pink to white along with the texture which becomes more firm and solid.

Causes attributed to the regression of the corpus luteum are the development of a new follicle or functional endometrium (Anderson et al., 1961) of the uterus. Recent views are that the endometrium is necessary for the regression of the corpus luteum and that the uterus (not the pituitary or the gonad as is the traditional belief) determines the ovarian cycle. Hystorectomy prolongs the life of the corpus luteum.

Corner (1921) observed the following features of change in the regressing corpus luteum:

1. In the early stages of retrogression, the granulosa lutein cells are seen in transitional forms from a fair state of preservation to almost complete degeneration.

2. Thick collagenous fibers form the thick reticulum of the corpus luteum with advancing retrogression. The fibrous tissue is synthesized probably by the endothelial cells of the capillaries which differentiate.

3. The theca lutein cells survive the blow of degeneration and appear somewhat like foam cells. They are seen enmeshed in scar tissue acquiring dense stores of pigmented fat.

In the succeeding cycle of ovulation the size of the corpus luteum is reduced to about 6 mm and by the second ovulation 3 to 2 mm. Kupfer (1920) found that they could be traced through a second interestral period but finally became so obscure that they cannot be distinguished from atretic follicles. Corner (1919) mentioned in his paper that the corpora lutea of pregnant and nonpregnant sows were very similar in microscopic appearance and did not differ at all in their mode of retrogression.

Blood and lymphatic vessels

After the rupture of the follicle and its subsequent collapse, capillaries from the theca interna grow in towards the granulosa and the arteries and veins of the theca externa also send small branches through the interna. According to the studies of Anderson (1926) growth is rapid opposite the small new arteries coming from the externa so that long tongues of granulosa tissue are formed with a strand of interna tissue down the center of each. The position of the arterioles, venules and lymphatics is in the center of the tongue. On reaching the end the arteriole branches,

returns along the margin very much diminished in size, and ends near the base.

As the granulosa proliferates the capillaries form a network instead of single loops. The longues grow in and meet in the center enabling the capillaries to fuse also. The corpus luteum enlarges and proportionally venules and arterioles bear thinner walls.

Lymphatics pursue the arteries in growth from the externa along the strip of recently grown tissue of the theca interna. Anastomosing lymphatic vessels from the externa unite and give off a single branch accompanying the arteriole entering the lutein substance. After running a distance of two or three valves it branches into a network of single, layered capillaries which spread among the cells of the interna. The granulosa is invaded after a period of 4 days.

In the regressing corpus luteum the lymphatic system is the first to disappear, followed by the capillaries. The arterioles, the last of the vessels left, collapse by the time the next follicles mature (Anderson, 1926).

Age changes

Yamauchi (1963) in his studies of 3 aged cows (28, 21, and 17 years) ovaries observed no follicular growth and did not find primordial follicles. In the ovary of the 17 year old cow progressive degeneration of oocytes appeared along with the atretic follicle. He postulates that oocytes disappeared from the ovaries at this age. In the other two cases ovarian cysts were found but the jelly-like contents of the cyst were not

analyzed for hormonal activity. Folliculoid structures with granulosa and cystadenoma were found. Granulosa cell tumors contained colloid bodies of various sizes composed of polysaccharides containing lipoidal substance. A folliculoid containing true call – Exner bodies was also found.

In the mouse the parenchyma involutes with age causing the dysfunction or malfunction of follicular maturation, ovulation and leutinization. Atretic degeneration resulting in an increase of interstitial elements resembles or is comparable to the stromal hyperplasia of the human ovaries. Functionally this phase involves unbalanced estrogen secretion with cornification of the vaginal epithelium in mice and rats. Bourne (1961) referring to Bloch, states that this stage can be compared to the human endometrial hyperplasia during climacteric and menopausal ages. Proliferation of germinal epithelium and the development of rete tubules can also be favorably compared in aged mice and women. These features seem to be a passing phase. These proliferations may be influenced by the pituitary hormones while the mass of normal receptor tissue declines (Bourne, 1961).

In their studies of age changes in the ovaries of the pig Hadek and Getty (1959) observed that the germinal epithelial cells appeared in every ovary of the pig from birth to 33 months of age. Primary follicles occupied the entire cortical area and a large part of the medulla leaving little space for the cortical stroma. The proliferation of primary follicles increased to 4 weeks of age decreasing thereafter and becoming static at the age of 6 months. The first appearance of vesicular follicles was noticed at 5 to 6 weeks of age.

The tunica albuginea became graudally distinct at 3 months.

In his studies of the dog ovary from the 2-day postnatal stage to the age of six months, Raps (1946) concluded that true primary follicles could be recognized at about 15 days although primordial oocytes with an epithelial covering were observed by him in the 4-day old ovary.

Further proliferation of the primary follicle became apparent at 15 weeks and at six months of age antrium formation in the follicle was observed. Germinal epithelial activity was cyclical. Tunica albuginea appeared in early life and the tissue composing of this layer was contributed to by the medullary stroma.

The germinal epithelium does contribute towards the formation of primary follicles. Even at sexual maturity cords of cells protruded through the spaces in the albuginea. Small nests of ova were seen perpendicular to the surface epithelium and continuous with the cell cords.

Mody (1963) has reported on the structure of the ovarian tissue with advanced age in IF mice. Pigment containing cells were found in adult and old ovaries of all groups, generally increasing with age. Further, he has observed two structures termed "anovular buds" and "dark staining epithelial cells". These structures have been regarded as separate entities arising from invaginations of proliferating germinal epithelium, especially in old ovaries.

When spontaneous pseudopregnancy is prevented by removal of the olfactory lobes, the ovaries become precociously senile. The atrophic and somewhat shrunken ovaries have a loose folded germinal epithelium and contain many atretic follicles. Intact corpora lutea are also lacking.

MATERIALS AND METHODS

The ovarian tissue samples were obtained from sows bred at the swine nutrition farm, Iowa State University, through the Department of Animal Sciences.

The breeding, age, and dietary records of these sows have been kept well documented in the Department of Veterinary Anatomy.

The animals were in normal health as observed by the usual standards of antemortem examination and supplemented by breeding records. Weights of animals were recorded before killing. The animals were killed by electrocution. A heavy rubber covered 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 volt alternating current were applied for about one minute.

The animals were exsanguinated immediately after death by exposing and cutting the axillery artery and vein. As soon as the bleeding ceased, the animals were eviscerated and the ovaries, both right and left, were collected and weighed.

The ovaries were then fixed in 10 percent buffered formaline solution. Forty-three specimens were utilized for the study. Age and breed of the sows are presented in Table 1.

Blocks of ovarian tissue were made by embedding them in paraffin according to the usual procedures. Sections of 6 microns in thickness were prepared. In the birth to 4 year age group four sections from each block were stained with the following stains:

Table 1. Age and breed of sows

Pig no.	Breed	Age	Total
3	Yorkshire	1 day	1
2	Yorkshire	1 day	1
1243	Yorkshire	1 week	1
1260	Yorkshire	1 week	1
1322	York. and Ch. White	2 weeks	1
1329	York. and Ch. White	2 weeks	1
1652	Duroc	1 mo 1 week	1
1244	Yorkshire	1 mo 3 weeks	1
3083	Pol. China	1 mo 3 weeks	1
1651-S	Duroc	2 mos 2 weeks	1
1680	Duroc	2 mos 2 weeks	1
Y650	Yorkshire	3 months	1
W708	Ch. White	3 months	1
2250-S	PC-York-L.R.	4 months	1
1292	Yorkshire	6 months	1
9310-S	PC-York-L.R.	6 months	1
3430-S	York-L.R.	1 yr 2 months	1
5930-S	York-L.R.	1 yr 2 months	1
2021-S	York-L.R.	1 yr 5 months	1
6333	York-L.R.	1 yr 7 months	1
6154	York-L.R.	2 yrs	1
6153	York-L.R.	2 yrs 1 month	1
2156	Pol. China	2 yrs 6 months	1
ISU-951	Landrace	2 yrs 7 months	1
1040	Ch. White	2 yrs 10 months	1
1361	York-L.R.	3 yrs 1 month	1
ISU-31	Landrace	3 yrs 7 months	1
4878	Landrace	3 yrs 6 months	1
4919	Ch. White	4 yrs	1
ISU-4966	Landrace	4 yrs	1
BB-2	Farmer's Hybrid	4 yrs 6 months	1
BB-3	Farmer's Hybrid	4 yrs 6 months	1
5898	Yorkshire	4 yrs 11 months	1
6015	Yorkshire	4 yrs 11 months	1
BB-5	Farmer's Hybrid	5 yrs 6 months	1
5895	Landrace	6 yrs	1
6043	Landrace	6 yrs	1
5815	Landrace	6 yrs 3 months	1
312	York-L.R.	6 yrs 9 months	1
13	Chester White	7 yrs 9 months	1
1	Chester White	7 yrs 9 months	1
LJF-1	Yorkshire	8 yrs	1
1175	Landrace	8 yrs 3 months	1
			43

Table 1b. Table of pig records

Pig no.	Breed	Age	Average weight of ovary in grams	Weight of body in kilograms
3	Yorkshire	1 day	0.02	1
1243	Yorkshire	1 week	0.04	2
1260	Yorkshire	1 week	0.02	2
1263	Yorkshire	1 week	0.03	2
1320F	York and Ch.White	2 weeks	0.03	4
1322	York and Ch.White	2 weeks	0.04	3
1329	York and Ch.White	2 weeks	0.07	4
1652	Duroc	1 mo. 1 week	0.03	6
480	Yorkshire	1 mo. 2 weeks	0.06	14
518	Yorkshire	1 mo. 2 weeks	0.04	12
527	Yorkshire	1 mo. 2 weeks	0.09	12
1244	Yorkshire	1 mo. 3 weeks	0.08	10
3083	Pol. China	1 mo. 3 weeks	0.06	12
3105	Pol. China	1 mo. 3 weeks	0.05	12
68	Chester White	2 mos.	0.11	20
69	Chester White	2 mos.	0.06	12
92	Chester White	2 mos.	0.11	18
627	PC-York,L.R.	2 mos.	0.08	14
592	PC-York,L.R.	2 mos.	0.05	16
S-1171	Yorkshire	2 mos. 1 week	0.09	22
S-1178	Yorkshire	2 mos. 1 week	0.09	19
S-1188	Yorkshire	2 mos. 1 week	0.11	14
1651-S	Duroc	2 mos.2 weeks	0.16	20
1682	Pol.Ch.Landrace	2 mos. 2weeks	0.85	14
1234-S	Duroc	2 mos. 3 weeks	0.60	20
1237-S	Duroc	2 mos. 3 weeks	0.76	23
Y650	Yorkshire	3 mos.		
W708	Chester White	3 mos.	0.12	24
W716	Chester White	3 mos.	0.52	24
9753-S	PC-York.,L.R.	4 mos. 1 week	1.78	61
2250-S	PC-York., L.R.	4 mos. 1 week	2.21	57
9713-S	PC-York., L.R.	4 mos. 1 week	2.08	52
1292	PC-York., L.R.	6 mos.	3.24	92
6022-S	PC-York., L.R.	8 mos.	3.30	111
634-S	PC., L.R.	8 mos. 1 week	2.52	121
5093-S	York., L.R.	9 mos.	2.00	116
9442	York., L.R.	1 yr.	14.20	156
7973	York., L.R.	1 yr.	7.46	163
5930	York., L.R.	1 yr. 2 mos.	10.75	186
3430-S	York., L.R.	1 yr. 2 mos.	10.06	174
2944-S	York., L.R.	1 yr. 3 mos.	10.82	204
2360-S	York., L.R.	1 yr. 4 mos.	11.06	177
2021-S	York., L.R.	1 yr. 5 mos.	39.66	202

Table 1b. (Continued)

Pig no.	Breed	Age	Average weight of ovary in grams	Weight of body in kilograms
3521	Yorkshire	1 yr. 8 mos.	8.68	156
3204	Yorkshire	1 yr. 9 mos.	10.53	193
6154	York, L.R.	2 yrs.	12.84	167
6153	York.,L.R.	2 yr. 1 mo.	8.95	168
2442	York., L.R.	2 yr. 2 mos.	11.83	220
2443	York., L.R.	2 yr. 2 mos.	8.14	195
5911	York., L.R.	2 yr. 3 mos.	7.74	195
5913	York., L.R.	2 yr. 3 mos.	9.53	200
5931	York., L.R.	2 yr. 3 mos.	15.85	190
5933	York., L.R.	2 yr. 3 mos.	11.10	195
6020	York., L.R.	2 yr. 3 mos.	9.81	193
6024	York., L.R.	2 yr. 3 mos.	13.30	188
5910	York., L.R.	2 yr. 4 mos.	8.96	190
ISU-951	Landrace	2 yr. 6 mos.	8.02	236
632	Landrace	2 yr. 7 mos.	10.76	161
3202	York., L.R.	2 yr. 7 mos.	10.60	175
3195	York., L.R.	2 yr. 9 mos.	11.34	209
3196	York., L.R.	2 yr. 9 mos.	13.68	188
1362	York., L.R.	2 yr. 11 mos.	14.10	186
1040	Ch. White	3 yr.	11.25	209
5335	Ch. White	3 yr. 5 mos.	9.70	197
ISU-31	Landrace	3 yr. 6 mos.	12.54	218
4876	Landrace	3 yr. 6 mos.	10.64	240
4878	Landrace	3 yr. 6 mos.	9.14	234
5132	Landrace	3 yr. 10 mos.	9.50	170
4919	Ch.White	4 yrs.	12.80	218
ISU-4966	Landrace	4 yrs.	6.38	177
6308	Landrace	4 yrs.	15.52	254
4915	Ch.White	4 yrs. 1 mo.	48.25	190
BB1	Farmer's Hybrid	4 yrs. 6 mos.	13.88	318
BB2	Farmer's Hybrid	4 yrs. 6 mos.	8.00	327
BB3	Farmer's Hybrid	4 yrs. 6 mos.	23.96	327
392	Landrace	4 yrs. 7 mos.	8.12	211
BB5	Farmer/s Hybrid	5 yrs. 6 mos.	14.00	356
6043	Landrace	6 yrs.	9.74	220
4583	York., L.R.	6 yrs.	10.54	240
5815	Landrace	6 yrs. 3 mos.	14.64	204
221	Landrace	6 yrs. 5 mos.	34.22	268
312	York.,L.R.	6 yrs. 9 mos.	14.45	228
13	Ch.White	7 yrs. 9 mos.	13.65	174
[illegible]	Ch. White	7 yrs. 9 mos.	11.41	201
LJF1	Yorkshire	8 yrs.	9.01	204
54-1175	Landrace	8 yrs.	12.88	181

1. Hematoxylin and Eosin
2. Gomori's silver impregnation method for reticular fibers.
3. Crossman's modification of Mallory's triple stain (details given below).
4. Dr. Getty's liver technique using the combination of Weigert's, Heidenhain's and Van Giesen's stains which gave excellent results for the study of elastoid tissue and blood vesewls.

Above the age of 4 years and six months, Von Kossa's stain for calcium salts was used in older animals to study the deposition of calcium in the ovarian tissue. Staining procedures for Hematoxylin-Eosin or Gomori's silver impregnation technique and Von Kossa's stain were in accordance with the Manual of Histologic and Special Staining Techniques, published by the U.S. Armed Forces Institute of Pathology (1960). The details of stain preparation and staining procedures of Dr. Getty's Liver Technique and Crossman's modification of Mallory's Triple are as follows:

Dr. Getty used the following three variations of this technique on 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	5 cc.
(Acid fuchsin 0.5 gram, distilled water 100 cc.)	
Picric acid, saturated aqueous solution about	100 cc.

The following Heidenhain's iron hematoxylin may be used instead of the above Regaud's Heidenhain but slides must be left 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.

Staining procedure:

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, will 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.

Crossman's modification of Mallory's Triple Stain:

A. Method of fixation:

Fix tissue specimens in neutral formalin, mercury formal, Helly's, or Zenker's fixative for a period of 48 hours to one week. Tissues in Helly's should be kept refrigerated to inhibit reduction of chromium by formaldehyde. This fixation is essential if correct action of oragne G is to be obtained, i.e., erythrocytes and Myelin should be stained orange. After

fixation in 10% formalin the various cellular elements lack the proper affinity for acid fuchsin and orange G.

B. Washing and dehydration method:

1. All tissues which have been fixed in fixative containing a heavy metal salt (mercuric chloride) should be washed in distilled water (several changes) at least 4 hours. (Tap water overnight.)

2. Place in 70% alcohol overnight (do only if storing).

3. Following morning, place in 1st change of dioxan for 4 hours.

4. Second change of dioxan for 4 hours.

5. Third change of dioxan, overnight.

C. Method infiltration and embedding:

1. Infiltrate tissues using vacuum method for 1.5 hours to 2 hours, using Biloid 56 - 58° MP. If no vacuum, 3 changes overnight.

2. Embed in fresh paraffin mixture.

D. Section

Preparation of stains and solutions:

Weigert's iron hematoxylin

Solution A

1. Hematoxylin ---------- 1 gram
2. Alcohol 95% ----------100 ml.

Solution B

1. Ferric chloride (29% aq. sol. stock) ----------4.0 ml.
2. Distilled water ----------95.0 ml.
3. Hydrochloric acid ----------1.0 ml.

For use mix equal parts of A and B solutions. The mixture will turn a deep black and is best prepared fresh each time, although it will keep and can be used for several days.

Acid fuchsin orange G solution:

1. Acid fuchsin ----------1.0 grams
2. Orange G ----------0.4 grams
3. Distilled water ----------300.0 ml.
4. Thymol ----------0.2 grams
5. Glacial acetic acid ----------3.0 ml.

Anilin blue solution:

1. Anilin blue ----------4.0 grams
2. Distilled water ----------200.0 ml.
3. Glacial acetic acid ----------4.0 ml.

Iodine solution:

1. Iodine ----------4.0 grams
2. Alcohol ----------200.0 ml.

Sodium thiosulfate 2%:

1. Sodium thiosulfate ----------4.0 grams
2. Distilled water ----------200.0 ml.

Phosphotungstic acid:

1. Phosphotungstic acid ----------6.0 grams
2. Distilled water ----------200.0 ml.

Acetic acid solution:

1. Glacial acetic acid ----------4.0 ml.
2. Distilled water ----------200.0 ml.

After 5 hrs. the staining performance is at a peak and this peak level is maintained for 4 to 8 weeks.

Staining procedure:

1. Xylene ---------- 5 min.
2. Xylene II ---------- 5 min.
3. Absolute alcohol I ---------- 3 min.
4. Absolute alcohol II ---------- 3 min.

5. Alcohol 95% ---------- 3 min.

6. Alcohol 80% ---------- 3 min.

7. Alcohol 70% ---------- 3 min.

8. Iodine and alcohol ---------- 5 min.
   Leave in this solution about 5 min. or until microscopic examination shows complete removal of mercuric crystals. Too long immersion of sections in iodine tends to hinder nuclear staining.

9. Sodium thiosulfate (2%) ----------30 sec.
   Transfer to this solution for 30 sec. or until iodine is completely decolorized

10. Wash sections in running tap water ---------- 5 min.

11. Weigert's hematoxylin solution ---------- 3 to 30 min.
    Time of staining is dependent upon examination. It will vary from 3-30 minutes. Following staining, excess of the dye solution can be rinsed from the section and slide by immersing them in distilled water for 5 second.

12. Distilled water (see above) ---------- 5 sec.

13. Wash (running tap water) ----------10 min.

14. Examine sections with scope. Nuclei should be blue. Background color can be removed completely by destaining in the following solution:

    a. Distilled water ----------50 ml.
    b. Ethyl alcohol 95% ----------50 ml.
    c. Sulfuric acid ---------- 0.18 ml.

    Then wash again in running tap water or place in a neutralizing solution of:

    a. Distilled water ----------50 ml.
    b. Alcohol 95% ----------50 ml.
    c. Sodium bicarbonate ---------- 0.5 grams

15. Transfer to distilled water for 5 minutes.

16. Stain in acid Fuchsin-Orange G mixture:

    A suggested initial staining time is one minute. Sections are then transferred to distilled water (Step 16) for examination.

If the time has been optimum the nuclear stain of hematoxylin will be properly differentiated and the rest of the section quite homogeneously stained red.

According to Mallory's original formula, Orange G is a copious ingredient of his secondary stain, a mixture of Orange G and Anilin blue. Putting it with the acid fuchsin, as suggested here materially lessens the amount of dyestuff needed and more consistently stains erythrocytes and myelin yellow.

Failure to stain yellow is usually due to too short an immersion time in acid fuchsin-Orange G mixture.

The purpose of adding acetic acid to the stain is to enhance the staining power of the solution and to assist in prevention of loss of stain in succeeding solutions.

16. Rinse in 3 changes of distilled water, thoroughly.

17. Transfer to 3.0% phosphotungstic acid in distilled water. Sections are left in this reagent until the connective tissue is completely decolorized.

Examination of an arteriole readily found in most sections is usually the criterion for perfect routine differentiation.

The tunica media, consisting chiefly of smooth muscle, retains the acid fuchsin while collagenous fibers in the tunica adventitia are colorless. The process of decolorization must be controlled according to the histological elements in the sections because the various connective tissues differeconsiderably in their tendency to become decolorized.

Prolonged treatment with phosphotungstic acid effects no other change than complete removal of the acid fuchsin from the connective tissue. Connective tissue, including bone, is uniformly colored by the secondary stain, Anilin Blue, only when previously completely decolorized.

For best results fresh solution should be used for each second rack of slides because phosphotungstic acid rapidly loses the power of decolorizing connective tissue.

If destaining with phosphotungstic acid is proceeding very slowly the process can be speeded up by the following step:

Dip slides into 50% alcohol for several seconds and then return to phosphotungstic acid for several minutes.

18. Rinse in distilled water. Sections should be removed immediately so that the mordanting effect of the phosphotungstic acid for the connective tissue is not lost.

19. Transfer to anilin blue. Since it is difficult to examine the slides in this stain a suggested time is 15 seconds. Anilin blue is mordanted to the connectide tissue by the phosphotungstic acid.

20. Rinse in distilled water, removing the sections immediately to prevent too great a loss of the connective tissue stain.

21. Transfer to 2% acetic acid to remove anilin blue loosely bound to other than connective tissue: .... 1.0 to 5.0 minutes.

22. Rinse in distilled water, removing sections immediately.

23. Dehydrate in 4 changes of absolute alcohol, 1.0 minute each the first two, and 3.0 minutes each the second two.

24. Clear in 4 changes of xylol.

25. Coverslip.

Results:

1. nuclei ----------blue-black
2. parenchyma ----------red
3. secretory granules ----------red or orange
4. neurokeratin network ----------orange
5. elastic fibers ----------pink
6. collagenous fibers ----------blue

As mentioned already in this section, the age of the sows, the weights of the right and left ovaries and the bodyweights of the animals before exsanguination have been recorded and filed in the Department of Veterinary Anatomy, Iowa State University, (Table 1b). It was decided to analyze these data and incorporate in the present investigation, the effect of age on the weight of the ovaries.

With a significance test of "t" it was found that there was no difference in the weights of left and right ovaries of the sow. Therefore average weight of both the ovaries was calculated to study the influence of age on the ovaries. For convenience, the age of the animals was measured in months taking a month as equivalent to 30 days.

Data was coded and punched on IBM cards and processed at the computation center of the Iowa State University.

Preliminary investigations by various regression techniques revealed that increase in ovarian weight above the age of one year was rather constant. This was confirmed by significance "t" test. Regression of age on weight of the ovaries resulted in regression coefficient B = 0 (no slope).

As age increases from birth to one year the variability of ovarian weights increases accordingly. Due to unequal variance over age during this age span, it was decided to convert ovary weights to Log. grams.

Since the relationship of ovary weight to age is not linear, the nature of relation was investigated by fitting a quadratic regression.

RESULTS

One day post natal (Pig no. 3 and Pig no. 2): In a day old pig the ovary appeared trilobed, macroscopically, subdivided by two discernible fissures. Cortical areas in each lobe could be clearly distinguished by the occupation of primary follicles covered on the external free surface by germinal epithelium. The medullary area was more extensive in the central lobe which lay between the two lateral lobes. In the lateral lobes, it was more centrally placed and was continuous with the medulla of the central lobe.

Germinal epithelium: Most of this epithelial sheet was made up of a simple cuboidal epithelium with a tendency to become low columnar in some places. Near the medullary region of the central lobe it became stratified to two layers in thickness.

Cortex: In the day old pig the cortex was primarily composed of primary follicles (Figures 1 and 2). Some secondary follicles were observed centrally. Each primary follicle in the cortex appeared to be surrounded by one or more layers of stroma cells arranged in a swirly manner. Very little collagen could be seen. Layers of reticular fibers, however, surrounded the primary follicles and were also seen extending from the follicles to form a sheet beneath the germinal epithelium. There was no discernible connective tissue layer beneath the germinal epithelium that could be called tunica albuginea. In the cortex some of the primary follicles appeared to coalesce to form multiovular follicles. At other places close to the medullary areas cell nests appeared cordoned by connective tissue. Whether the multiovular follicles had the morphology of a true

follicle is an academic question. (The word follicle means a sac.)

Medulla: In the central lobe, mostly composed of connective tissue and devoid of primary follicles, the blood vessels were quite distinct. The arteries especially had thin walls. The internal elastic lamina of these arteries consisted of a single layer of elastic tissue, against which rested a layer of endothelial cells. The tunica media of these arteries was composed of about two layers of smooth muscle cells arranged in a circular manner. The tunica adventitia was demarcated by a very fine layer of elastic tissue. The adventitial tissue was more cellular than fibrous.

In arteries somewhat larger than those described in the preceding paragraph, the cells of the tunica media appeared almost at a right angle to the tunica intima (Figure 4). A few vessels, on the contrary, had smooth muscle cells of one to two layers arranged in a longitudinal manner in the tunica media. The nuclei of the smooth muscle cells appeared much larger than that of the smooth muscle seen in the tunica media of the arteries of normal adult pigs. A few endothelial cells appeared to be in the process of migration into the tunica media of the arteries (Figure 3).

One week to two weeks post natal (Pig no. 1243 and Pig no's. 1260, 1322, 1329): In the specimens of the tissues studied in this age group, the germinal epithelium did not differ much from the one day old ovary. Most of the ovarian tissue in this age group was cortical tissue occupied by mostly primary and a few secondary follicles. Close to the germinal epithelium, some multiovular follicles were seen (Figure 5). The secondary follicles were located mostly towards the medulla.

In the medulla were also seen some venous sinuses lined with endo-

thelial cells and devoid of any tunica media (Figure 8).

The arteries were not very different from those of a day old pig. The internal elastic lamina was wavy giving a serrated appearance. Some endothelial cells were seen trapped in the serrations and at right angles to the elastic lamina. In some cases half of the cells appeared to be in the tunica media and the other half still in the lumen of the vessel. In short, it appeared that the endothelial cells of these medullary arteries were migrating into the tunica media through the internal elastic lamina (Figures 3 and 7).

There were very small arterioles in the cortex. Their media was one to two cells thick but these small vessels did not have a significant lumen.

One month, three weeks post natal (Pig no's. 1652 and 1244): The only significant differences observed between the ovaries of this age group and the previous group were that:

1. The secondary follicles had enlarged and moved closer to the medulla;
2. There was an increase in size of the venous sinuses of the medulla; (Figure 8) and
3. The connective tissue stroma appeared to be invading the cortex which was mainly composed of primary follicles.

Two months, two weeks post natal (Pig no. 1651-S and Pig no. 1680): At this age the ovary appeared to have grown in volume. The primary follicles were found concentrated beneath the germinal epithelium. The primary follicles had probably not decreased in number but were dispersed due to growth in the volume of the ovary.

At this age maturing graafian follicles along with some atretic follicles were observed (Figure 6).

Many secondary follicles were present. The ovarian stroma appeared to have grown in considerable proportions to have invaded the cortex, thus scattering the primary and secondary follicles.

Medulla: The venous sinuses, veins and arteries appeared to have increased three to four times the size of their corresponding vessels. The tunica media of the arteries had thickened about four times the size of the arteries of the preceding age group. It is therefore quite logical to conclude that rapid growth of the arterial wall takes place between one to two months of age.

The internal elastic lamina, composed of a single layer of elastic tissue, appeared wavy and serrated as in the younger age groups. In the folds of this elastic lamina were wedged the endothelial cells disposed at acute or right angles to the tunica media.

Three months post natal (Pig no's. Y650 and 708): In this age group, as was also seen in the preceding age groups, the primary follicles appeared to have been pushed very close to the germinal epithelium. The morphology of the cells of the germinal epithelium did not vary much from the younger age groups.

Also observed near the cortex was the growth of small arterioles composed of one to two cells. These cells resembled the large cells of the endothelium of somewhat larger vessels.

In the medulla the large venous sinuses, along with lymphatic sinuses and the arteries, appeared similar in structure to those of the younger age groups.

Four months post natal (Pig no. 2250-S): The ovary was considerably enlarged at this age. Many developing follicles past the stage of secondary follicles were seen. Atretic follicles were present. Some healing atretic follicles or atretic follicles being replaced by connective tissue were also present.

In these atretic follicles - the follicular cavities were filled with mainly two types of cells - Pyontic granulosa cells and large giant cells.

In the medulla the arteries appeared to be still in the process of growth. Their morphology was the same as that of the previous age group.

The number of smaller arterioles around the developing graafian follicles increased. The venous sinuses were much larger than those of the previous age groups.

Six months post natal (Pig no's. 9310-S and 1292): No fissures, as described in the ovary of a day old sow, were seen. There were many maturing graafian follicles. The presence of these follicles indicated that the sows (no. 1292) were probably in the late proestrus or estrus stage of the cycle when sacrificed. No ruptured follicle was seen and no corpus luteum as such or one beginning to form was observed. Some follicles were protruding over the surface of the ovary.

The granulosa cells of the maturing follicles in this stage of development were 7 to 10 layers in thickness. A hyperemic vascular wreath appeared around the granulosa (Figure 37). No vessels were seen to enter the granulosa layer. The cells of the theca interna appeared slightly hypertrophied. A lesser hyperemic wreath of vessels was seen between the theca interna and externa.

Along with the normal developing follicles an equal number of atretic follicles were seen. It appeared that this breakdown in the number of follicles at a certain stage is a normal process. Many types of cells were seen in the cavity of these cyst-like atretic follicles.

Type I: This type consisted of a comparatively small cell with degenerated nuclei (pycnosis, Karyrheris). Some cytoplasm appeared somewhat granular.

Type II: This type of cell seen in the atretic follicle was of a larger size with a large nucleus and vacuolated cytoplasm which appeared to be foamy.

Type III: Cells resembling type "2" described in the above paragraph were seen close to the innermost lining of the theca interna towards the follicular cavity. Their function appeared to be of a phagocytic nature to clear the cell debris in the follicular cavity. Some of these cells also showed degenerative changes (Figure 13).

Another type of degeneration appeared in follicles. Almost 1/3 of the layer of the granulosa cells towards the center of the follicular cavity were detached and exhibited degenerative changes similar to type I cells. At this junction no phagocytic cells like type 3 were seen.

In other types of atretic follicles, the cells of the cumulus oophorus appeared loosely detached with some degenerative changes. The yolk in the oocyte was granular, vacuolated and woven with fibrinous material. The zone pellucida persisted surrounded by the cells of the corona radiata, most of which appeared normal (Figure 12).

Germinal epithelium: The morphology of these epithelial cells

varied from region to region depending on their close relationship to the growing follicle beneath its surface or its relationship to the thick cortical region. When the epithelium was covering the cortical areas, devoid of maturing follicles beneath its surface, it tended to become a simple cuboidal, columnar or stratified layer. When a maturing follicle appeared below the germinal layer the cells assumed a low cuboidal to a simple squamous type of morphological appearance (Figure 10).

A dense connective tissue layer which can be called the tunica albuginea lay under the germinal epithelium. This layer consisted chiefly of dense collagenous connective tissue composed mostly of fibrocytes. A layer of reticular fibers was seen under the germinal epithelium. Most of the cortical area was dwarfed by maturing follicles and it was towards the medulla that a few primary and developing follicles could be seen. The tissue between the maturing follicles was mainly dense collagenous with a rich supply of vessels.

Medulla: This area was almost densely packed with blood vessels. The larger arteries were mostly muscular arteries with a thick muscular tunica media. The internal elastic lamina appeared to be split in some of these larger vessels and some smooth muscle cells were noticed between the split layers of the elastic lamina (Figure 9).

In the medium sized arteries the reticular fibers formed the supporting framework of the smooth muscle of the tunica media. In the larger vessels finer collagen and elastic tissue was present.

Large venous and lymphatic sinuses were observed in the medulla along with the arteries. These venous sinuses had very wide lumens, much larger

than the arteries. These were lined by endothelial cells only as they completely lacked a muscular coat. In this respect the basic structure of these veins (if they were veins in the true sense) resembled the capillaries (Figure 8). The only difference was the extreme variation of size - i.e. diameter of the lumen, which was about 1-2 mm. The only way to differentiate a venous sinus from a lymphatic sinus is by the presence of red blood cells in the venus sinuses. There is no blood in the lymphatic sinuses.

One year post natal (Pig no. 5930 and Pig No. 3430-S): In these ovarian tissue samples, functional or blooming corpora lutea were present (Figure 11). These animals could have been in a very early stage of pregnancy or in the diestrus period of the sexual cycle when sacrificed.

Cortex: A layer of tunica albuginea similar to that of the preceding age group was seen. Some developing follicles were also present. In some distended follicles approaching the germinal eipithelium, the tunica albuginea became thin and could be distinguished from the layer of thecal cells. Vascular supply to this part, between the developing follicle and the germinal epithelium, appeared to be slightly diminished.

The cortical tissue overlying the corpora lutea bulging on the surface of the ovary seemed to be entirely tunica albuginea. A definite pattern of blood vessels (small arterioles) surrounded the corpus luteum in this area.

In addition to the developing follicles and blooming corpora lutea many atretic follicles were also present exhibiting the former granulosa cells dispersed in the follicular cavity in degenerative stages. Large phagocytes, engaged in the removal of the granulosa cell debris, were observed in the follicular cavity (Figure 13).

In one of the sections regressing corpora lutea were seen. Early regressive stages showed hypertrophy of the cells. Advanced stages exhibited a hyaline mass of tissue with scattered cells and former luteal arterioles. The arterioles appeared as helical vessels with a longitudinal arrangement of smooth muscle cells in the tunica media and elastic tissue.

Corpus luteum: The blooming or functioning corpus luteum appeared like any other endocrine gland with its hypertrophied thecal and granulosa cells. These cells, however, could not be distinguished very clearly from each other.

The cytoplasm of the luteal cells exhibited vacuoles. Mostly a circumferential vacuolated area was noticed at the periphery of these cells (Figure 11). The lipids of these cells appeared to be stored more toward the peripheral area abutting on the luteal capillaries. A few arterioles were present amidst the luteal cells. The reticular fibers were seen weaving around the luteal cells and supporting the capillaries. The whole of the corpus luteum was encapsulated with dense collagenous connective tissue in which were embedded a considerable number of blood vessels surrounding the corpus luteum. This collagenous tissue merged with the ovarian stroma.

In the rest of the cortex nothing significant was discernible except a few primary, developing and atretic follicles.

Medulla: the medullary area appeared more spongy due to the presence of numerous sinuses, and many types of arteries.

In some of the smaller arteries the tunica intima appeared slightly thickened. In the split internal elastic lamina, one to two layers of smooth muscle cells were seen arranged longitudinally or parallel with the long axes of the vessels and interwoven with elastic tissue. In the rest

of the tunica media the smooth muscle of these vessels had a circular arrangement.

The adventitia lacked elastic tissue.

One year, seven months post natal (Pig no. 6333 and Pig no. 2021-S):

Germinal epithelium: There was not much change in the morphology of the germinal epithelium from the preceding age groups. The morphology still depended on its relations with the maturing follicle on the blooming corpus luteum.

Cortex: The ovaries studied in this age group had uniformly regressing corpora lutea and developing graafian follicles. Processes of follicular growth and luteal regression appeared to be simultaneous. Therefore, the animal was in the beginning of the estrus stage. The follicles in the sections looked almost mature.

The regressing corpora lutea presented a homogenous substance sparsely studded with cells. Some primary, developing and atretic follicles were seen resembling similar ones described in the preceding age groups.

Medulla: The larger arteries of the medulla appeared to be almost normal except that some splitting was seen in the internal elastic lamina. The intima appeared to be one or two cell layers thick. There was a considerable amount of collagenous intercellular substance in the tunica media of these large vessels (Figure 20).

The medium sized vessels, with a wall thickness of 40-50 microns, and lying towards the cortex, showed considerable thickening of the tunica intima. In some, the thickened intima approximated 1/3 of the total arterial wall.

The smaller arterioles with longitudinal smooth muscle in the media also had a considerable amount of elastic tissue.

However, there appeared little deviation from the normal morphological features of the medullary vessels.

Two years post natal (Pig no. 6154 and Pig no. 6153 - Diestrus stage): There was not much change in the morphology of the germinal epithelium. In these tissue samples functional as well as regressing corpora lutea were observed. In one of the specimens a corpus luteum had a central core or cavity with considerable hemorrhage. In this central cavity formation of fibrous tissue was noticed.

In some of the regressing corpora lutea in the early stages three different cell types were seen:

(1) Extremely large cells with large pale nuclei;

(2) Spindle shaped fibroblasts; and

(3) Small cells simulating granulosa luteal cells.

The blood supply still persisted with small arterioles and somewhat dilated capillaries.

Atretic follicles presented a picture similar to that of younger age groups. In a healing atretic follicle fibrous tissue build-up was seen advancing from the periphery towards the center of the follicular cavity.

Vessels: Arterioles in an advanced regressing corpus luteum (about one cycle old) were typical. They survived while the corpus luteum involuted. These vessels had longitudinal smooth muscle cells in the tunica media with a collagenous adventita. In some corpora albicans these vessels appeared in the form of colonies - probably brought closer to each other as a result of the shrinking of the lutea during involution. The tunica media of these arterioles was very rich in elastic tissue.

Medulla: In the medulla the larger vessels appeared normal although an accumulation of collagen was seen in the tunica media of these vessels. In some of the medium sized vessels whose wall thickness ranged from 50 to 80 microns, marked thickening of the tunica intima was noticed (Figure 15). A considerable number of smooth muscle fibers lying in apposition to the internal elastic lamina of the intima was seen.

Two years, six months post natal (Pig no. 2156 and Pig ISU, 951):

Cortex: The overall picture of the cortex of these tissue samples was the same as for the younger age groups except that there were more maturing follicles and some regressing corpora lutea. Animal no. ISU,951 was in the proestrus stage of the cycle while animal no. 2156 appeared to be in late diestrus.

In the cortex the small arteries with a wall thickness ranging from 40 to 80 microns had a uniformly thickened intima (Figure 22). The tunica intima was composed of longitudinally arranged smooth muscle fibers showing elastic fibers as the main type of formed intercellular substance. This intercellular substance was positive to P.A.S. stain and was more in the thickened intima than in the tunica media. Some vacuoles could be seen close to the intima in the lumens of these vessels. These vacuoles could represent lipid like substances in the plasma of the living animal.

In the medulla, however, the larger vessels appeared normal, with a few exceptions, where some focal thickening of the intima, like a plaque or mound, was noticed. Elsewhere the intima showed only slight thickening and splitting of the internal elastic lamina.

Three years post natal (Pig no. 1361 and Pig no. 1040):

Cortex: In the cortex were present, blooming corpora lutea, developing and primary follicles, atretic follicles and corpora alpicans. These animals were in diestrus stage of the cycle.

The medulla was composed, as usual, of connective tissue stroma, with arteries and venous and lymphatic sinuses.

The intimal thickening in the medium sized vessels in this age group was slightly more pronounced than in the previous age groups.

Three years, six months (Pig no. 4878 and Pig no. ISU, 31): There was no deviation in the morphology or constituents (e.g., primary and developing follicles, corpora lutea, and atretic follicles) from the normal as described for the preceding age groups.

Colonies of helical arteries, that probably were the former vessels of a functioning corpus luteum, appeared closer to the medulla (Figure 35). Other helical vessels did not show much elastic tissue in the media of the walls in which the smooth muscle was disposed in a longitudinal pattern (Figure 23).

The larger vessels were normal with a slight intimal thickening. Atherosclerotic tendency appeared more in smaller vessels.

Four years post natal (Pig no. 4919 and Pig no. ISU-4966): With blooming corpora lutea in the cortex this animal was in the diestrus stage when sacrificed for tissue collection.

In another specimen - No. 4919 - formation of incomplete corpus luteum was not observed in the tissue samples as in the younger age group. The central cavity appeared empty and the luteal cells were arranged in a thin margin surrounding the central cavity.

In other areas of the ovary some of the smaller arterioles showed an increased amount of intimal thickening. In most of these vessels, the tunica intima appeared almost equal to or thicker than the tunica media.

In the walls of the larger medullary vessels collagen formed increasing the amount of intercellular substance of the tunica media. In these vessels also the tunica intima appeared much thicker than the intima of the corresponding vessels of previous age groups. The thickening of the tunica intima in these larger vessels was not uniform as compared to the smaller vessels. One side of the larger vessels showed thicker plaques than the opposing surface of the wall.

Four years, six months (Pig no. BB-3 and Pig no. BB-2): The cortical area in this age group had almost the same morphological appearance as described for the previous age groups except for the very early stage of the developing corpus luteum following the rupture of a mature graafian follicle. At this stage the granulosa cells which have become larger luteal cells appeared invaginating towards the lumen of the follicular cavity. Between the folds of the invaginating granulosa cells were seen the cells of the theca interna which invaginated along with the blood vessels. At this time the theca lutein cells were very clearly distinguished from the granulosa lutein cells (Figures 17 and 18).

In this age group, the smaller arterioles of the cortex exhibited significant intimal thickening so that the thickness of the tunica intima excelled the thickness of the tunica media.

In the larger vessels of the medulla, the thickening of the tunica intima appeared slightly more enhanced than in the corresponding vessels of the preceding age group (Figure 24).

Four years, eleven months post natal (Pig no. 5898 and Pig no. 6015): The cortex resembled that of the previous age group.

Some of the smaller arterioles in the cortex exhibited an extremely thickened tunica intima similar to the corresponding vessels described in the preceding age group. Pig no. 5898 was in diestrus stage of the cycle.

In the larger vessels of the medulla an increased amount of collagen in the tunica media was noticed which formed about 50% of the tunica media, the rest was composed of smooth muscle cells. The smooth muscle resembled fibrocytes. The intimal thickening of these vessels was not as significant as compared to smaller vessels.

Five years, six months (Pig no. BB-5): There was no significant variation in the morphology of the germinal epithelium from the younger age groups. A few atretic follicles and young developing follicles were present in the cortex. (One can distinguish between a regressed corpus luteum and a healing atretic follicle. A regressed corpus luteum has the remnants of small arterioles. On the contrary a healing follicular cavity is replaced by a hyaline loose fibrous connective tissue without blood vessels.)

A nest of oocytes was present in the cortex.

In specimen BB-5 studied in this age group, blooming corpora lutea, along with young growing follicles, gave evidence of a late diestrus stage of the cycle. It appeared that one of the corpora lutea was in the earliest stage of regression when this animal was sacrificed. The appearance differed from a normal corpus luteum in that these luteal cells did not show any vacuoles in their cytoplasm. The nuclei stained pale with H and E stain. These luteal cells appeared detached from each other and an area of intercellular space could be seen. Cells towards the periphery e.g., the thecal

border, were similar to a characteristic blooming luteal cells.

Medulla: As usual the medulla had a spongy appearance due to the presence of large venous and lymphatic sinuses.

The walls of the large vessels had about half collagen as its intercellular substances. The cells of the media appeared more like fibrocytes than smooth muscle.

The smaller arterioles, as in the preceding age group, showed significant thickening of the tunica intima (Figure 26).

Six years post natal (Pig no. 5895 and Pig no. 6043): There was no difference in the cortex of this age group from the younger age groups described. The only exception was what should be called an incomplete corpus luteum with a large central cavity filled with a colloid-like substance. The functional luteal cells occupied 1/4 of the peripheral area. The cells of the theca interna seemed to have participated in the formation of this incomplete corpus luteum along with cells of the membrana granulosa (Figure 19). Almost mature follicles were observed. Possibly this animalwas in the proestrus stage of the cycle, or late diestrus. In the ovarian tissue of pig no. 6043, many ruptured follicles beginning to form corpora lutea were noticed. Folds of granulosa and theca interna were seen along with blood vessels invaginating into the follicular cavity. A similar situation was observed in the ovarian tissue of the 4½ year age group. Animal no. 6043 was just past the estrus stage of the cycle and in the beginning of diestrus.

In a few exceptional follicles which were not ruptured, blood capillaries were found within the membrana granulosa cells (Figure 28). This appears to be an exception.

In some of the smaller arterioles approaching the cortex, the amount of intimal thickening was so marked that the intima appeared to have invaded almost all of the tunica media, e.g. the tunica intima was about three times the size of what might have been the original tunica media. The elastoid intercellular substance was diffusely disposed between the smooth muscle cells of the intima. With Mallory's triple stain the larger arterioles showed more fibrous tissue in the thickened intima. This fibrous tissue was also positive to P.A.S. stain.

Six years, nine months post natal (Pig no. 312 and Pig no. 5815):

Cortex: Germinal epithelium changed at places from a simple cuboidal to stratified cuboidal type. There were many nests of oogoma. The presence of blooming corpora lutea and absence of developing graafian follicles suggest that the animal was in the diestrus stage of the cycle.

Medulla: In this age group some of the larger medullary vessels showed extensive intimal thickening. This thickening of the tunica intima was so severe that the lumens of these vessels appeared to be almost completely occluded. The cortical vessels appeared to be all affected with atherosclerosis. In one of the helical arteries cut in longitudinal section the tunica intima appeared more thickened at the bends (Figure 27).

Seven years, nine months to eight years post natal (Pig no. 13, Pig no. 1175, and Pig no. LJF-1): The cortex appeared to be the same as that of younger age groups. In some of the developing follicles many cells with eosinophilic granular cytoplasm appeared in the theca interna. Blooming corpora lutea in these animals indicated that they were in diestrus stage of the cycle.

In the cortex of these specimens studied, the vessels had similar

thickenings of the tunica intima.

The medullary vessels, however, exhibited a more advanced stage of atherosclerosis (Figures 31 to 34). The lumen of some vessels appeared to be almost completely occluded as a result of the internal proliferation (Figure 29).

In the ovary of specimen no. 54-1175 were present blooming corpora lutea which indicated the estrus stage of the ovarian cycle. A vein with a muscular coat appeared with an intimal plaque in this age group also. This was a rare observation (Figure 30).

The results of the data analyzed, as mentioned in the previous section, indicated that the ovarian weight increased steadily up to the age of one year. Above one year the ovarian weight (relatively) was constant. This was confirmed as calculated by significance test of "t".

As the age of the sow increased from birth to one year, the variability of ovarian weights also increased accordingly. This was inevitable because the weight of the ovaries is influenced by the estrus cycle. Therefore the ovarian weight was converted into Log. grams in order to work out the quadratic regression. The following equation shows the quadratic expression of relationship between ovarian weight and age from birth to one year.

$$\text{Log } Y = a + bX + cX^2$$

where

a = Y intercept

b = regression coefficient and

X = age in months and C is constant.

Since the initial regression analysis for ages one year and older indicated

that ovarian weight is relatively constant during this period, a restriction was imposed upon the quadratic expression in order that the slope of the function reach a maximum at one year of age and have a slope of zero (B = 0) for ages one year and older.

The restriction imposed was:

$$\frac{d \log Y}{d \log X}_{X=12} = 0$$

Setting the first derivative of the quadratic expression equal to zero and solving for c, we have

$$\frac{d \log Y}{d X}_{X=12} = b + 2c(12) = 0$$

or

$$c = \frac{-b}{24}$$

Substituting for c in the original expression, we have

$$\text{Log } Y = a + b\left(x - \frac{x^2}{24}\right)$$

Letting Z represent the transformed variable,

$Z = X - \frac{1}{24} X^2$ when X is equal to 12 months, and

$Z = 12 - \frac{1}{24}(12)^2$ when X is equal to or greater than 12 months (Graphs 1 and 2).

Ovarian weight from birth to 12 months can be predicted approximately substituting the computed values of regression coefficient.

$$\text{Log } \hat{Y} \text{ (Y hat)} = 0.47108 \left(X - \frac{X^2}{24}\right) - 1.76745 \quad \text{or}$$

$$\text{Log } \hat{Y} = 0.47108X - 0.01963X^2 - 1.76745, \text{ where}$$

Y = predicted weight of ovary and X = age in months.

Log grams can be converted into grams of ovarian weight by consulting the log tables.

DISCUSSION

According to the morphological changes taking place in the ovaries under the influence of age, the changes can be grouped under approximately three age periods. The first period of growth, during which significant changes take place in the ovarian tissue constituents, is from birth of the pig to 4 months of age. During this period the ovaries exhibit a steady growth.

The first report on age changes of the ovary of the sow was by Hadek and Getty (1959) who observed the following changes from birth to 33 months of age. At birth, the ovary appeared trilobed with discernible fissures. The presence of germinal epithelial cell clusters appeared to be a regular feature. The entire cortex was occupied with primary follicles with scarce stroma (Figure 1). The present observations on the morphology of the one day old ovary of the sow are in agreement with the findings of Hadek and Getty (1959). They did not mention the morphology of the vessels in the day old pig ovary. The present investigator observed the arterial walls in the day old pig to be composed of one to two layers of smooth muscle cells, with a layer of internal elastic lamina. Small venous sinuses lined with endothelium but devoid of other layers were seen in the medulla.

In the growing pig Hadek and Getty (1959) reported the presence of multinuclear ova as well as polyovular follicles up to 5 weeks of age (Figure 5). Beyond 5 weeks vesicular follicles appeared; and the number of primary follicles increased up to 4 weeks after which they appeared to decrease. The present investigator concurs with the findings of these authors regarding the presence of polyovular follicles and the appearance

of vesicular follicles (referred to as graafian follicles) beyond 5 weeks of age. Regarding the decrease in the number of primary follicles, it appears that the cohesion of primary follicles was interrupted by the proliferation of connective tissue stroma which dispersed these follicles. The follicles were also seen in various stages of involution.

In specimen No. 2250-S, which was 4 months old, many developing follicles along with atretic follicles were present. Some healing atretic follicles exhibited the membrane of Slavjansky, composed of hyaline connective tissue. It therefore appeared that atresia of the follicles occurred earlier than the age of 4 months. Hadek (1958), in his studies on the morphology of the ovary of sheep, reported that the atresia of follicles was a seasonal process. The number of atretic follicles was large during diestrus and small during proestrus. In the sow it appeared that atresia of the follicles can set in before the beginning of the ovarian cycle. The age of puberty in the sow ranges from 3 to 7 months. Thung (1966) remarked that Slavjonsky's membrane remained larger in the healing atretic follicle of primates, especially women, than in mice (Figure 14). It stayed even longer in the whale. The progress of healing of the follicle depended on the metabolic rate of the particular species. According to the present observations, atresia of the follicles appeared to be a constant feature in the ovary of the sow up to 8 years of age (the oldest animals), so far studied. Zuckerman (1962) described that 99.9% of the oocytes of the original stock in women are destined to undergo atresia. Age seemed to influence the degeneration of oocytes in the ovaries of very young animals. Simkins (1932) suggested that in human ovaries, the number of oocytes is

reduced from 300,000 at birth to 20,000 at 14 years of age. Since the decline in the number of germ cells is due to atresia, it follows that the number of oocytes which becomes atretic per unit of time varies with age. Corner (1919b) commented in his study on the origin of corpus luteum that atresia of the graafian follicle may set in at any time in the life of a follicle, even in the last stages of the follicular development. The present findings are in agreement with Corner as atresia has been observed in follicles in different stages of development. Causes and morphology of the atretic follicles will be discussed later. From the age of one month onward, it was observed that the walls of the ovarian arteries grew to the normal adult size by the age of 4 to 6 months. It appeared that rapid proliferation of the arterial wall took place between 1 to 2 months of age. The method of smooth muscle proliferation by cell division has not been reported so far. 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 experiments with ligating the carotid arteries in rabbits, mentioned the resemblance between endothelial cells which changed into fibroblasts and smooth muscles cells. He also quoted other authors who also suggested a transformation of endothelium into smooth muscle. In the present studies it was observed that the endothelial cells were disposed at right angles to the internal elastic lamina in arteries studied from ovaries of young animals. In a few arteries it was observed that part of the endothelial cell was embedded in the media of the wall and the remaining part in the lumen of the vessel (Figures 1, 4, and 7). Secondly, the endothelial cells lying parallel to the smooth

muscle were very similar in morphological appearance, the only difference being the position of these cells. The present investigator is therefore inclined to concur with the contention of the above authors that the endothelial cells may migrate into the media and undergo a certain degree of metaplasia. The smooth muscle cell of the arteries of pigs below 1 month of age are much larger as compared to the smooth muscle cells of the arteries of animals of the 1 year age group.

Regarding the structure of veins, it was observed in the present studies that most of the venous drainage in the ovaries of the sow was by peculiar large venous sinuses lined by continuous endothelium and devoid of a muscular wall (Figure 8). There were, however, a few veins present with walls composed of smooth muscle and elastic tissue. Burr and Davies (1951), in their studies of the vascular system of the rabbit ovary, described 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. The veins of the sow's ovary were, therefore, morphologically similar to the veins of the rabbit's ovary with the exception of those veins with a smooth muscle wall (Figure 30).

From the age of 4 months to 8 years the following ovarian constituents exhibited a regular feature:

Growing and maturing graafian follicles: These growing follicles along with the primary follicles in the cortex were observed in all the tissue specimens of the sow's ovaries. From the growing follicles and almost mature follicles it could be deducted that the animal was in proestrus or estrus periods of the cycle. The mature follicle had the following features.

The granulosa cells of the maturing follicles were 7 to 10 layers in thickness. Around those follicles in an advanced stage of growth two wreaths of hyperemic capillaries were observed. The inner wreath of vessels was between the theca interna and granulosa and the other wreath between the theca interna and externa (Figure 37). Similar vascularization of the mature graafian follicle has been reported by Anderson (1926) and also by Yamashita (1959). Yamashita (1959) further classified the normal follicles into 6 types according to their ripening process. It is hard to conceive how growing follicles can be classified on morphological appearance as growth is a continuous dynamic process. Secondly it is possible that a single follicle can exhibit most of the 6 types of morphological appearances as described by Yamashita. Thirdly, growth is not triggered off simultaneously in all the follicles destined to grow normally. However, the present morphological findings relating to normal graafian follicles are in accordance with the general overall description of Yamashita. Corner (1919b) as well as Yamashita (1959) have described mitosis in the theca interna and granulosa. Corner (1919b) also reported the extension of vascular loops at the base of the discus proliferus pushing before them cells of the basal columnar layer. Such vascular loops penetrating the granulosa were not reported previous to Corner's work (1919b). In the present studies it was observed in specimen No. 6043 in the 6 year age group that vessels were well within the granulosa layer (Figure 28). This was possibly a rare exception as such a characteristic was not observed in other ovarian tissue specimens studied.

Atretic follicles: Yamashita (1959) described cellular elements in atretic follicles of the sow and he further classified these follicles into 6 types. He described large normal cells and degenerating cells and further subdivided these cells into two types - according to the granular contents in the cytoplasm and vacuoles in the cytoplasm. In the present studies similar large cells with vacuolated cytoplasm and phagocytic properties were observed (Figure 13). It is felt that the subclassification of this large cell is unnecessary as a slight difference in morphological appearance could be a manifestation of a functional stage of the cell. Smaller cells conforming to Yamashita's description and subclassification were also present in the atretic follicles. The present investigator feels that these small cells are degenerating cells of the membrana granulosa. In some large and almost mature follicles which appeared to be in the very early stages of atresia because the granulosa cells were normal and showed no signs of degenerative changes, it was observed that there was no hyperemic wreath of capillaries between the granulosa and the theca interna. This situation can be interpreted as atresia taking place due to a decrease in the normal amount of blood supply to the follicle.

Corpora lutea: In the 4½ year as well as the 6 year age group ovaries, ruptured follicles beginning to form corpora lutea were observed (Figures 17 and 18). Folds of tongue-like invaginating granulosa cells were seen with the cells of the theca interna, which invaginated along with the blood vessels. These observations are in agreement with the observations of Anderson (1926) and Corner (1919b). The theca interna cells appeared to be round and could be distinguished from the granulosa lutein cells. Therefore, there is little doubt about the participation of thecal cells in the

formation of the corpus luteum (Figures 17 and 18).

In a blooming corpus luteum however it was observed that theca luteal cells could not be clearly distinguished from granulosa lutein cells. Peripheral vacuoles were seen in the luteal cells (Figure 11).

Corner (1919b) has described four stages in the development of corpus luteum:

(1) Ruptured graafian follicle, (2) invasion of the membrana granulosa, (3) a completely developed blooming corpus luteum, and (4) retrogressive stage of the lutea. In the present studies, the ruptured follicle in the early stages of its transformation into corpus luteum was observed. Sections taken from ovaries of the 4½ and 6 year specimens exemplify this stage (Figures 17 and 18).

Retrogression of the corpus luteum at early and subsequent stages was also recognized in various tissue specimens beyond 1 year of age (Figures 15 and 16). However, it has not been possible to study a step by step retrogression of the luteal tissue. An advanced stage of a regressed corpus luteum is represented by a colony of tortuous arterioles - referred to as vascular bodies by Yamashita (1959). He has observed that these vascular bodies of the ovaries of the sows are the scar tissues of old corpora lutea, which correspond to the fertilized ova in the past gestation. To substantiate this hypothetical conclusion, breeding records of the sows and studies of both the right and left ovaries by serial sections to count the number of bodies should be undertaken. The present investigator has found that the weight of the right and left ovaries of the sow have no statistically significant difference and are, therefore, equally functional. Secondly

all the ova shed by the ruptured follicles are not likely to be fertilized; some may involute or be absorbed in the uterus. Therefore, even the number of piglets born by the sow would not give sufficient proof nor necessarily agree with the same number of vascular bodies. The probable chance, therefore, is that there could be more vascular bodies or colonies in both the ovaries than the number of piglets born by the sow (Figure 35).

Van der Kaay and de Vink (1939) experimented with the ovaries of the bitch by injecting varying doses of gonadotrophic hormones and induced the formation of corpora lutea from unruptured graafian follicles. They obtained good results in tertiary follicles by injecting 10,000 to 20,000 R.E. units of Pregnyl. The granulosa cells transformed into luteal cells following invasion of the granulosa by the blood vessels. In higher doses it appeared that even the thecal cells transformed into luteal cells. But these induced cells were not very close to a functional luteal cell of a blooming corpus luteum. These induced, incomplete, corpora lutea of Van der Kaay and de Vink retained the oocyte.

In the present investigation a normally occurring incomplete lutea similar to the one induced by injection of gonadotrophins by Van der Kaay and de Vink in the bitch was found in the ovary of sow No. 5895 in the 6 year age group (Figure 19). It cannot be said whether this formation of incomplete corpus luteum is due to failure of the follicle to rupture or by a disturbance in the follicle in the equilibrium of gonadotrophic hormones of the sow's endocrine gland system.

Germinal epithelium: Hadek and Getty (1959) described the presence of germinal epithelium varying from a simple cuboidal to a stratified

cuboidal type in the sow. Raps (1946) described the probable development of new follicles throughout life from the germinal epithelium in the ovary, in the bitch. In the present studies no evidence of new follicular development from the germinal epithelium of the sow was observed. Morphology of the germinal epithelium of the sow's ovary was influenced by the growth of a graafian follicle beneath its surface and also the corpus luteum (Figure 10). When a growing follicle or a corpus luteum caused bulging of the ovarian surface the germinal epithelium was simple squamous and at other places where no such relationship existed, the epithelium varied from simple cuboidal, columnar to even stratified types. The change in the shape of the part of the epithelium induced by the growing follicles can be due to the following factors: (1) Mechanical stretching due to growth of the follicle and corpus luteum, (2) hormonal effects and (3) reduced blood supply.

Blood 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 with advancing age has been reported in recent years by Skold and Getty (1961), Getty (1965a), Skold et al. (1966) and French et al. (1963). Gottlieb and Lalich (1954) were the first to emphasize that lesions in the aorta are common to swine and that the incidence increases with age. Most of these studies have been of the aorta and the coronary arteries in swine. 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 in the Department of Veterinary Anatomy at Iowa

State University and have been reported previously by Getty (1965a) for the hog and for the dog (Getty 1965b).

In the present investigation an attempt was made to examine the blood vessels of ovaries in the very early age period as this has received scant attention. The descriptions have been based on month by month observations according to the sequence of events on the structure of the ovarian arteries up to the age of 4 months, and approximately at half-yearly intervals from 6 months to 8 years of age.

The early development of the arterial wall has been discussed in the previous paragraphs of this section. It is obvious from the results of the present studies that the arteries in the ovaries of the sow exhibit a thickening of the tunia intima with advancing age. In relating these observations, efforts have been made to give descriptions of the vessels representing the average morphology of a number of sections studied. It should also be borne in mind that there are wide variations in microscopic structure in the different parts of the same vessels and that a given change is seldom uniform even throughout the microscopic section. It has been observed that progressive changes in the tunica intima are not constant. This discussion will represent the average changes taking place in a section studied.

There is extensive variation in individual vessels. Therefore, most of the published material loses significance as the individual variations cannot be considered along statistical lines.

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 should hold good in case of the domestic pig also.

As age changes involve thickening of the tunica intima of the arteries, as well as alterations in other layers, it should be defined as to what part of the arterial wall exactly constitutes the tunica intima.

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 border line between the intima and media and accordingly retained the name lamella elastic interna (internal elastic lamina).

In the present studies it was seen that the internal elastic lamina was present at birth in the medullary arteries of the sow. The first signs of splitting of this elastic lamina appeared at the age of six months (Figure 9). At the age of one year smooth muscle cells appeared in the tunica intima between the split layers of the elastic lamina. This did not happen in all of the vessels but occurred in only a few vessels of the cor-

tex and medulla. Below the age of six months, however, there was no subendothelial layer. The endothelium, with its basement membrane, lay directly against the internal elastic lamina. In some arteries fenestrations were seen in the internal elastic lamina at 4 months of age. Movat et al. (1958) observed in their studies of human aorta that the earliest stages of the development of the subendothelial layer was represented by splitting of the internal elastic lamina and the accumulation of small pools of mucopolysaccharides. Most new born 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 most 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 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 media as well as the endothelial cells migrate 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. According to Rhodin (1962) fine intracellular fibrils were commonly seen in the endothelial cells. The functions of these fibrils, although not well understood, would lend some support to endothelial differentiation to smooth muscle.

Intimal thickening, whether it is a result of the migration of smooth muscle cell or of endothelial cells or both into the intima, results because the internal elastic lamina and 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. In the present observations it was also noticed that the continuity of the internal elastic lamina was interrupted in the age group from birth to one year. It also appeared, in animals approximately one year of age, that the smooth muscle cells synthesized the elastic tissue which appeared in the intracellular spaces of arterioles of vascular bodies left behind by the regressed corpus luteum. 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 homogeneous. 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 clearly whether the filaments are distributed throughout the entire elastic fiber or only occur at the surface.

If the elastic membranes are constituted of amorphous matrix with or without fibrils, the present investigator is inclined to postulate that these membranes cannot form any effective barrier to cells that may pass through them at any part of their surface, whether fenestra are present or not. If the cells can pass through the elastic membranes only through their fenestra, then only those cells closest to the fenestra would be capable of going through and other cells may have to make considerable ameboid effort to reach the fenestra. If the elastic lamina of the arteries are closer to the structure of the basement membrane, and not like the typical elastic fiber of the ligamentum nuchae or the elastic cartilage, there is no reason why cellular elements could not pass through. Cells, as lymphocytes or macrophages, can pass through the endothelium of the capillaries and their surrounding basement membranes. It is not impossible that smooth muscle or endothelial cells could open a fenestra in the elastic membranes of arteries.

To prove this hypothesis, more work should be done in this field. Moreover the fine structure of elastic fibers does not seem to have reached its final description as yet, mostly because of difficulties in preservation, staining and sectioning. The fenestrations in the elastic lamina have been confirmed by Pease and Paule (1960) in their studies with the election 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 the gaps. Collagen fibrils were also seen in the fenestra by these workers.

In the present studies it was also observed that the endothelial cells

in the one day old ovary were migrating into the tunica media (Figures 3 and 4). However, the present investigator is inclined to believe that these endothelial cells probably differentiated into the smooth muscle cells and in this manner brought about the proliferation of the media in a growing artery of the young. On the effect of aging of the elastic fibers, Blumenthal et al. (1944) reported that elasticity was lost and the fibers were subsequently infiltrated with mineral salt. No mineral deposit of any kind was seen in the vessels in the present investigation in the age groups studied.

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 the coronary arteries which have been studied quite extensively. Gottlieb and Lalich (1954), Getty (1965), Skold et al. (1966) and French et al. (1963) have also reported thickening of the tunica intima in the large arteries of the domestic pig. Smooth muscle appears to play an important role in the thickening of the intima. The pluropotent properties of the smooth muscle cells of the vessels have been recognized and, as such, this cell deserves a special status in the family of undifferentiated cells of mesodermal origin. Haust et al. (1960) and Pease and Molinari (1960) have recognized the ability of the smooth muscle to form extracellular structures such as elastic, collagen and mucopolysaccharides in the intimal plaques. Buck (1962) called these cells, which were predominant in the thickened intima of the aorta and which developed during cholesterol feeding in the rabbit, myointimal cells.

So far the study of arteriosclerosis has been made on the large arteries such as the abdominal aorta, coronary arteries, or femoral arteries etc., which have been studied in a collapsed condition and not in the natural state while they 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 vessels studied with his device as compared to the relaxed vessels. It is possible that the description in the literature of intimal thickening, as observed by workers on relaxed vessels, could be slightly exaggerated.

In the present studies considerable progressive thickening of the tunica intima was noticed with advancing age in the arteries of the ovary of the sow. For further manifestations of aging effects on the arteries of the ovary of the sow, more work needs to be done beyond 8 years of age.

SUMMARY AND CONCLUSIONS

In the studies of the morphological changes in the ovaries of the sow, it has been observed that significant changes take place in the following age periods:

1. Birth to three months
2. Four months to three years
3. Above three years to eight years.

Birth to three months: 1. Primary follicles close to the medullary area grow into secondary and vesicular follicles. 2. The ovarian stroma grows and invades the cortex separating the follicles which appear dispersed close to the germinal epithelium. 3. Rapid growth of the tunica media of arteries takes place during one month to two months of age probably as a result of migration of endothelial cells into the media. 4. Atretic follicles were seen along with some healing atretic follicles at four months of age even before the ovary came under the influence of the estrus cycle.

Four months to three years age period: In this age group there was no significant change in the ovarian constituents except for changes in the arterial walls in which some increase in intercellular collagenous substance was noticed. Stray arterioles exhibited some intimal sclerosis. Other regular features observed were as follows:

1. The venous blood of the ovaries was drained by the large venous sinuses in the medulla. These venous sinuses were lined by endothelial cells and were without a muscular coat. They were present in ovaries of all age groups studied from birth to eight years.

2. The morphology of the germinal epithelium appeared to be influenced by the cyclical changes in the ovary.

3. Normal graafian follicles in various stages of development, atretic and cystic follicles in different stages of regression appeared to be a normal feature in the ovaries of the sow beyond four months of age.

4. Blooming corpora lutea and corpora albicans were also a normal feature after the sow attained puberty which occurs approximately at the age of four months.

Above three years to eight years age period: Beyond three years of age, the arteries of the ovarian tissue appeared to be affected by intimal thickening. It appeared in two noticeable forms and progressed with advancing age in a number of vessels.

In the medium sized vessels, it was of a uniform nature affecting the whole tunica intima. At five years of age, the intimal thickness equaled almost twice that of the tunica media.

In the eight year age group the tunica intima approximated 2/3 of the cross section diameter of the entire arterial wall of the affected arteries.

In the larger vessels, the intimal thickening was not very uniform. It appeared in the form of plaques in parts of the arterial walls and in other areas it was diffuse.

Morphological studies revealed that thickened tunica intima resulted from the migration of endothelial cells and the smooth muscle cells form the tunica media.

As investigated by statistical means, the difference in weights of the right and the left ovaries was insignificant.

The ovarian weight showed a steady increase up to the age of one year. Above the age of one year, the weight of the ovaries was relatively constant.

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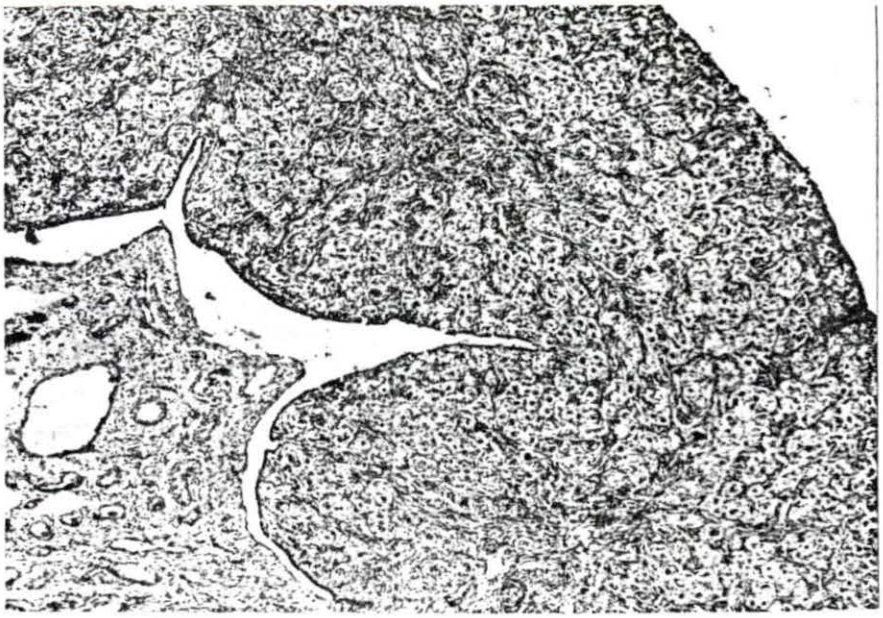
Lastly the author wishes to thank his wife Harbhajan and his children, Upinder, Jyotinder, and Sarvinder for patience and understanding through the days of separation during the author's absence from his home and country, and through many trying hours.

APPENDIX

Figure 1. Pig no. 3. Cortex and medulla of the ovary of 1 day old pig. The cortex is almost full of primary follicles. Medulla shows small developing vessels, H and E stain, 40X.

Figure 2. Pig no. 3. Age 1 day old. Cortex with follicles and germinal epithelium, H and E stain, 100X.



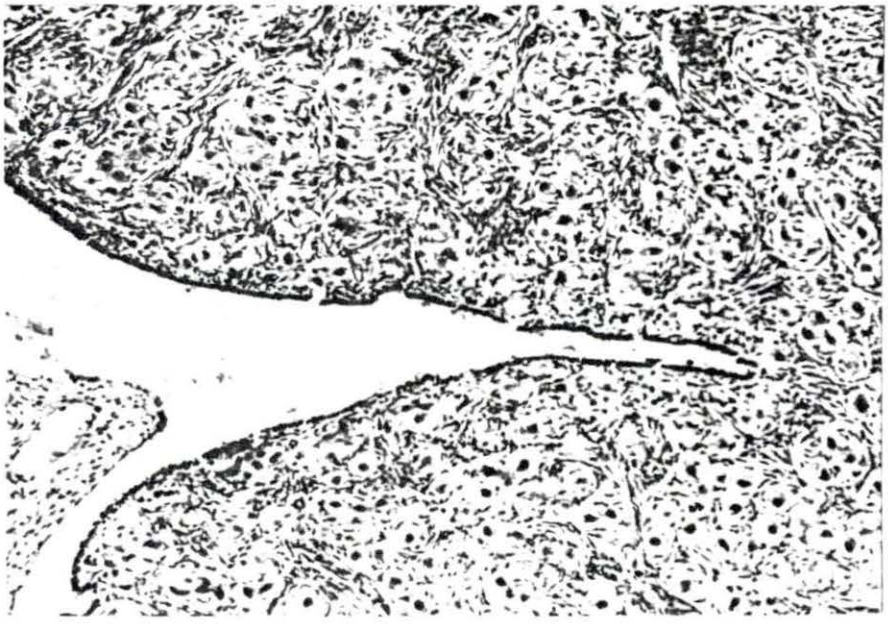
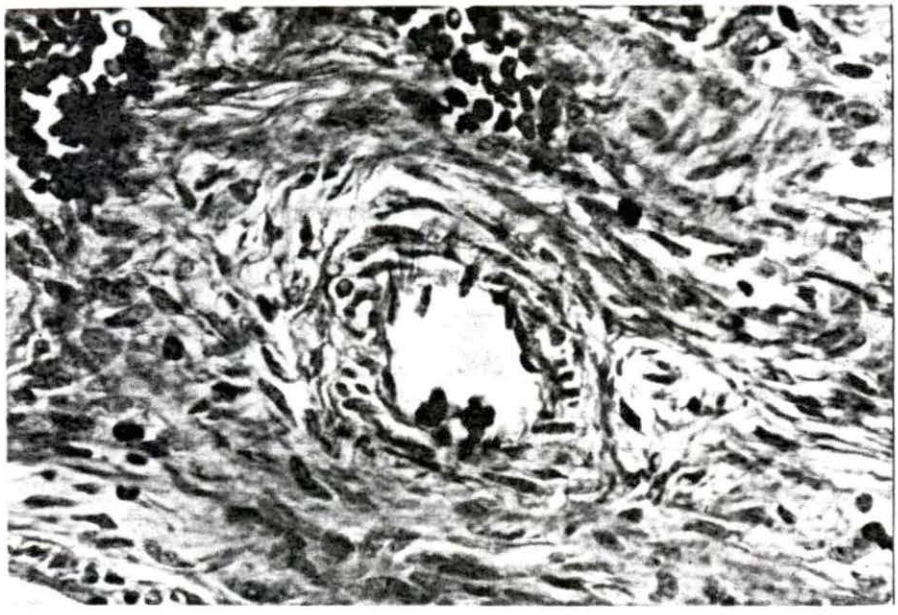


Figure 3. Pig no. 3. Age 1 day old. Artery in the medulla showing migrating endothelial cells into the tunica media. Weigert's, van Gieson's and Heidenhain's stain, 400X.

Figure 4. Pig no. 3. Age 1 day old. Artery in the medulla showing the disposition of muscle cells in the tunica media at right angles to the lumen. Weigert's, van Gieson's and Heidenhain's stain, 400 X.



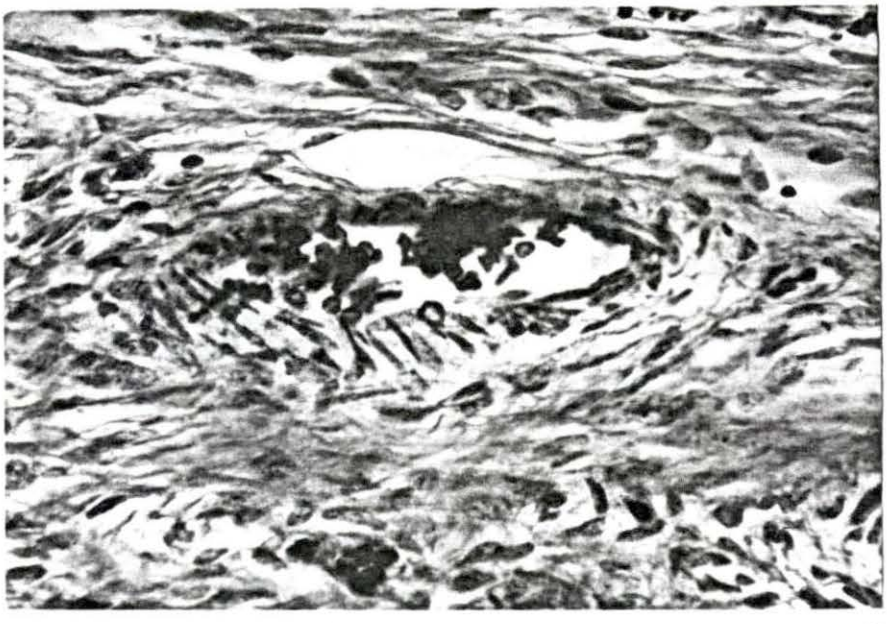
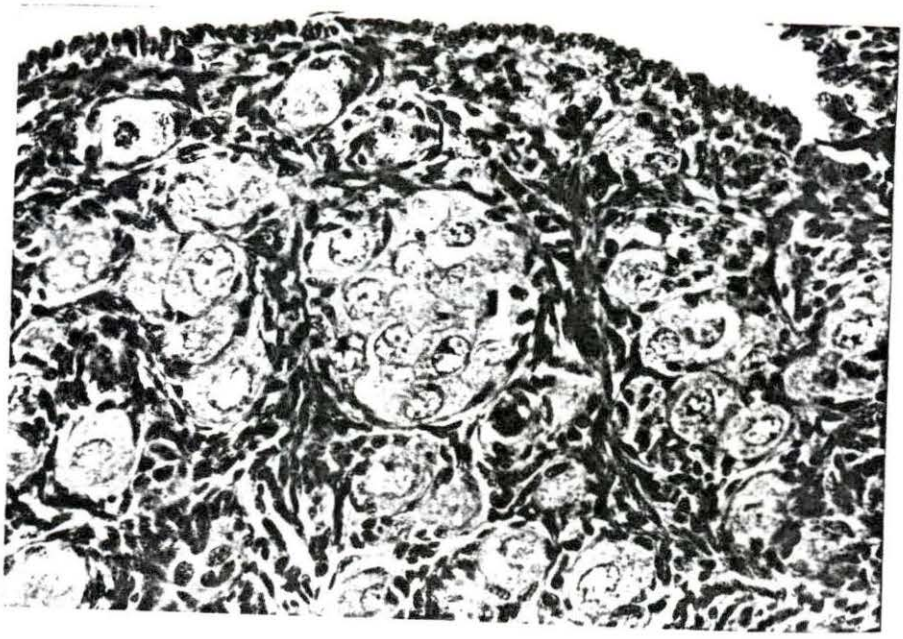


Figure 5. Pig no. 1260. Age 1 week. Cortex of the ovary showing germinal epithelium, polyovular follicle and primary follicles. Weighert's, van Gieson's, and Heidenhain's stain, 250X.

Figure 6. Pig no. 1244. Age 1 month, 3 weeks. Section showing development of a vesicular follicle. Weigert's, van Gieson's and Heidenhain's stain, 100X.



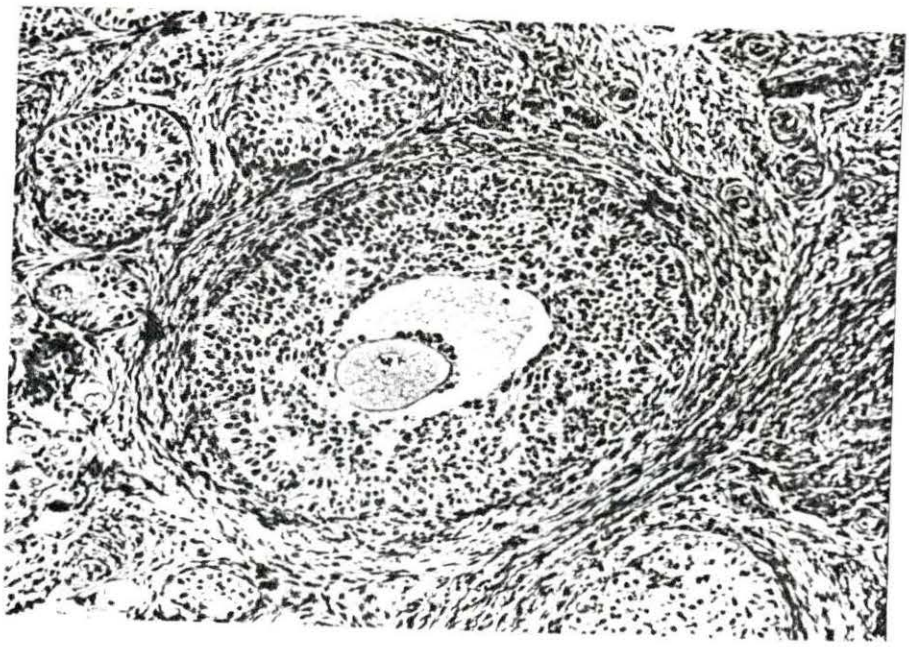
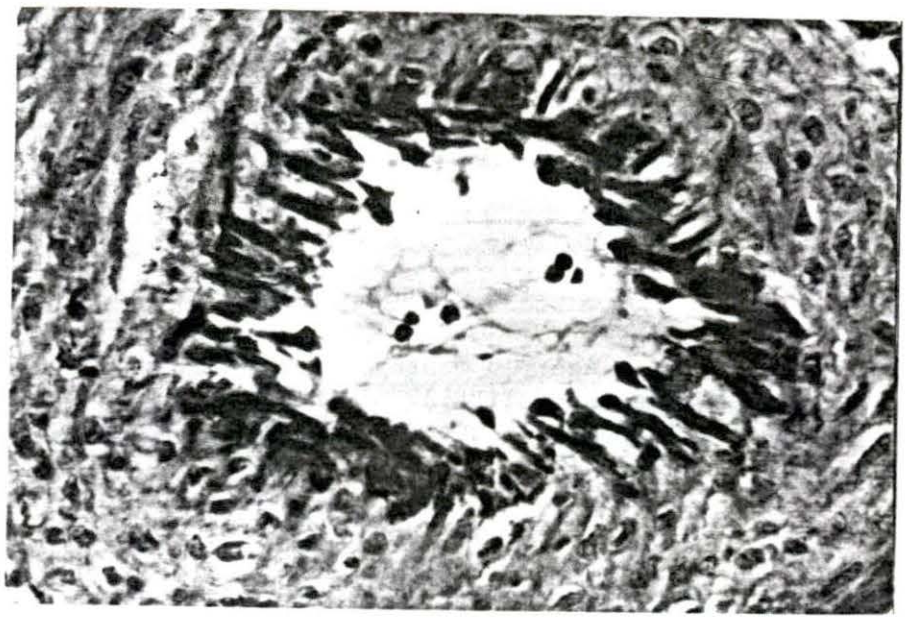


Figure 7. Pig no. 680. Age 2 months, 2 weeks. Section showing the disposition of the endothelial cells to the arterial wall. Some cells simulating the darkly stained endothelium can be seen penetrating the vessel wall close to the lumen. Weigert's, van Gieson's and Heidenhain's stain, 400X.

Figure 8. Pig no. 1244. Age 1 month 3 weeks. Section showing a venous sinus lined with endothelial cells. The wall is constituted of fibrous tissue around the endothelium. Weigert's, van Gieson's and Heidenhain's stain, 400X. (This is seen at all ages.)



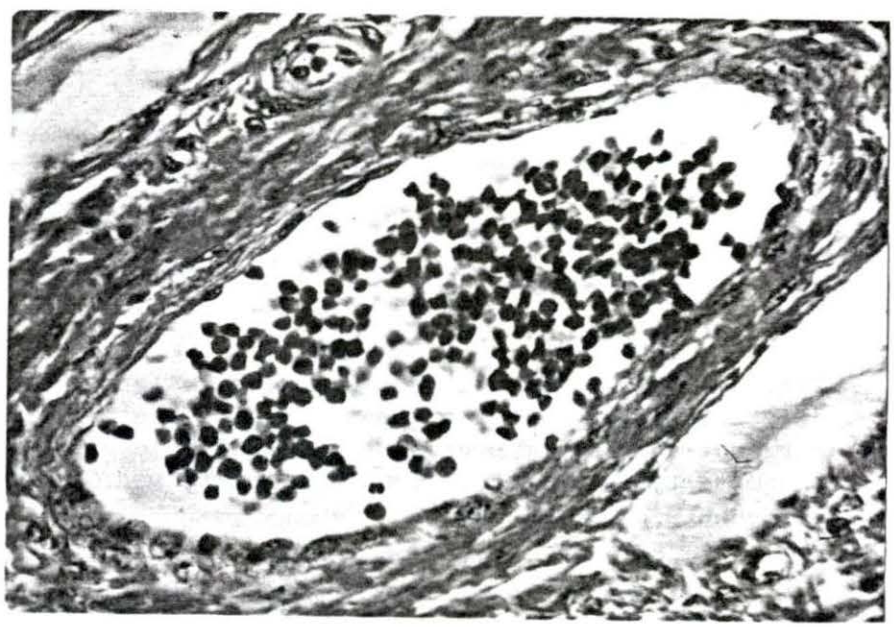
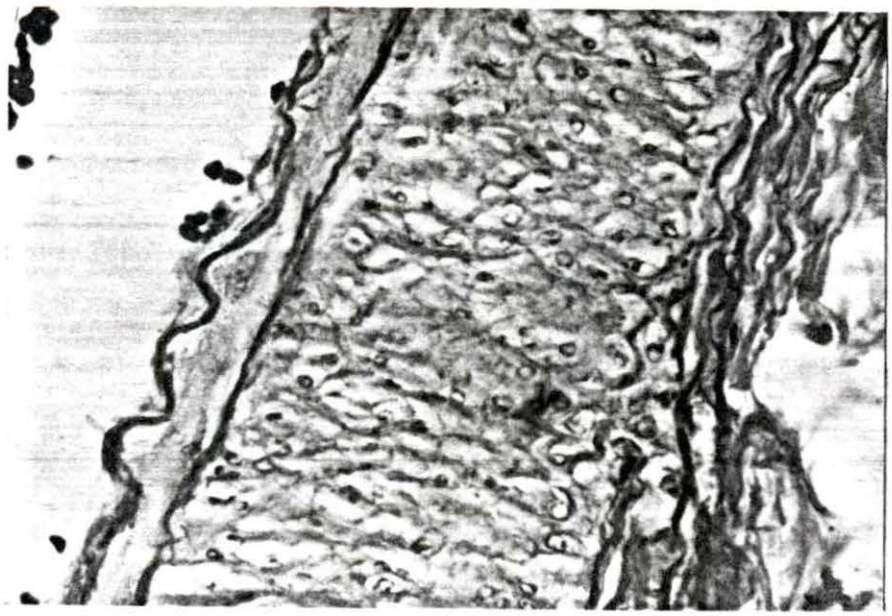


Figure 9. Pig no. 1292. Age: 6 months. Artery in the medulla of the ovary showing the splitting of the internal elastic lamina. Weigert's, van Gieson's and Heidenhain's stain, 400X.

Figure 10. Pig. no. 5930. Age: 1 year 2 months. A graafian follicle near maturity bulging on the surface of the ovary. The germinal epithelium overlying this follicle has become simple squamous type H and E stain. Weigert's, van Gieson's and Heidenhain's stain, 100X.



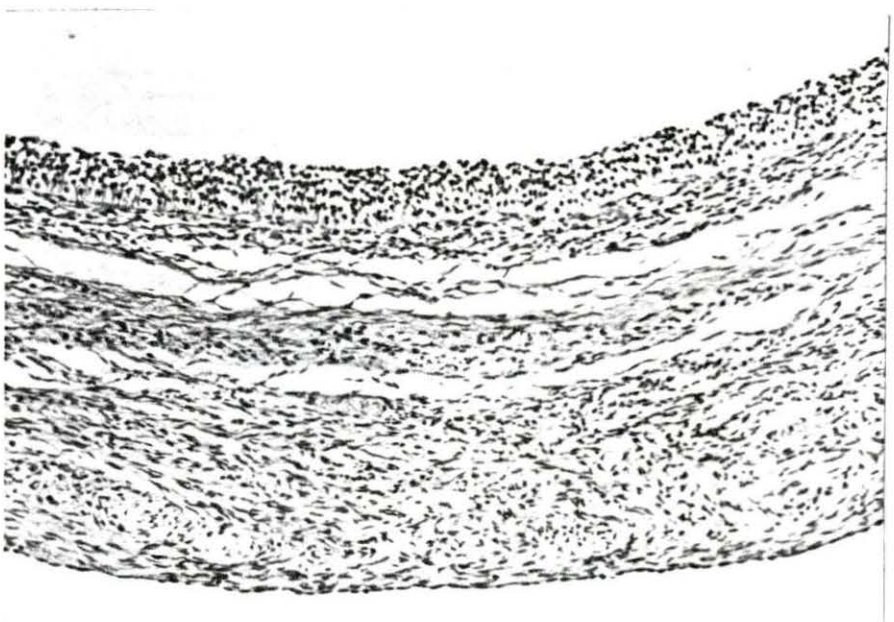
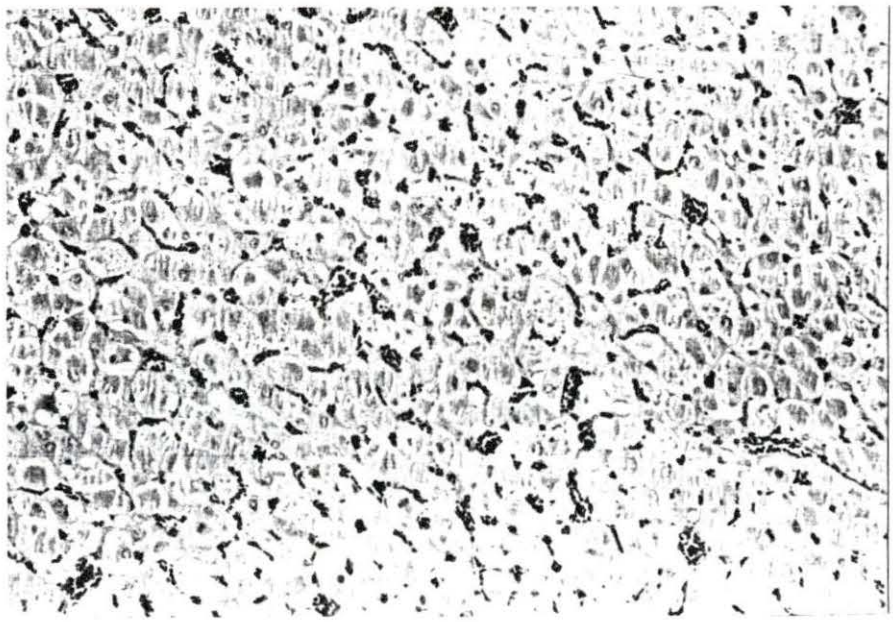


Figure 11. Pig no. 3430S. Age: 1 year. Concentric vacuolated area around the luteal cells adjacent to capillaries. H and E stain, 250X.

Figure 12. Pig no. 2021. Age: 1 year 5 months. Atretic follicle showing detached granulosa cells and the oocyte. H and E stain, 100X.



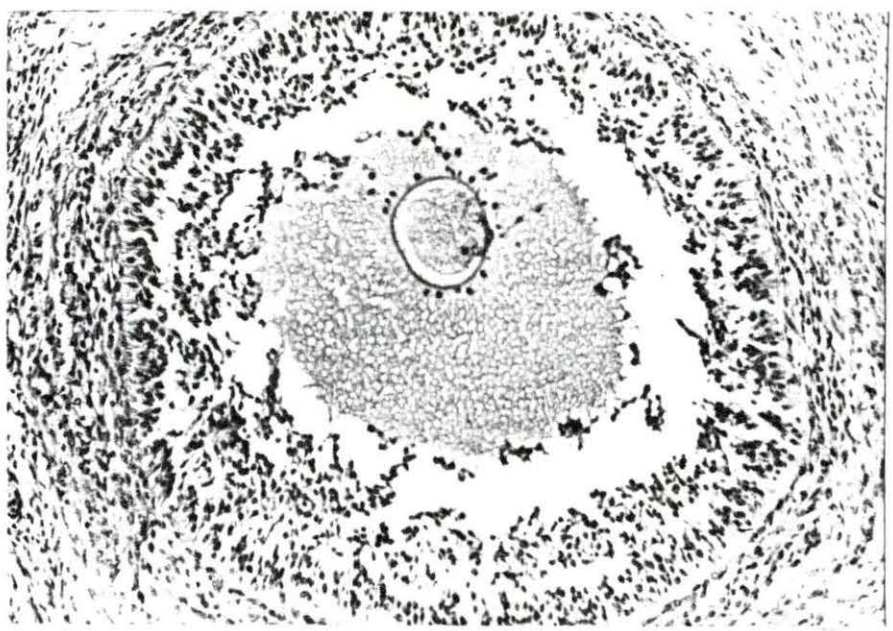
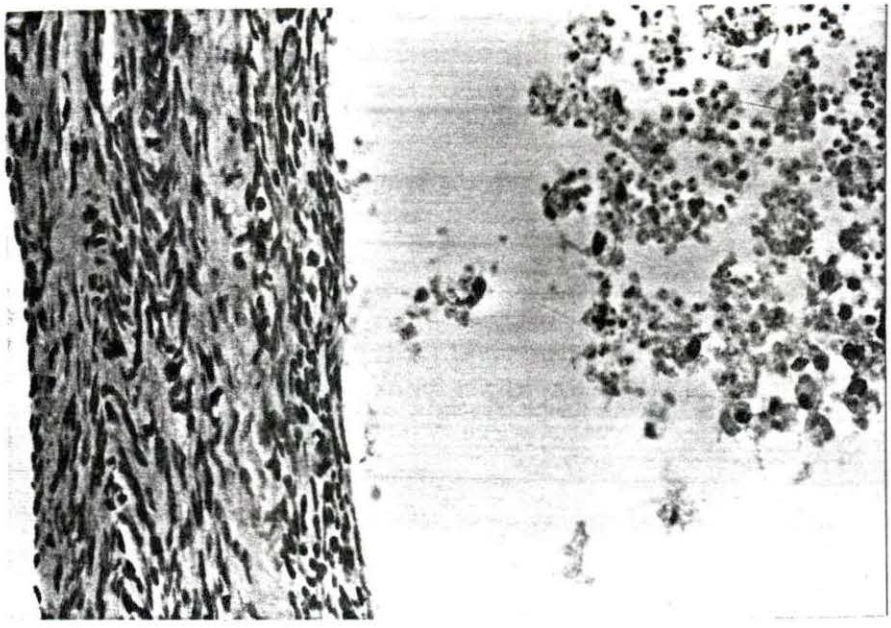


Figure 13. Pig no. 13. Age: 7 years 9 months. Atretic follicle showing cell debris consisting of large and small cells. The larger cells appear to be phagocytosing the small cells, H and E stain, 100X.

Figure 4. Pig no. L.J.F. 1. Age: 8 years. A healing atretic follicle showing the Slavjonsky's connective tissue membrane at periphery of atretic follicle. Mallory's triple stain, 100X.



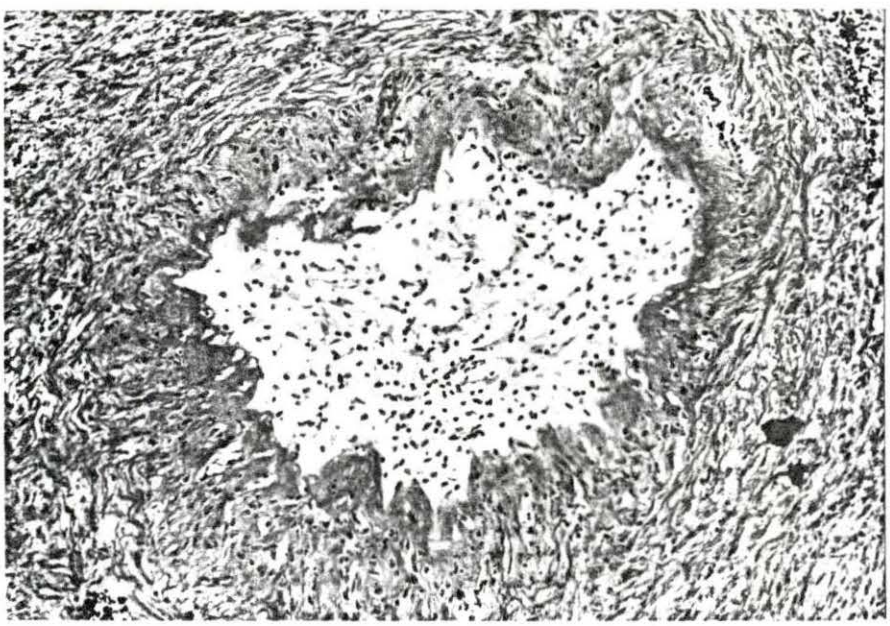
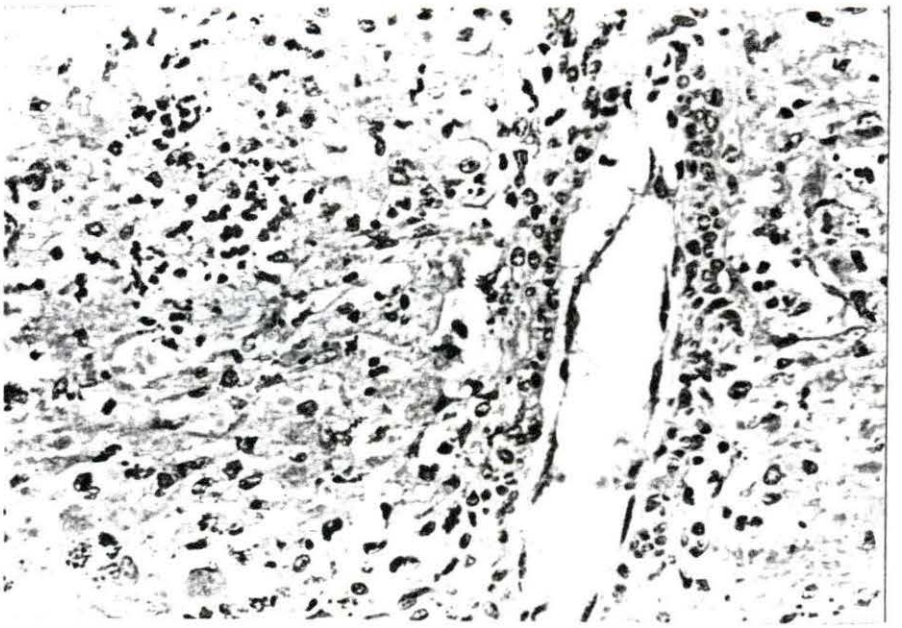


Figure 15. Pig no. 6153. Age: 2 years. An early stage of regressing corpus luteum. H and E stain, 250X.

Figure 16. Pig no. 2021. Age: 1 year 5 months. An advanced stage of regression in corpus luteum as compared to Figure 15. H and E stain, 250X.



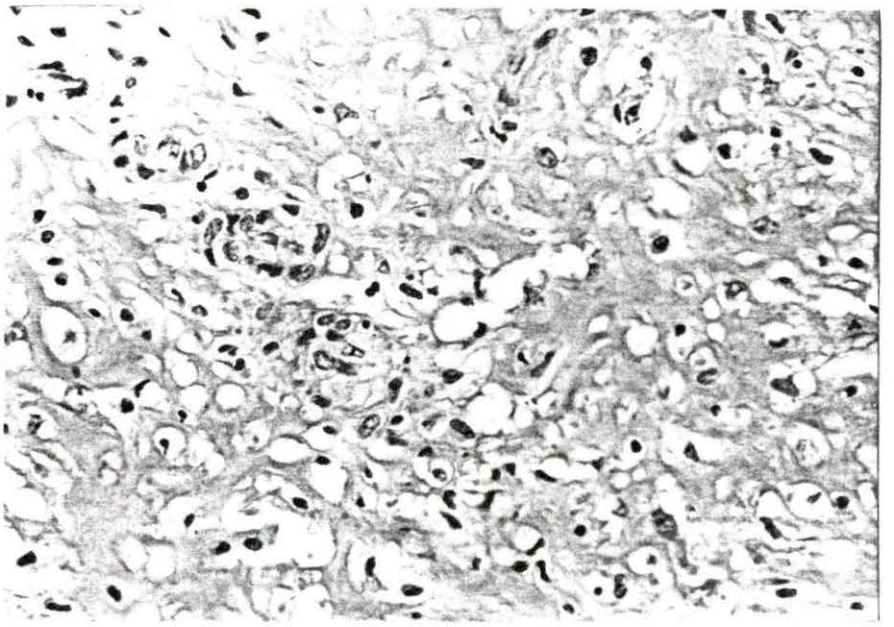
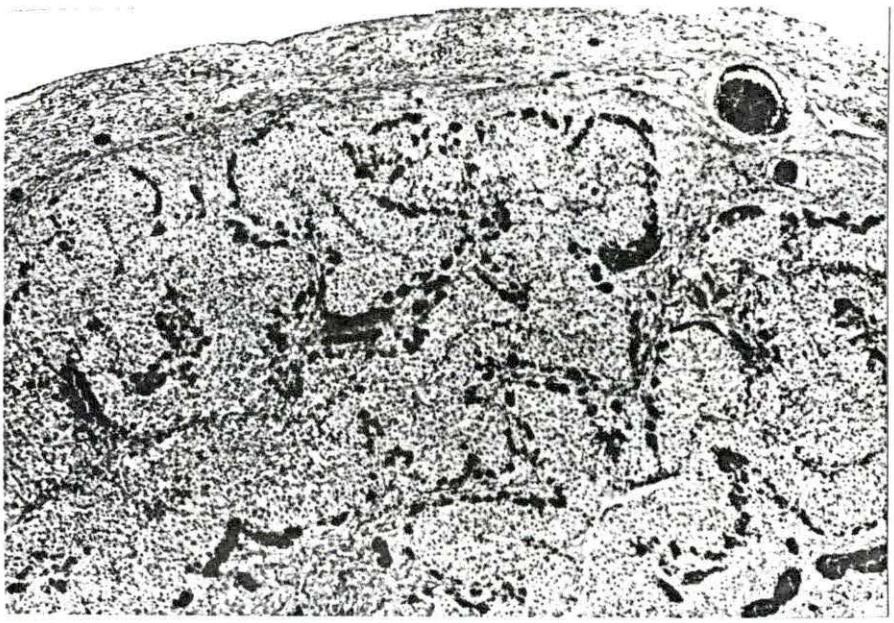


Figure 17. Pig no. BB2. Age: 4 years 6 months. A ruptured graafian follicle showing the invagination of blood vessels and theca interna into the folds of granulosa. Mallory's triple stain, 40X.

Figure 18. Pig no. BB2. Age: 4 years 6 months. Same as Figure 17, magnified to 100X.



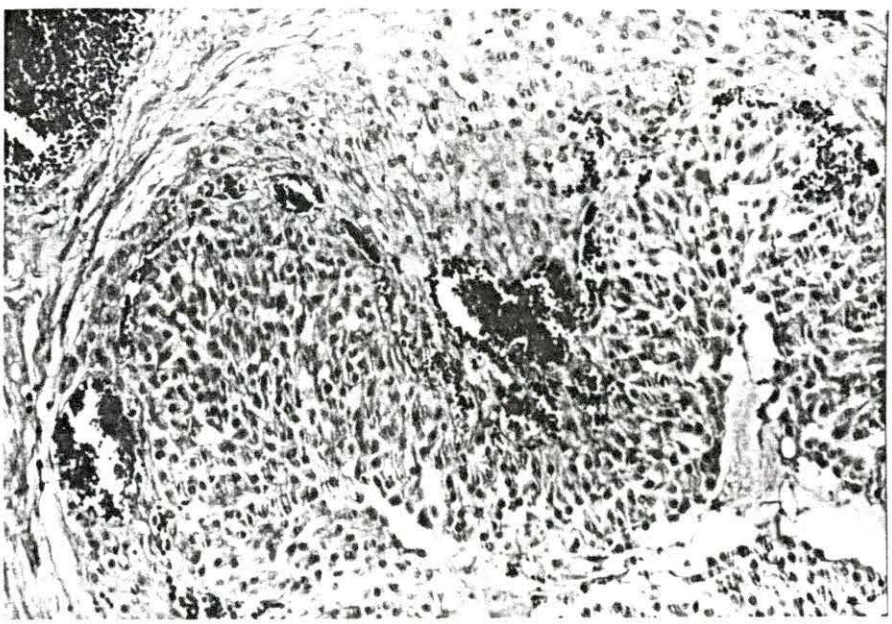
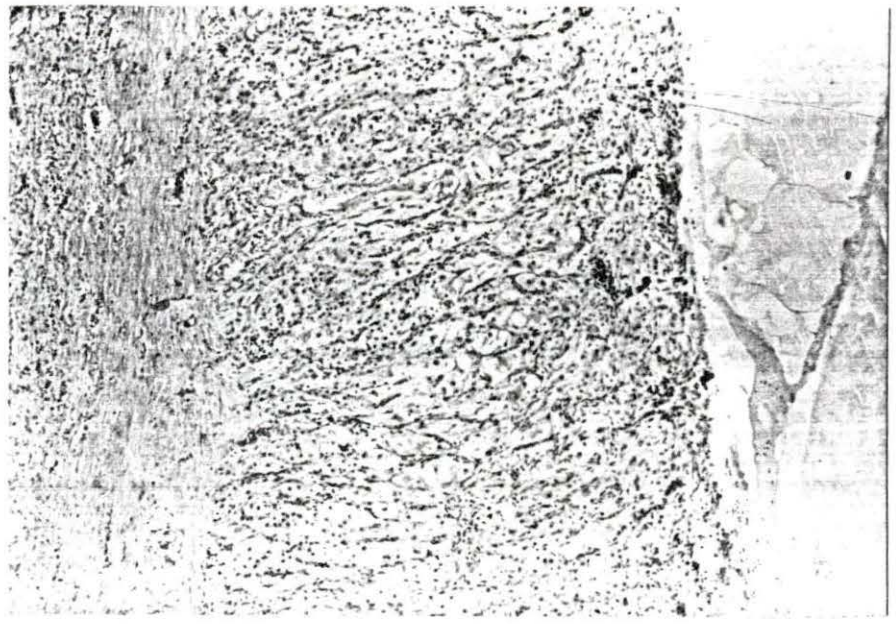


Figure 19. Pig no. 5895. Age: 6 years. An incomplete corpus luteum with a large central cavity. H and E stain, 100X.

Figure 20. Pig no. 6333. Age: 1 year 6 months. Arteries showing slight thickening of the tunica intima. Weigert's, van Gieson's, and Heidenhain's stain, 250X.



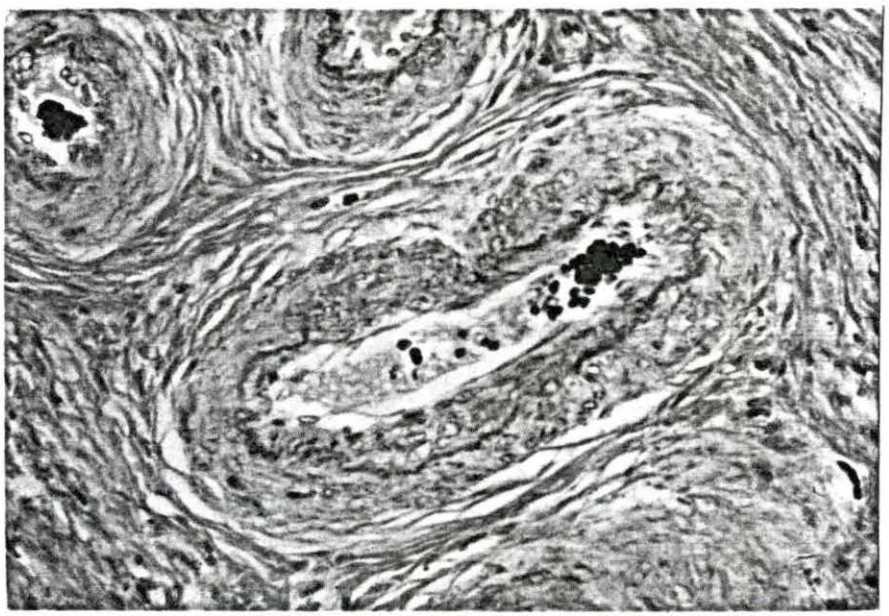
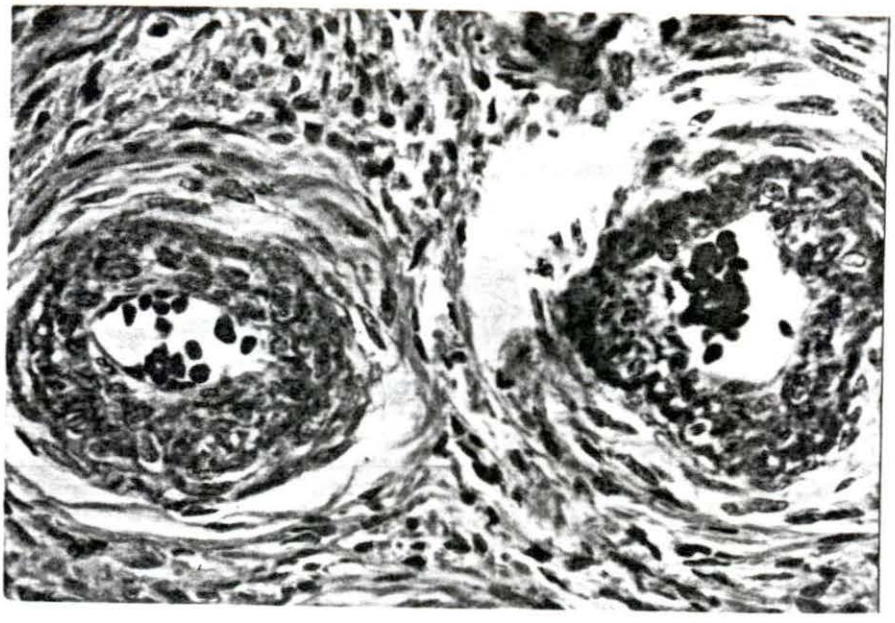


Figure 21. Pig no. 6153. Age: 2 years. Small arterioles with elastoid tissue in the thickened intima. Weigert's van Gieson's and Heidenhain's stain, 400 X.

Figure 22. Pig no. 951. Age: 2 years 5 months. An artery with split internal elastic lamina and thickened intima. Weigert's, van Gieson's and Heidenhain's stain, 400X.



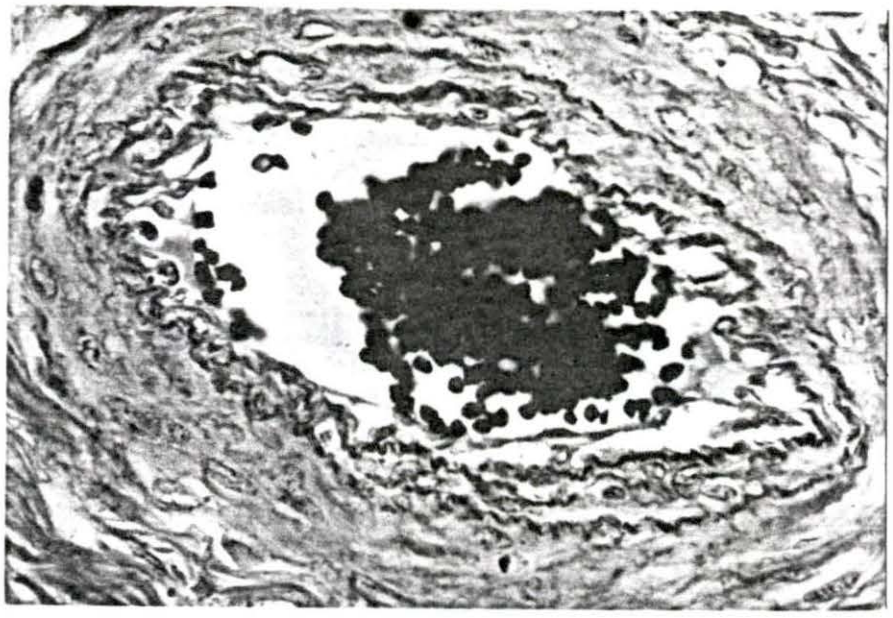
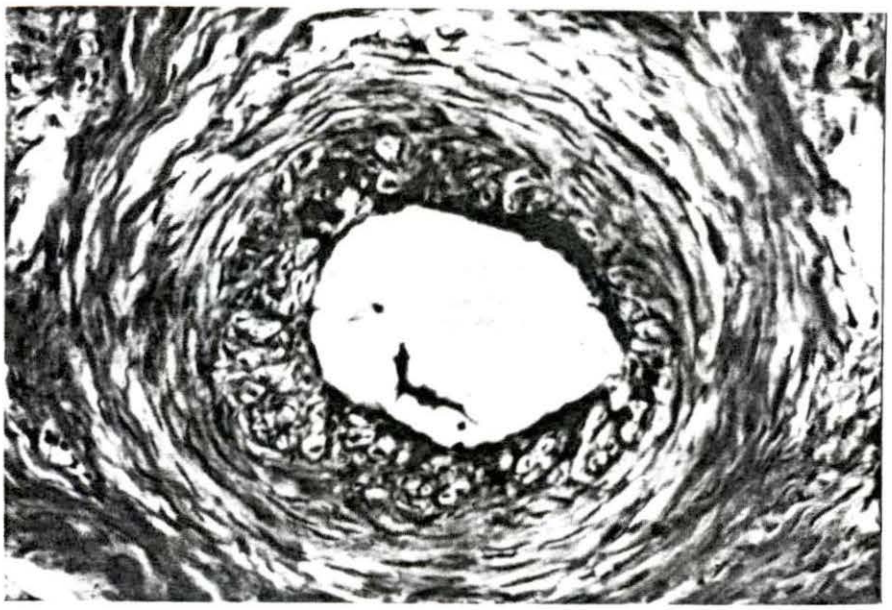


Figure 23. Pig no. 1040. Age: 3 years. An artery with thick intima showing longitudinal arrangement of smooth muscle and circular arrangement in the tunica media. Weigert's, van Gieson's and Heidenhain's stain, 400X.

Figure 24. Pig no. BB2. Age 4 years, 6 months. Arteries with thickened tunica intima. The smooth muscle in the intima is disposed in a longitudinal pattern. 250X.



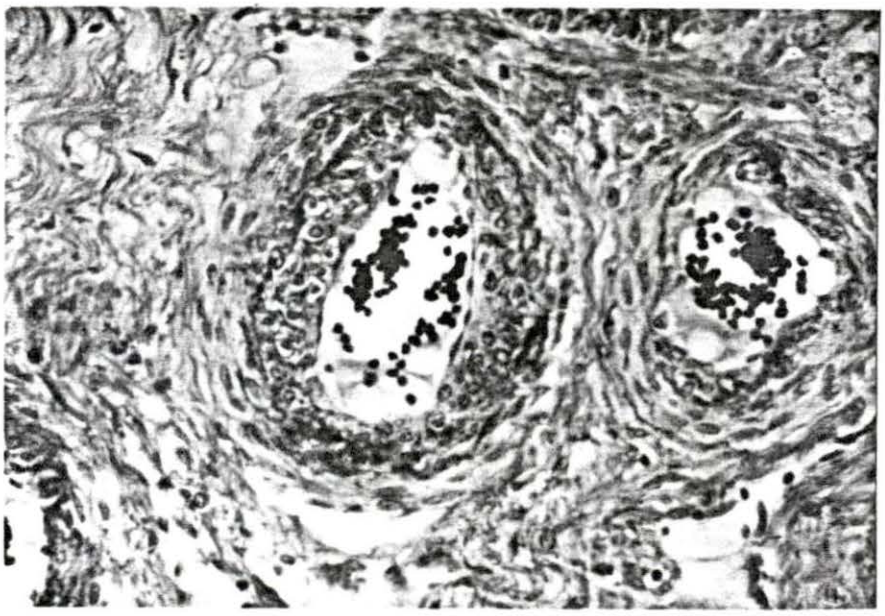
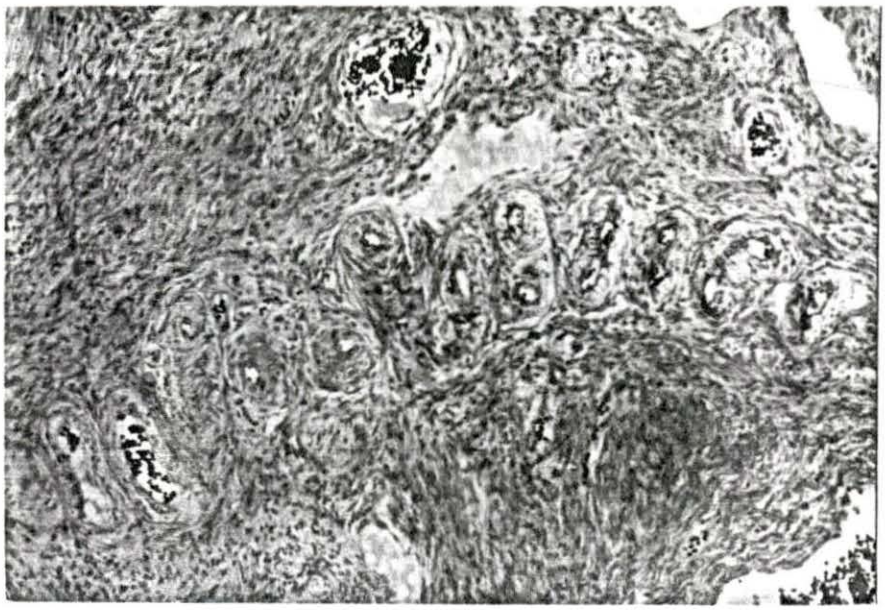


Figure 25. Pig BB2. Age: 4 years 6 months. A helical artery cut in a tangential section. Weigert's, van Gieson's and Heidenhain's stain, 100X.

Figure 26. Pig no. 5898. Age: 4 years 11 months. Artery with an extensive thickening of tunica intima almost equal to half the cross-sectioned diameter of the tunica media. Weigert's, van Gieson's and Heidenhain's stain, 250X.



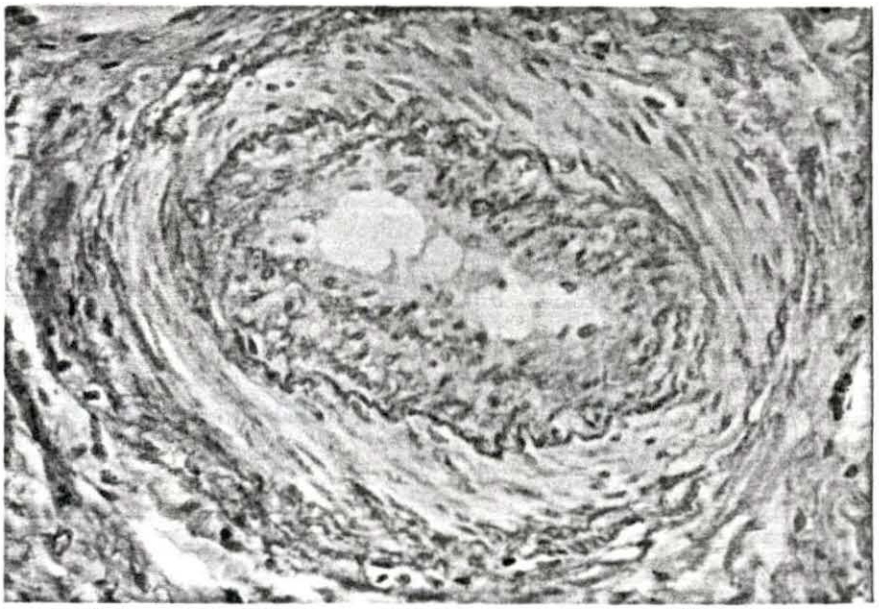
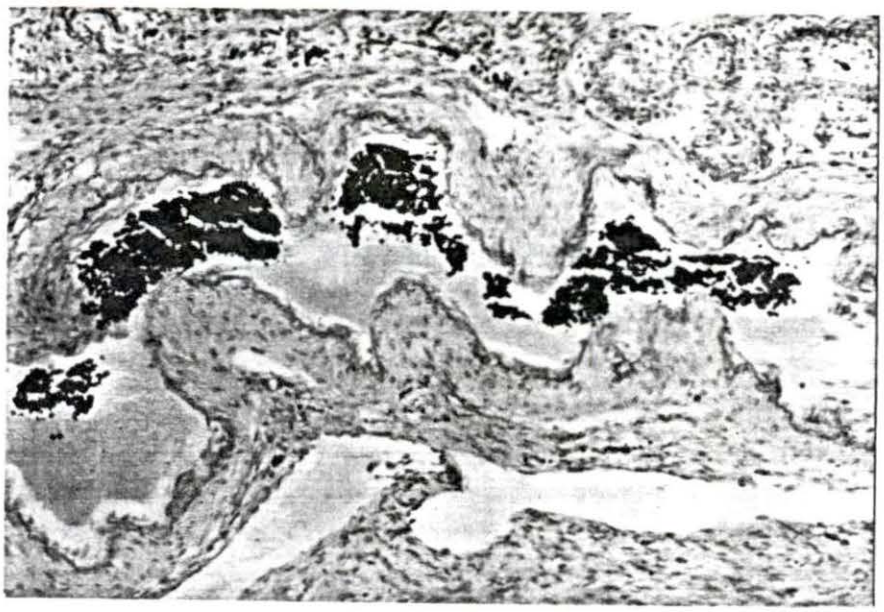


Figure 27. Pig no. 312. Age: 6 years 9 months. A helical artery cut in a longitudinal section. Intimal plaques appear at the bends. Weigert's, van Gieson's and Heidenhain's, 100X.

Figure 28. Pig no. 6043. Age: 6 years. Section of a graafian follicle with vessels within the membrana granulosa. Mallory's triple stain, 250X.



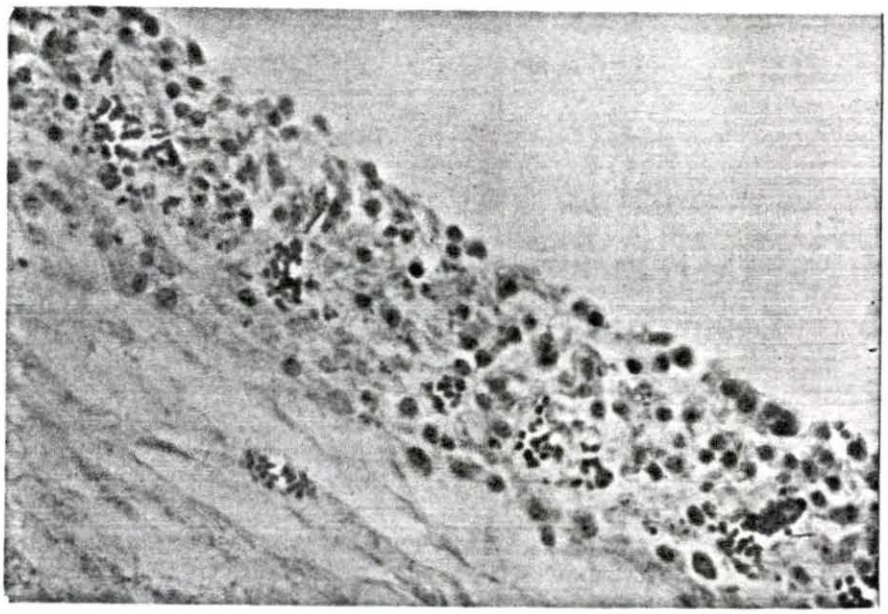
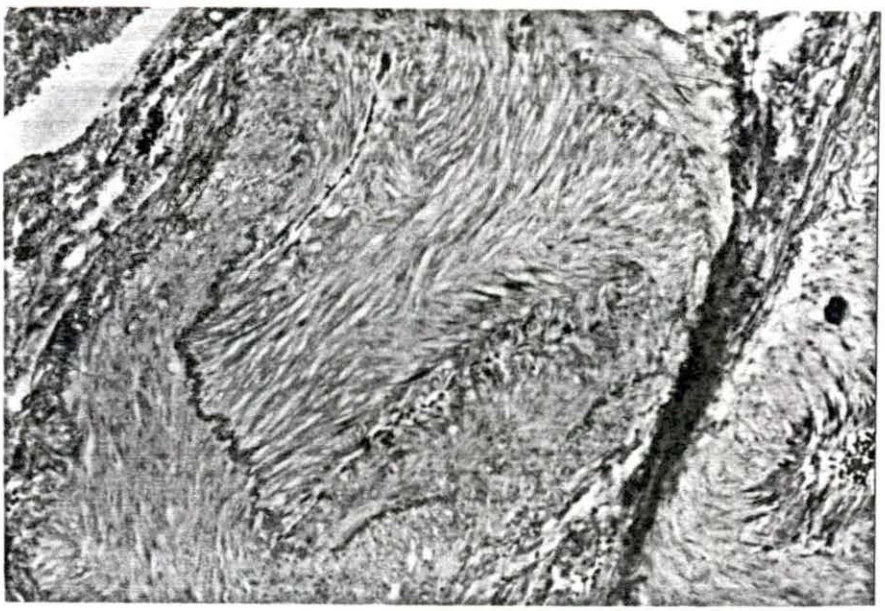


Figure 29. Pig no. 13. Age: 7 years, 9 months. Section showing extensive intimal thickening almost occluding the lumen of the vessel. Weigert's, van Gieson's and Heidenhain's stain, 100X.

Figure 30. Pig no. LJF 1. Age: 8 years. Section of a vein showing an intimal plaque. Weigert's, van Gieson's and Heidenhain's stain, 100X.



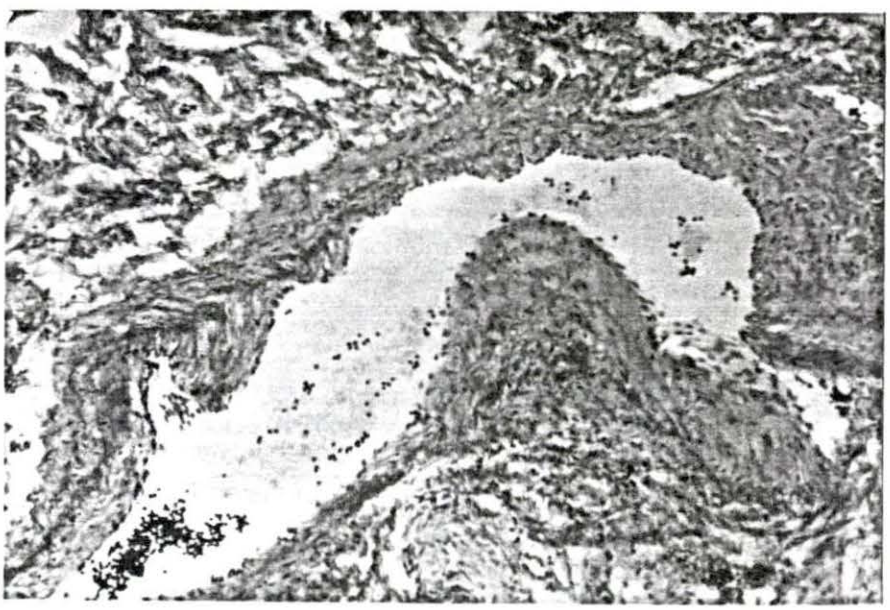


Figure 31. Pig no. LJF. 1. Age: 8 years. A large medullary vessel showing thickened intima. Weigert's, van Gieson's and Heidenhain's stain, 100X.

Figure 32. Pig no. 1175. Age: 8 years. A group of arteries showing thickened intima. Weigert's, van Gieson's and Heidenhain's stain, 100X.

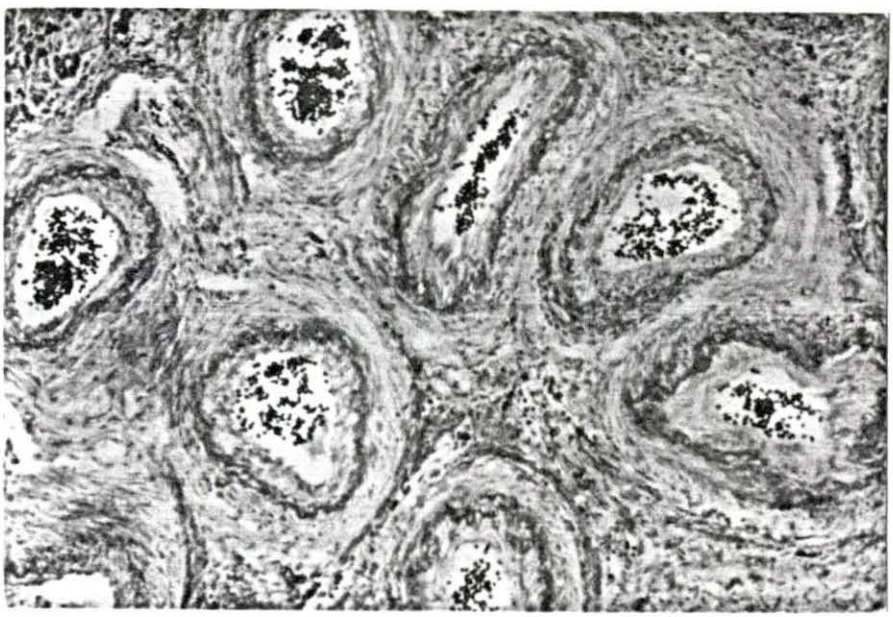
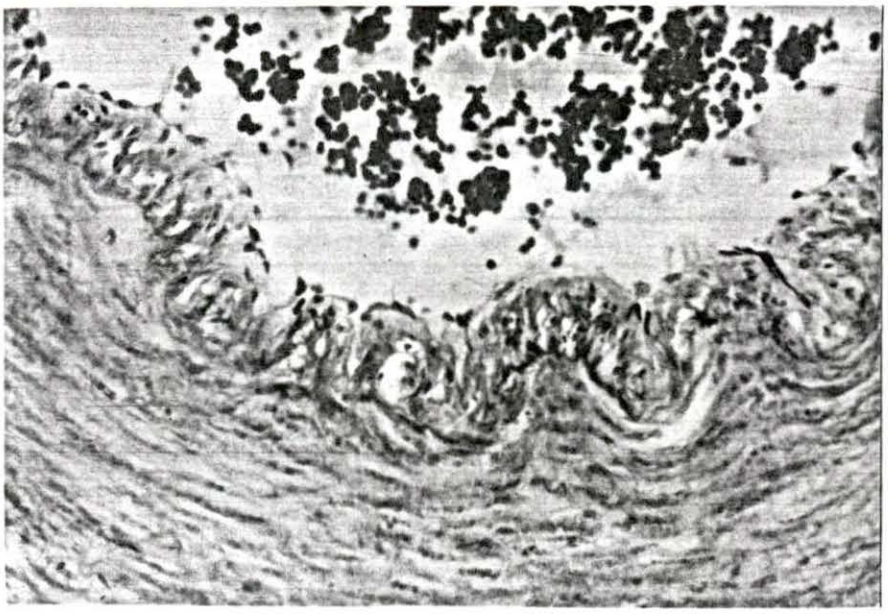


Figure 33. Pig no. 1175. Age: 8 years. Intima of a large artery showing increased amount of collagenous fibers. Mallory's triple stain, 250X.

Figure 34. Pig no. 1175. Age: 8 years. Section of an artery with extensively thickened tunica intima and a narrow lumen. Weigert's, van Gieson's and Heidenhain's, stain 250X.



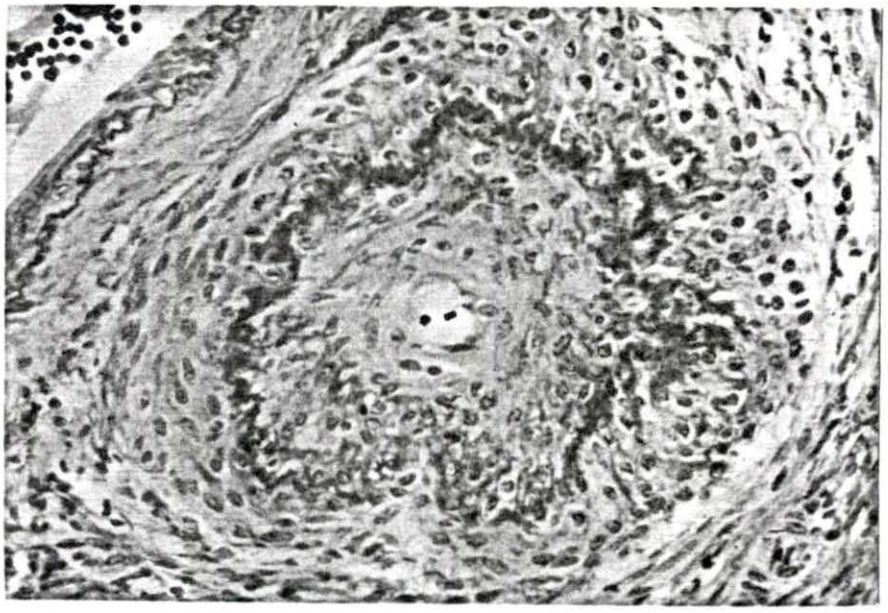
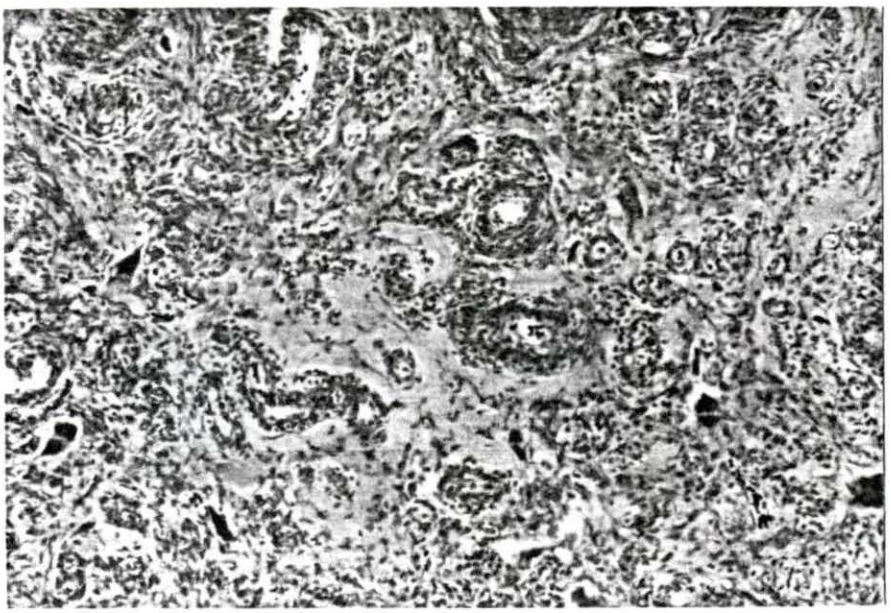


Figure 35. Pig no. 13. Age: 7 years 9 months. A regressed corpus luteum with vascular colonies. Weigert's, van Gieson's and Heidenhain's stain, 100X.

Figure 36. Pig no. 5895. Age 6 years. Cells of the incomplete corpus luteum. H and E stain, 250X.



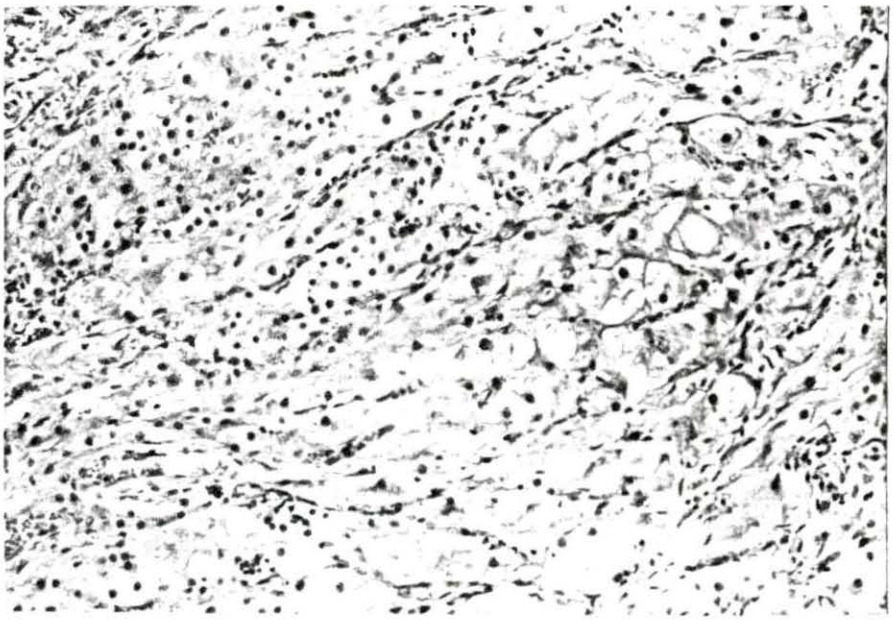
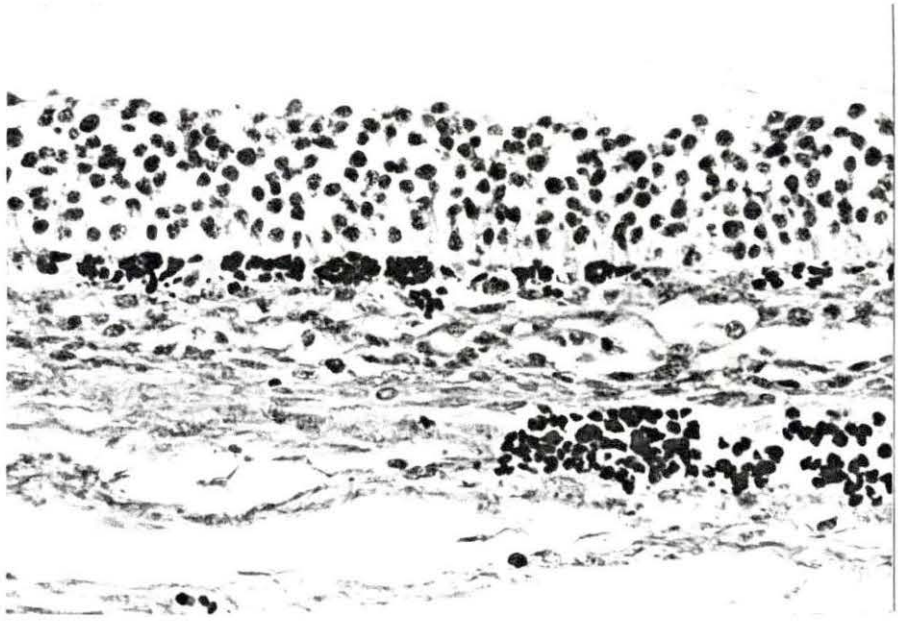
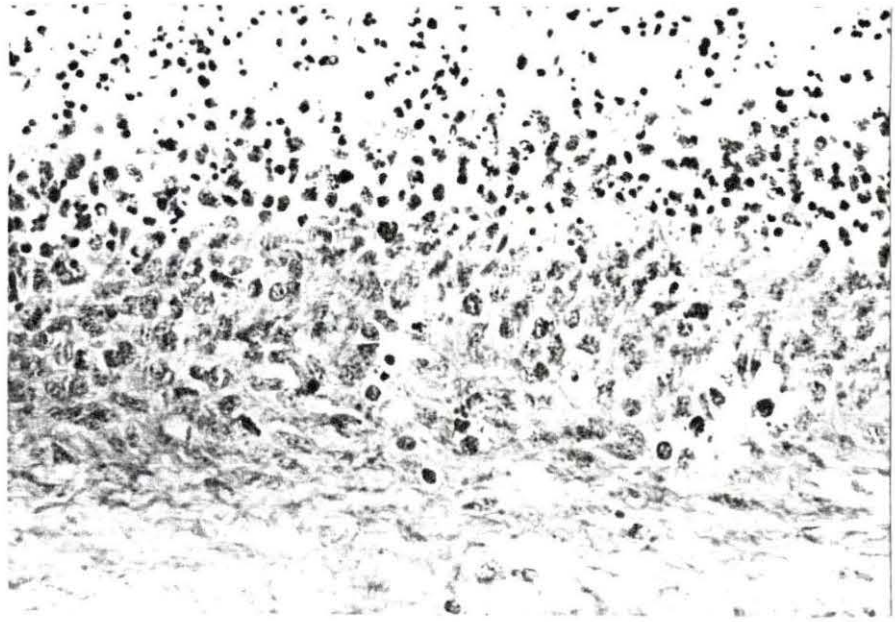


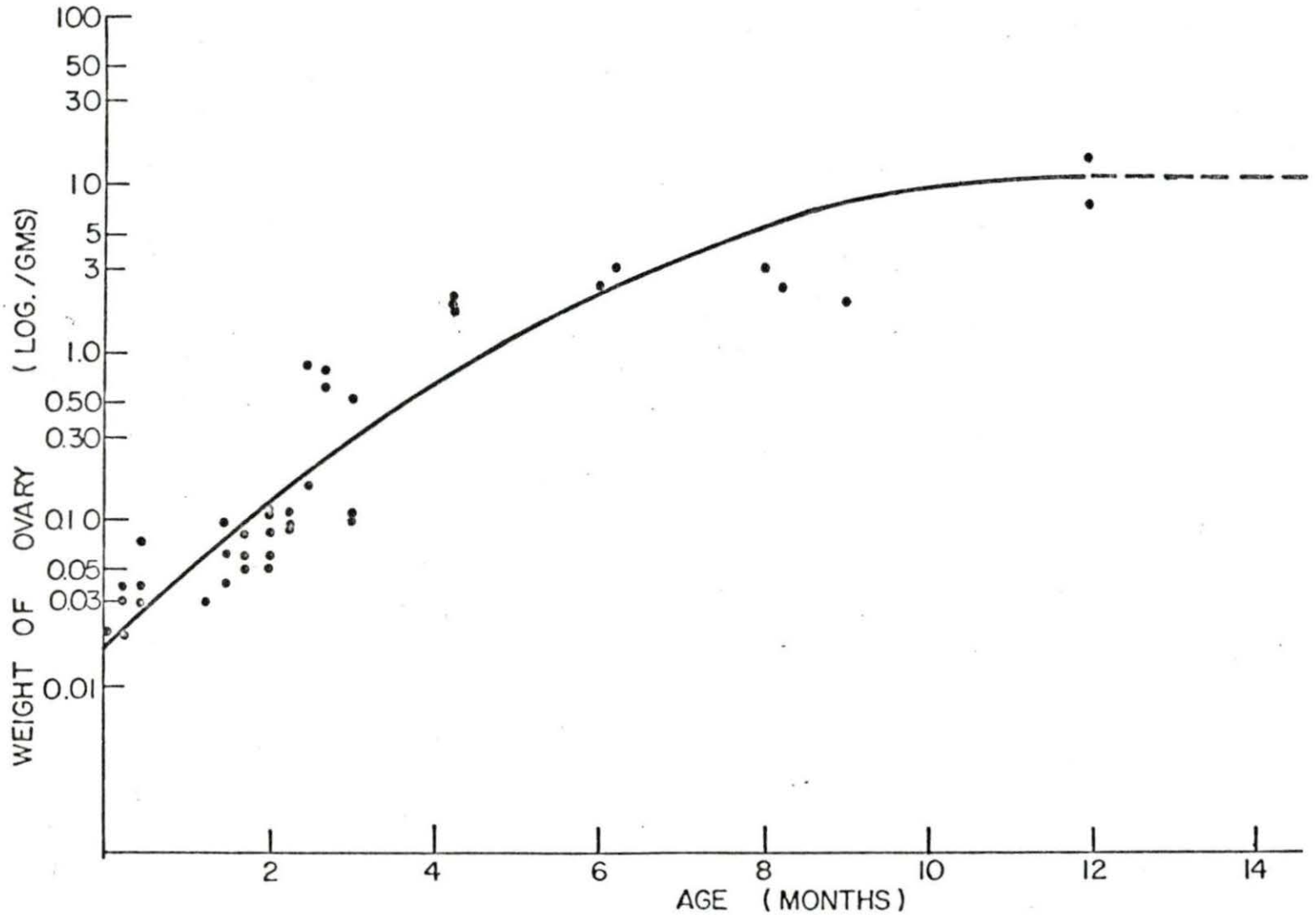
Figure 37. Pig no. 1292. Age: 6 months. Section of a graafian follicle showing the pattern of vascularization. An inner wreath of capillaries is seen between the membrana granulosa and theca interna and an outer wreath of vessels outside the theca interna. Mallory's triple stain, 250X.

Figure 38. Pig no. 1292. Age: 6 months. A section of the graafian follicle in the early stage of atresia. No vascular wreath is seen between the theca interna and the desquamating granulosa cells. Mallory's triple stain, 250X.





Graph 1. Increase in weight of the ovary of the sow from birth to 12 months.


100
50
30
10
5
3
1.0
0.50
0.30
0.10
0.05
0.03
0.01
WEIGHT OF OVARY (LOG./GMS)
2
4
6
8
10
12
14
AGE (MONTHS)

Graph 2. Increase in weight of the ovaries from 12 months to 96 months (1 year to 8 years).

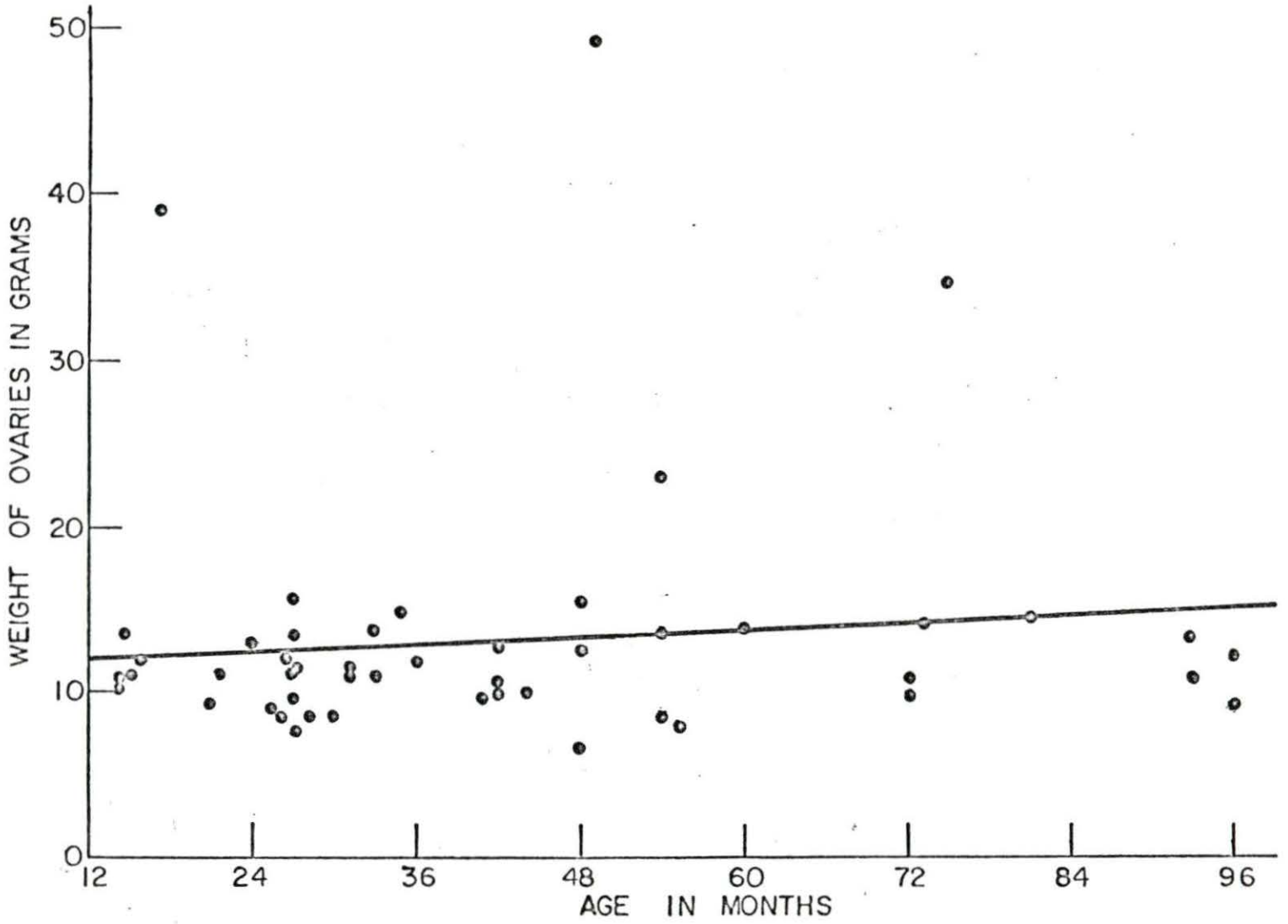

WEIGHT OF OVARIES IN GRAMS
0
10
20
30
40
50
12
24
36
48
60
72
84
96
AGE IN MONTHS

Figure 39. Pig number 1292. Age 6 months. Section of the ovary showing normal arteries. H and E stain 50X.