MORPHOLOGY OF THE FEMALE REPRODUCTIVE ORGANS OF SEA OTTERS (*ENHYDRA LUTRIS* L.)

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by

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The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

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a candidate for the degree of Doctor of Philosophy

and hereby certify that in their opinion it is worthy of acceptance.

The author wishes to dedicate this Dissertation

as a memorium to his grandfather

Mr. Akhouri Nityanand Sinha (1876-1952).

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CHAPTER I

INTRODUCTION AND ACKNOWLEDGEMENTS

The purpose of this study was to investigate the gross and microscopic anatomy of the reproductive organs of the female sea otter (Enhydra lutris L.) and relate them to the reproductive cycle. Emphasis has been given to ovarian histology.

Although the value of the sea otter has been known to fur hunters for over two centuries, very little is known about this animal. Barabash-Nikiforov (1947) noted that sea otters have lenticulate shaped ovaries. Pearson (1952) studied a single pregnant sea otter and noted that the ovaries have irregular surface and subsurface fissures, besides the small Graafian follicles and a corpus luteum. The uterus was bipartite. Lensink (1962) briefly described reproduction in sea otters, based upon field observations.

Previous investigations were greatly hampered by the limitations of the material. For a decade reproductive tracts have been collected in the Amchitka and Aleutian Islands. Karl W. Kenyon of the United States Department of the Interior, Fish and Wildlife Service, collected most of these tracts and provided the field reports on them. Some of the reproductive tracts were collected and provided for the present studies by Dr. C. M. Kirkpatrick of the

University of Purdue. My thanks are due to Mr. Kenyon and Dr. Kirkpatrick for the ample supply of the material. Dr. Clinton H. Conaway deserves credit for initiating this project.

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CHAPTER II

LITERATURE REVIEW

There are relatively few descriptions of the complete morphology of the female tracts of the Mustelidae. Marshall (1904), described the reproductive tract of the female ferret in the following terms, "The uterus of the ferret is typically bicornuate, each of the uterine horns passing forward into a slender fallopian tube, which is very much coiled at its anterior end, passing several times around one side of the ovary. The mouth of the fallopian tube encloses the ovary, so that the ova on being discharged pass into a sac and consequently are not shed into the body cavity." The female tract is of the usual carnivore type (Marshall, 1904; Hamilton and Gould, 1940).

Deanesly (1935), describes the reproductive tract of the female stoat as follows; "The ovaries are flattened bodies enclosed in a capsule; their surface is smooth and neither follicles nor corpora lutea project conspicuously. The uterus is small and bicornuate, and the uterine canals run side by side for about 1.5 mm. and then fuse at the top of the cervix. The vagina is thin and terminates in a vulval swelling, which enlarges in size just before the oestrous." The tract of the female stoat resembles that of the ferret (Marshall, 1904).

The reproductive tracts in weasels (Deanesly, 1944), minks (Hansson, 1947; Enders, 1952) and wolverines (Wright and Rausch, 1955) do not differ greatly from that of the generalized carnivore or Mustelid type.

Barabash-Nikiforov (1947) states that the ovaries in sea otters are lenticulate organs, lying with their posterior edges adjacent to the oviduct funnels. Pearson (1952) said the uterus in the sea otter is bipartite like other carnivores; whereas it is bicornuate in river otters (Lutra canadensis) (Hamilton and Eadie, 1964).

Ovarian Morphology

Robinson (1918) states that in ferrets and in the ferret-pole cat hybrid the ovaries are irregular, ovoid, carnio-caudally longer than dorso-ventrally. The ovary of the left side as a whole is larger than that of the right side, but this is of no importance. Tanaka (1962) said that the difference in size of the left and the right ovaries in 66 dogs, was statistically insignificant ($P \leq 0.05$).

The shape and the size of the ovaries vary in different animals and during the different periods of the estrous cycle. The ovaries are ovoid in ferrets (Robinson, 1918), flattened in stoats (Deanesly, 1935), bean shaped in minks and goats (Hansson, 1947; Enders, 1952; Harrison, 1948), lenticulate or compressed oval in sea otters (Barabash-

Nikiforov, 1947; Pearson, 1952), ovoid in Rocky Mountain pikas (Duke, 1952), and almond shaped in porcupines (Mossman and Judas, 1949).

The ovaries are smaller in size during the anestrus period than during estrus or pregnancy in the ferret and ferret-pole cat hybrid (Robinson, 1918; Hamilton and Gould, 1940), in cat (Foster and Hisaw, 1935), in rat (Long and Evans, 1922), in weasel (Deanesly, 1944), in stoat (Deanesly, 1935), in mink (Enders, 1952; Hansson, 1947), and in Rocky Mountain pika (Duke, 1952).

Loeb (1911) established that the cyclic changes in guinea pig ovaries correspond to the cyclic changes taking place in the uterine mucosa. Long and Evans (1922) state that the estrous cycle is characterized by regular, periodically co-ordinated histological changes in every portion of the reproductive tract of the rat.

Deanesly (1944) states that the ovary of weasel is small, compact and generally enlarges during the breeding season, owing to the development of the follicles and corpora lutea. This phenomenon in general holds true for the ferret (Robinson, 1918; Hamilton and Gould, 1940), mink (Enders, 1952), rat (Long and Evans, 1922), guinea pig (Loeb, 1911), Rocky Mountain pika (Duke, 1952), and many other mammals.

Ovarian Histology

Robinson (1918) states that the cortex and medulla in ferrets and ferret-polecat hybrids are sharply defined. The cortex is surrounded by the germinal epithelium, internal to which is a definite tunica albuginea. The interstitial tissue is the main mass of the cortex. The tunica albuginea has spindle shaped cells and fibrils. The medulla is reticular in character and it is continuous with the extraperitoneal connective tissue at the hilum. Mainland (1928) observed in ferrets a definite narrow band of tunica albuginea lying close to the germinal epithelium. Hamilton and Gould (1940) noticed a thick cortex and small medulla in the resting ferret ovary. The epithelium of the ovary is thickened here and there, and the thickened portions project for some distance into the cortex.

Hansson (1947) observed in mink a well developed tunica albuginea from which connective tissue lamellae ran into the cortex and divided it into irregular columns and nests, giving it a labyrinthine appearance. The medulla is confined to the parts around the hilus of the ovary like that of the ferret (Robinson, 1918), and it is highly vascularized in the sexual period. Enders (1952) said that the germinal epithelium is simple cuboidal, strongly basophilic with scanty cytoplasm. The main mass of the

cortex consists of the interstitial cells like that of the ferret (Robinson, 1918). The tunica albuginea is in abundance during pregnancy. The medullary region is compact, composed chiefly of small cells with strongly basophilic nuclei and it contains a few germ cells, which are of the 'primitive type' in the 70 days post partum animal. In the adult animal the medulla forms a small part of the ovary. Within the medulla and close to its hilar margin, the rete canals have been noticed. The rete tubules have a simple squamous epithelium, irregular and tortuous lumina, free intercommunications, and generally converge at the hilus. The rete canals are similar in other mammals. In the adult animal, rete canals are reduced in size and number. Mossman (1937) said that in pocket gophers rete tubules were common near the hilum, often they were cystic, equalling the diameter of the antra of mature follicles.

Deanesly (1935) said that the stoat's ovary is similar histologically to that of the ferret (Robinson, 1918). The ovaries of the weasel (Deanesly, 1944), and the wolverine (Wright and Rausch, 1955), are like those of the ferret. Mossman and Judas (1949) state that the internal structures of the porcupine ovaries are in general similar to those in other mammals, as far as rete, medullary cords, stroma, and follicles are concerned.

Harrison (1962) states that all mammalian ovaries are

covered by a continuous sheet which is usually a single layer of cuboidal or low columnar epithelium and which invests both the smooth and lobed contours.

Sub-surface Crypts or Folds

Harrison and Mathews (1951) reviewed the literature on this subject. They state that the formation of crypts is a phenomenon by no means restricted to a few species. They are found in all Pinnipeds and many Fissipeds, besides other unrelated mammals. The evidence indicates that crypt formation can be induced by the injection of gonadotrophin or estrogenic substances into ferrets. The ferret's ovary ordinarily does not exhibit marked crypts formation; of course, occasional clefts have been found in pregnant animals. The terms cleft and crypt may be defined as follows:

1. A crypt is a hollow tubular or slit like invagination of the germinal epithelium, which penetrates into and passes through the tunica albuginea.

2. A cleft simply subdivides the cortex and the tunica albuginea and follows its contour.

They discussed sub-surface crypt formation in the cortex of mammalian ovaries to a considerable extent. The covering germinal epithelium often invaginates into the subjacent tunica albuginea to form small folds, pits or

sub-surface crypts. Bonner (1955) said that the sub-cortical crypts are slight in juvenile elephant seals compared to that observed by Harrison and Mathews (1951). These formations are particularly developed in Pinnipedia (Harrison et al., 1952; Laws, 1956). In seals the germinal epithelium dips down at regular intervals into the peripheral ovarian stroma forming a tube with a conspicuous lumen (Mathews and Harrison, 1949).

Pearson (1952) observed the presence of irregular surface fissures in both ovaries of a sea otter. These fissures may be as much as 3.5 mm. deep in the ovaries, and are reminiscent of cerebral fissures. The tunica albuginea follows these clefts into the interior of the ovary and is not penetrated by them.

Harrison and Neal (1956) said that the intra-cortical epithelial tubes in badgers sometimes extended from the openings on the surface of the ovary to the hilum; where they might anastomose with the rete ovarii. These are reminiscent of sub-surface crypts of the covering epithelium (Harrison and Mathews, 1951). The tubules of epithelial cells dip from the cortex and form a network throughout the cortex and medulla (Neal and Harrison, 1958). The intra-ovarian tubules occasionally branch or communicate in the badger.

Mathews and Harrison (1949) state that the size and extent of the crypts and proliferations of the epithelium

appear to be related to the reproductive activities of seals. Harrison and Mathews (1951) noticed the cyclical phenomenon of crypt formation in Pinnepedia, which might be associated with the estrous cycle. Harrison (1962) indicated that the significance of crypts and their connections with the intra-ovarian tubules are difficult to assess. The subsurface crypts in fur seals are invaginations of the germinal epithelium which encapsulates the ovary in a single celled layer (Craig, 1964). They extend in tubular networks through and parallel with the tunica albuginea. Smaller branches extend into the tunica and form rounded diverticula.

Follicular Growth

Brambell (1928) has given a detailed description of the relationship between the ovum growth and the follicle growth in mice. He divided the growth of follicles into two phases.

In the first phase the ovum grows rapidly until almost adult size, while the follicle increases only slowly in size.

In the second phase the ovum remains practically stationary in size, although the follicle grows rapidly chiefly by enlargement of the liquor filled antrum and by the formation of the theca interna.

Subsequently the relationships between ovum and follicle growth have been studied in rats (Parkes, 1931),

in mice (Pincus, 1936), in guinea pigs (Dempsey, 1937), in rabbits (Pincus and Enzman, 1937), in cats (Dawson and Friedgood, 1940), in minks (Hansson, 1947), in goats (Harrison, 1948; Brambell, 1956), and in the opossum (Martinez, 1942). They supported the above relationship between the ovum and follicle (Brambell, 1928).

Hartman (1929) said that the diameter of the oocyte did not show any relationship with the body size of the animal, however, the ultimate size of the follicle at the end of the second phase is directly related to the animal's body size, and the major part of the follicular growth is associated with the enlargement of the follicular antrum (Parkes, 1931).

Mathews (1939) observed two components in the first phase of the follicular growth of the spotted hyaena: an initial one of slow growth of a single layer follicle followed by a period of faster growth when the follicle is multilayered. Pincus (1936) said that the ovum might grow to full size without a vestiture of follicle cells, as attested by the frequent presence of such ova in the ovaries of dwarf mice (Pincus unpublished data). The growth of the follicle beyond the antrum stage is independent of the growth of the ovum. This fact has been amply supported from the data of Brambell (1928), Parkes (1931), Pincus and Enzman (1937). Pincus (1936) said that the curve of Pincus and Enzman (1937), might be taken as a representation of the growth curve of the

ovum. Harrison (1962) said that if the y = the mean diameter of the oocyte, and the x = that of the follicle, the two phases of growth can be expressed by the linear regression; y = a + bx; where 'a' and 'b' are constants. He also added that the phenomenon of follicular growth holds good for all mammals examined, which have varied from the mouse to the whale. The number of follicles, which reach the end of the second phase and ovulate, varies widely in different mammals.

Follicular Cycle

Robinson (1918) postulated that in the ferret and in the ferret-polecat hybrid there are successive waves of growth and decline in the follicles. The follicles develop and retrogress in overlapping crops, until ovulation in the breeding season of the rabbit, the ferret and the mink (Hartman, 1939; Enders, 1952). In the mink and the ferret new groups of follicles appear throughout the functional life of the ovary (Enders, 1952; Mainland, 1928). Deanesly (1935) said that the early stages of follicular growth in the stoat were similar to that of the ferret.

Robinson (1918) said that the presence of the corpus luteum has no detrimental effect on the growth of follicles. Enders (1952) observed the same phenomenon in mink, where primary follicles appear at all times of the year. The

formation of small follicles is not completely inhibited by the presence of follicles ready to rupture, or by the corpus luteum, or by early pregnancy. Ringrose (1962) observed an increase in the number of follicles during pregnancy. On the contrary in Callorhinus ursinus cynocephalus, the corpus luteum of pregnancy suppresses follicular growth to a remarkable extent (Enders et al., 1946; Craig, 1964). But the opposite ovary has pronounced follicular growth during the pregnancy, and at the parturation it contains numerous growing follicles. These findings have been confirmed in the fur seal, Northern European and Antarctic seals (Harrison et al., 1952). The age at which the Graafian follicles first develop in the ovaries of the fur seals is variable. The limits may be set at 2-6 years of age (Craig, 1964).

Hammond and Marshall (1930) observed follicles during the anestrus period of the ferret. The ovaries are inactive with small follicles (Hammond and Walton, 1934). Evans and Cole (1931) noticed many small and medium sized follicles in the cortex of the dog's ovary during proestrus. In estrus only a few small follicles are present, most of them have already degenerated.

Evans and Swezy (1931) said that in rat and guinea pig, the follicular cycle normally coincides with the estrous cycle. In the cat, it may or may not coincide. The follicles

in the ovaries of the goat change little in size during anestrus and metestrus, but rapidly increase in size at proestrus (Harrison, 1948). In this species a wave of degeneration starts at or just before the onset of the estrus. Mossman (1937) noticed no obvious cycle with the small and medium sized normal follicles of the pocket gopher.

Deanesly (1944) states that the adult weasel's ovary, unlike that of the stoat, shows growing follicles in anestrus. The lack of large follicles and the inactivity of wolverine ovaries indicate the absence of an estrous period shortly after parturition (Wright and Rausch, 1955). This condition has also been found in the fisher (Hall, 1942). Nine weeks after parturition the European badger shows an estrous period (Neal and Harrison, 1958). Ovulation might occur during delayed implantation in the badger. Parkes (1931) observed that the size of the mature follicles varied greatly from species to species. Hill and Parkes (1933) said that the diameter of the largest follicles in anestrus varies greatly in the ferret at different periods of the time in year. The follicles in the cat during estrus have a diameter between 2-3 mm. like that of the ferret. The proestrus follicles of the cat range in diameter from 1 to 1.5 mm. with a large antrum (Dawson and Friedgood, 1940). The follicles in the striped skunk reach their greatest size

at estrus, as do the sex cords and interstitial cells (Leach, 1960). An inverse relationship between the follicle size and sex cords, has been observed during proestrus. The follicles, sex cords, and the interstitial cells of the skunk are larger in the post-partum animals than in pregnancy. Provost (1962) said that follicle size in the beaver was related to the number of follicles in the ovary.

Hisaw (1947) said that ovulation in mammals marks the end of the follicular phase of the estrous cycle and the beginning of the luteal phase.

The changes in the follicles during pregnancy are identical with those found in the non-pregnant cycle of the guinea pig (Loeb, 1911).

Structure of the Follicles and Ova

Robinson (1918) reviewed the earlier works on the structure of follicles, which do not deal with the progressive changes in follicular development. He presented a detailed description of follicular growth during the vesicular stages, which is given in brief at this point.

The primitive ovarian follicles consist of an ovum and single layer of flattened cells, which are progenitors of the follicular epithelium. As the follicles reach full growth the follicular epithelial cells appear columnar, conical or spindle shaped.

The first indications of antrum formation is a fluid-filled meshwork seen at the poles of the follicles. The fluid of the early antrum is called "primary liquor folliculi". The formation of the "secondary liquor folliculi" is seen first at the base of the cumulus oophorus. It is formed more rapidly and it is of more fluid consistency than the "primary liquor folliculi". It also takes part in the final distension of the follicle, which precedes rupture. Earlier workers had not drawn attention to the formation of the "secondary liquor folliculi" in the ovarian follicles. Allen et al. (1930) demonstrated the presence of the "secondary liquor folliculi" in the cumulus region of the large follicles in human ovaries.

During the post-inseminal growth and the rupture of the follicles, portions of the membrana interna are forced from its anchorage and extruded. It carries with it long filamentous processes of the follicular cells, which frequently drag with them some of the internal follicular cells. These processes and the cells together with the rapidly exuded fluid form a granular coagulum, called the "tertiary liquor folliculi". It also plugs the rupture of the follicle and finally breaks into fine detritus. After rupture, the follicles redistend and this is associated with the transformation of the follicle cells into the lutein cells.

Kingsbury (1939) said that the follicular fluid is preserved as a flocculent precipitate of coarse mesh in the normal Graafian follicle.

Pincus and Enzman (1937) have identified nine types of follicles in the rabbit, each distinguished by the characteristic features of the developing ovum, granulosa and theca cells. Hisaw (1947) divided the follicular growth of the mink into nine stages, from the primitive follicle (which consists of on ovum surrounded by the disconnected, flattened cells) to fully grown preovulatory follicles. He has given a detailed description of each stage. The stages 2 to 5 correspond to the "secondary follicle stage" as described by Sand (1918; quoted by Hansson, 1947). The 6th stage is characterized by the formation of antrum in the poles of the follicle as suggested by Robinson (1918), Brambell (1928), Pincus (1936), Pincus and Enzman (1937). The normal vesicular follicles first make their appearance during proestrus and estrus, but not in anestrus. The 7th stage is characterized by the beginning of the vesicular phase, which almost coincides with the cessation of the increase in the size of the egg. This stage, thus indicates a physiological change in the activity of the granulosa cells. The follicles in stage eight have well developed cumulus oophorus, corona radiata and uniform liquor folliculi. The vacuoles are seen in the cumulus region which correspond to the

formation of the "secondary liquor folliculi" of Robinson (1918). In the last stage, the ovum floats in the liquor folliculi and is ready to ovulate. The growth of the follicles is very slight during the first six stages of development, but is rapid during the later stages.

Pearson (1952) said that the left ovary of a pregnant sea otter contained only small follicles, whereas the right one had many small follicles and a corpus luteum.

Leach and Conaway (1963) state that the medullary polyovular follicles in the striped skunk are similar to the cortical follicles.

Origin of the Liquor Folliculi

The breaking down of the follicle cells plays an important part in the formation of the liquor folliculi fluid (Alexando, 1891; Janosik, 1887; Schottlander, 1893; Nagel, 1888; Van der Stricht, 1912; and Sandes, 1903; all quoted by Brambell, 1956). Honoré (1900) said that the follicular fluid was of intercellular secretion. Robinson (1918) supported this view. Honoré (1900) said that the so-called bodies of Call and Exner were intercellular spaces, whereas Janosik (1887, quoted by Brambell, 1956) thought them to be vacuoles in the cells.

Ovum

Van der Stricht (1912, quoted by Robinson, 1918) said that the ovum of the cat is spherical or ovoid with a rounded and eccentric large nucleus and one or two nucleoli. The ovum of the ferret is similar to that of the cat (Robinson, 1918). A mature ovum of the badger is elliptical in section (Hamlett, 1932). Enders (1952) states that the ovum of the mink in large anestrus follicle has the nucleus peripherally or centrally located with an eccentric nucleolus. In the proestrus follicle the nucleus is at the periphery of the ovum.

Leach (1960) said that the striped skunk had several oocytes in the rete, a condition not reported earlier in the literature.

Mainland (1932) states that most of the ova showed radial striae in the zona pellucida, the later being 4-6 micra in thickness. The zona pellucida is homogeneous in the ovum of badger and the thickness varies from 3.6 to 5 micra (Hamlett, 1932). It is also homogeneous in the ferret (Hamilton, 1934). Dickmann (1963) states that the zona pellucida of recently ovulated rabbit eggs consists of two distinct concentric layers, the outer being granular and the inner being homogeneous. There is no difference between the structure of the zona of fertilized and unfertilized ova. The presence of a zona pellucida has been

recorded in most of the mammals so far studied.

Origin of the Zona Pellucida

There are two theories regarding the origin of the zona pellucida which have been debated for a long time.

The ovum forms the zona pellucida. This has been supported by many workers such as Beneden (1880; quoted by Hartman, 1926), O'Donoghue (1912), Hartman (1926) and Mainland (1928).

The follicle cells form the zona pellucida. This view has also been supported by many workers such as Waldeyer, Retzius (1870, 1912, quoted by Chiquoine, 1960), Van der Stricht (1923), and Chiquoine (1960). On the basis of the observations in the immature ovaries of the rats, guinea pigs, hamsters and cats, Chiquoine (1960) states that the zona pellucida is formed by the activity of the follicular cells.

Atresia

The literature on the atretic follicles and atresia, which is extensive, has been reviewed several times (Asami, 1920; Clark, 1923; Branca, 1925; Athias, 1920; Garde, 1930; Harman and Kirgis, 1938; Kingsbury, 1939; Brambell, 1956 and Ingram, 1962). In the present review atresia will be considered as representing the process or processes, whereby

oocytes are lost from the ovary other than by ovulation (Ingram, 1962).

The mammalian ovary has a greater proportion of ova and Graafian follicles which degenerate and disappear, leaving a small number of them to attain maturity (Waldeyer, 1870; Beigel, 1878; Schottlander, 1893; Henneguy, 1894; all quoted by Asami, 1920; Kingsbury, 1913; Clark, 1923; Pincus and Enzman, 1937; Mandl and Zuckerman, 1950). Pike et al. (1960) said that atresia in the fur seal is characterized by formation of the "glassy membrane" which is located between the theca interna and membrana granulosa and contributed to by fibroblastic elements of both.

Atresia of the Small, Medium and Large Follicles

In the atresia of medium and large follicles both the granulosa cells and ova are involved. Marshall (1903) states that the atretic follicles in sheep can be readily distinguished from the normal ones. Usually the degenerating ovum is present in the cavity of atretic follicles. In an atretic follicle of the ferret the ovum shrinks, granulosa cells degenerate and a few cells in the advanced stage of degeneration are seen scattered in the cavity (Marshall, 1904). The zona pellucida persists for a long time in the atretic follicles (Brambell, 1956). Leach and Conaway (1963) state that atresia of polyovular follicles in the striped

skunk is similar to the atresia of the cortical follicles. Kingsbury (1913) states that follicular degeneration occurs throughout life. Follicles of different sizes, both atretic and healthy, are found furing the estrous cycle of the rabbit (Asami, 1920). This condition has been noticed in the ferret (Hamilton and Gould, 1940), the guinea pig (Harman and Kirgis, 1938), and the stoat (Deanesly, 1935).

Clark (1923) states that there is no mammal in which continual degeneration of ova is not going on in the ovary, at least during the period of sexual activity. Branca (1925) regarded atresia as a normal feature and said that it occurred more commonly at certain periods than at others.

Neal and Harrison (1958) observed numerous atretic and luteinized follicles in adolescent badgers. Atresia in the mouse is very common at the time of weaning (Kingery, 1917; Brambell, 1927). Engle (1927) found variation in the number of atretic follicles corresponding with the estrous cycle in the mouse. Evans and Swezy (1931) concluded that all oocytes, including the primary ones, become atretic at estrus, the sole surviving follicles being those that are ovulated. Harrison (1948) found widespread atretic changes in all the follicles of the goat, commencing at the 40th day of the pregnancy.

Pincus and Enzman (1937) distinguished 4 types of atretic follicles. The percentage of atresia among the

young oocytes is low, about 10%; whereas in the large follicles it is about 60% at any given time.

Brambell (1956) said that the onset of anestrus, in mammals with a restricted breeding season, is marked by atresia of the larger follicles in the ovaries. The follicular atresia increases as a rule during pregnancy and more especially during lactation and anestrus. The formation of the corpora lutea atretica or false corpora lutea is of common occurrence in many mammals during the mid-gestation.

Mandl and Zuckerman (1950) states, "The estimations of atretic oocytes at various stages of the cycle are subject to considerable variation, since the diagnosis of atresia depends upon qualitative judgment." In each rat the total number of follicles in the right ovary is not statistically different from that of the left. Contrary to the views of several workers, they did not find systematic variation in the total number or in the healthy oocytes during the estrous cycle of the rat. Jones (1957, quoted by Ingram, 1962) was unable to find systematic fluctuations in the proportion of atretic oocytes.

Heape (1905) said that the small follicles in the rabbit were situated in the neighborhood of large follicles which deprived them of their nutrition. Asami (1920) concluded from his studies of the small follicles that they were by no means confined near the large growing follicles, but

might be at a distance from them. Small follicles might be atretic while others were normal. So on the basis of location of follicles, atresia cannot be explained.

Harman and Kirgis (1938) show a definite correlation between the condition of ovum and follicle. It seems that atresia of the follicle stimulates the egg to begin its maturation divisions. This is in agreement with the report of Pincus and Enzman (1937), in which the number of ova undergoing maturation increased following the inducement of follicular atresia in the rabbit.

The Sites and Signs of Follicular Atresia

Opinions differ as to the sites and signs of the earliest changes within the follicles. Asami (1920) believed that the granulosa cells are first affected; whereas Clark (1923) advocated that the ova are the first site of degeneration.

Granulosa Cells as the Site of Initial Atresia

Loeb (1901) said that the degeneration in the granulosa cells is the first sign of follicular atresia. In medium and large follicles degeneration is followed by the invasion of the follicular cavities by connective tissue cells of the theca, and thus all the follicles are eliminated (Loeb, 1911). The primary degeneration in granulosa cells is followed by the

degenerative changes in the ovum (Asami, 1920). Asami (1920) also said that the disappearance of the zona pellucida and contraction of organizing connective tissue constitute the end of the atretic process. Wilkerson (1926) supported Asami (1920) and said that the cumulus oophorus broke down quite early allowing the ovum to escape into the antrum of the follicle. Hamilton and Gould (1940), and Harrison (1948) supported this view. Tanaka (1962) said that the atretic follicles of dog had hyalinized zona pellucida and connective tissue.

Evans and Swezy (1931) found that usually degeneration started in the granulosa cells of the follicle of rat, guinea pig, dog and cat, but occasionally the first indications may be found in the ovum. Mossman (1937) noticed the first signs of degeneration in the granulosa cells of the follicles of the pocket gopher. Atresia always seems to lead to the differentiation of the theca interna into the interstitial cells (Mossman, 1937). In follicular atresia, the egg is eventually hyalinized and granulosa cells disappear completely. Hamilton and Gould (1940) state that the interstitial tissue helps in the collapse of the follicle, both by its slow proliferation and by the pressure it exerts. Atretic scars are least at the anestrus period of the ferret.

Harman and Kirgis (1938) state that the follicles with slight atresia show degeneration in the granulosa cells and

in the discus proligerous cells. These have an increased amount of chromatin material when compared to cells of normal follicles. In the completely atretic follicle of the guinea pig, much connective tissue is seen. Stafford et al. (1942) said that the first changes in the follicles marked for atresia were the absence of mitotic figures in the follicular epithelium and the thecal cells. The degenerated granulosa cells became detached from the follicular wall and floated in the antrum (Ingram, 1962).

Clark (1923) said that the earliest stages of the degenerations were not visible easily.

Ovum as the Site of Initial Atresia

Bonnet (1899, quoted by Ingram, 1962) said that divisions in the ova were the signs of degeneration. This view is also supported by Engle (1927) and Stockard and Papanicolaou (1917). In the guinea pig the atretic ova degenerate before the formation of the polar bodies (Stockard and Papanicolaou, 1917). Pincus and Enzman (1937) said that the nuclei of atretic primordial oocytes contained masses of dense granules. The first sign of atresia in the ovum of the Rocky Mountain pika is the achromatic condition of the nucleus, which finally disappears (Duke, 1952); whereas the first indication of atresia in the vesicular follicles is karyorrhexis of the granulosa cells. Clark (1923) states

that the fatty degeneration of the oocyte is the sign of atresia.

Hennequy (1894, quoted by Clark, 1923) observed that the degeneration of the ovum is marked by fragmentation; whereas Van der Stricht (1901, quoted by Clark, 1923) said that it was marked by the parthenogenetic division. Sansom (1920) distinguished between degenerative fragmentation and parthenogenetic development in the water vole. Clark (1923) supported the idea of degenerative fragmentation of the ova. He also said that the stages through which ova passed during degeneration were numerous and varied.

Mandl and Zuckerman (1950) estimated that the proportions of the oocytes to become atretic were very high.

Hennequy (1894, quoted by Garde, 1930) said that the epithelial cells invaded the zona pellucida and ovum. Loeb (1911) said that the granulosa cells phagocytized the ovum. The epithelial and the connective tissue cells occur in early follicles around the ovum, even before the formation of the antrum (Asami, 1920). He states that the foreign cells enter the ovum but do not phagocytize it, as long as it remains healthy. But the granulosa cells invade the ovum after it has undergone some degeneration, and the invading cells remain healthy and act as phagocytes. This view was supported by Branca (1925) and Garde (1930). Garde (1930) indicated that the mechanism of ovum destruction

veried, but the constant factor is the phagocytic role of the follicular epithelial cells. Some ova are absorbed *in situ* by the phagocytic epithelial cells, whereas others undergo atresia by a process, wherein the follicular wall ruptures and the contents pass either internally into lacunae of the medullary region or externally upon the surface of the ovary.

The epithelial origin of the phagocytic cells has been widely supported (Loeb, 1901; Marshall, 1904; Heape, 1905; Asami, 1920; Branca, 1925; and Hammond and Marshall, 1925).

Ingram (1962) states that the changes which occur during the atresia of primordial oocytes (surrounded by a single layer of granulosa cells) are less documented.

Some Other Signs of Atresia in the Graafian Follicles

Kingsbury (1939) said that the first demonstrable change in the Graafian follicle destined to undergo atresia appeared in the follicular fluid. The latter is preserved as a flocculent precipitate of coarse mesh, whereas in atretic follicles the fluid is a fine meshed coagulum. A considerable variation is exhibited within the atretic follicles. Harrison (1948) observed the fine mesh coagulum in large atretic follicles of the goat.

Glandular Tissue of the Mammalian Ovary

Mossman (1937) reviewed the current position of the glandular tissues in the mammalian ovaries. Mossman (1946) distinguished three types of glandular tissues in most mammalian ovaries which are present sometime during the reproductive cycle, i.e., the thecal gland developed from theca interna of ripening follicles; the interstitial gland developed from theca interna of atretic follicles; and the luteal gland developed from the follicular epithelium of ruptured follicles.

He also noticed additional types of the glandular tissues in certain species.

1. The interstitial gland derived from the epithelium of the medullary cords common in Mustelidae and certain bats.

2. Rete gland, which is a peculiar modification of the rete epithelium cells in bats.

3. Adreno-cortical-like glands, which are developed from the stroma cells near the epoophoroon and rete, varying greatly in amount in the different groups.

He concluded from his studies on the glandular tissues of the adult mammals that the theca interstitial, medullary cord interstitial, and the adreno-cortical-like gland tissues undergo changes correlated with the reproductive cycle of the same species, being apparently most active during estrus and early pregnancy.

Corpus Luteum

Volcherus Coiter, and Fallopius (1573, 1584, quoted by Asdell, 1928) described that the ovaries were filled with fluid and sometimes with the yellow bodies. These are probably the earliest references about the corpora lutea. Regnier de Graaf (1672, quoted by Asdell, 1928) said that the corpus luteum was like "conglomerate glandulus". Malphighi (1689, quoted by Asdell, 1928) gave a very accurate description of the corpus luteum.

There are three distinct theories regarding the origin of the luteal cells.

Connective Tissue (Theca Interna) Origin of the Luteal Cells

Von Baer (1827, quoted from Corner, 1915) believed that the lutein cells are derived from the connective tissue theca interna. The principal supporters of this idea are Leuckart, Gegenbaur, His, Minot, Nagel, Jankowshi, Delestre (1852, 1861, 1865, 1899, 1892, 1896, 1899, 1904, 1910, respectively; all quoted by Brambell, 1956).

The Membrana Granulosa Origin of the Luteal Cells

This theory has by far received the maximum support from the earlier and the recent workers.

Bischoff (1842, quoted by Corner, 1915) states that the luteal cells are formed exclusively from the cells of the

membrana granulosa. Among the earlier supporters are Pfluger, Waldeyer, Call and Exner, Sobotta (1863, 1870, 1875 and 1896 respectively; all quoted by Brambell, 1956). Sobotta (1896, quoted by Corner, 1921) clearly demonstrated that the granulosa cells of the follicle took a very important part in the formation of the luteal cells, while the theca interna was used up in the production of the connective tissue reticulum. Corner (1921) also supported this view.

Marshall (1903) observed that the connective tissue elements of the corpus luteum in the sheep were supplied, not only by the ingrowth from the theca interna, but also by the theca externa. This has also been observed by Craig (1964) in the fur seal. The luteal cells are formed from the granulosa cells, whereas the theca interna supplies the connective tissues and the blood vessels to the corpus luteum (Hammond, 1927). Corner (1932) suggested that the connective tissue trabeculae in the corpus luteum of the pocket gopher arose from the theca interna. Dawson (1941) noticed in cats that the theca interna cells persisted and eventually appeared as the marginal septa of the corpus luteum.

Robinson (1918) said that the theca interna took no part in the formation of the connective tissue reticulum, which appeared in the corpus luteum; on the contrary it was

ejected from the follicle at the time of the rupture.

Marshall (1904) stated that in the ferret the lutein cells were formed from the follicular epithelium as suggested by Sobotta. The granulosa origin of the luteal cells has been observed in the guinea pig (Loeb, 1911), the cow (Hammond, 1927), the pig (Corner, 1915, 1919, 1921, 1948), the mouse and the rabbit (Deanesly, 1930), the pocket gopher (Mossman, 1937), and the mink (Hansson, 1947; Westman, 1929, quoted by Hansson, 1947; Mossman, 1946; and Harrison, 1948 and 1962).

Westman (1929, quoted by Hansson, 1947) showed that when the entire granulosa layer was removed from a follicle, no corpus luteum was formed and there was no pregnancy reaction in the uterus. When a remnant of granulosa layer was left in the follicle, a partial corpus luteum was formed, and a corresponding reaction in the uterine mucosa was observed. Hence the granulosa layer forms the luteal tissue, which in turn is the site of progesterone secretion.

Mossman (1937) noticed difficulty in separating the theca interna and the granulosa cells, during the formation of the corpus luteum in the pocket gopher. He states that the lutein cells of the corpus luteum, which degenerate near the term of the pregnancy, are derived from the follicular epithelium. The lutein cells are separated by the trabeculae of vascular connective tissues which grew in them from the

theca interna. There is no evidence that the lutein gland cells are derived from the theca interna.

Corner, in a series of papers (1915, 1919, 1921, 1948) has dealt with the development of the corpora lutea in the pig. He supports the granulosa origin of the lutein cells. He noticed the presence of the 'theca lutein cells', first at the periphery and later in the corpus luteum. The theca lutein cells are smaller than the luteal cells. Corner (1932) also reviewed the varying roles of the theca interna and the granulosa cells in the establishment of the corpus luteum.

Harrison (1948, 1962) states that in the Eutherian mammals the granulosa cells become transformed into the luteal cells of the corpus luteum. The fate of theca interna cells is not yet clear although it may persist among luteinized cells of the corpus luteum of certain species. The observations of Harrison, Corner and Dawson correspond to the development of the corpus luteum of the fur seal (Craig, 1964).

The Membrana Granulosa and Theca Interna Origin of the Luteal Cells

Schron (1862, quoted by Corner, 1915) states that both the membrana granulosa and the theca interna layers of the follicle form lutein cells. This has been supported by Rabl (1898, quoted by Corner, 1915), Van der Strieht (1912, quoted

by Corner, 1915), Loeb (1906), Pederson (1951), and Leach (1960).

Pederson (1951) said that both the theca interna and the granulosa cells transformed into the typical lutein cells of the rat. Leach (1960) also noted the dual origin of the lutein cells in the striped skunk.

Malone (1957) used histochemical methods to show the dual origin of the lutein cells in the albino rat.

Structure of the Corpus Luteum

The histogenesis of the corpus luteum has been reviewed several times (Marshall, 1910; Corner, 1915, 1919; Pratt, 1935; Harrison, 1948, 1962; and Brambell, 1956). The study of the corpora lutea can be roughly divided into four categories.

Corpus Luteum of Ovulation

Corner (1915) said that the corpus luteum of ovulation lacked regularity of structure in the luteal cells, and had large fat vacuoles. He distinguished three stages of the corpus luteum, such as the formation, maturity and retrogression. Mayer (1911, quoted by Corner, 1915) had recognized proliferation and vascularization of the corpus luteum as the formative stages.

Robinson (1918) said that the ruptured follicles redistend,

which is the beginning of the corpus luteum formation. Hansson (1947) states that the proliferation activity of the granulosa cells, during the pro-ovulation stage, found in the follicle development of the mink, is probably the prelude to the formation of the corpus luteum. He differentiated six stages from the ovulation to the fully formed corpus luteum.

1. Proliferation phase of the granulosa cells, where the luteal cells are in a confused state inside the antrum.

2. Proliferation phase of the vascular system, where the vessels have split up the tissue into irregular nests.

3. Inactive phase, where the lutein cells are small, with dense and strongly basophilic nuclei. No progestational effect can be found on the endometrium. The female allows mating and ovulates. At this stage some involution can also be noticed.

4. Incipient incretion phase where the luteal cells increase in volume and have larger but less basophilic nuclei. The blood capillaries are dilated.

5. Incretion phase where the lutein cells are pear shaped, nuclei eccentrically located and the corpus luteum is highly vascularized. It seems to represent an active stage.

6. Immediately before parturition regression in the corpus luteum is visible. The lutein cells decrease in the volume and increase of the connective tissues is noticed.

The involution of the corpus luteum after partus has not been studied in mink.

Evans and Cole (1931) observed that the luteal cells in a fresh corpus luteum are in irregular columns, which often constitute an open lace-work unlike any other ovarian structures. The luteal cells are compact in late estrous. The luteal cells in the ferret are large and polyhedral, about 60 micra in diameter (Robinson, 1918). The luteal cells in the dog acquire fine fat droplets. During estrus, they are supported on a stroma of the connective tissues and the capillaries (Mulligan, 1942). Corner (1915) found irregularity in the structure of the corpus luteum. The luteal cells in seals are spherical, oval or polygonal in shape (Harrison et al., 1952). This was also noted by Craig (1964). Hansson (1947) states that the histological picture of the corpus luteum varies somewhat from body to body of the corpus and within a body from periphery to the center.

Robinson (1918) said that the size of the corpora lutea varied greatly in the same and in different ferrets. The size and the state of development of the corpora lutea in mink are related to the length of the day rather than to the age (Enders, 1952). The corpora lutea of the ovulation are small. Brambell (1956) states that the size of the corpus luteum is related to the size of the Graafian follicle from

which it was formed and indirectly also to the body size. This relationship may not be precise in different species and at different periods of pregnancy. Harrison and Neal (1959) observed the similarity in the appearance of corpora lutea in 19 badgers. They could not differentiate the corpora lutea into age groups. Neal (1957) noticed variations in the size of the corpora lutea of the badger. Deanesly (1944) reported the continuous growth of the corpora lutea in the weasel.

Hamlett (1935) found corpora lutea in marten, but no vesicles during the summer and hence concluded that it was a case of delayed implantation. Wright (1942), and Watzka (1940, quoted by Hansson, 1947) suggested that the so-called unfertile matings associated with the smaller corpora lutea, indicated delayed implantation.

Neal (1957) said that the youngest corpora lutea were enclosed in a thin capsule; whereas the older ones had organized central core of the connective tissue and thicker capsule in the non-lactating and non-pregnant adult badger. The failure to recover ova or blastocysts from the animals suggests that the corpora lutea are either the result of infertile matings or of the spontaneous ovulation. The corpora lutea were poorly developed to be those of the previous pregnancies and were of an age beyond which the unfertilized ova could not survive. The inactive corpus

luteum of the wolverine has small cells according to Wright and Rausch (1955). In the fur seal the luteal cells continue to hypertrophy until the time of the implantation (Craig, 1964).

Mossman and Judas (1949) found accessory corpora lutea in large numbers in the porcupine. They are in close correlation with the cyclic occurrence of the primary corpus luteum. The lutein cells are formed from the granulosa cells of the atretic follicles, or of the ovulated follicles and also from the embryonic stromal cells of the luteal remnants or capsule of the corpus luteum. The thecal gland cells never form true lutein cells and may remain as 'thecal lutein cells' in the definitive corpus luteum.

Deanesly (1935) said that the luteal cells are small in the corpus luteum of the stoat. It has a restricted breeding season, in which the animal is seasonally polyestrus, hence all the corpora lutea belong to the same cycle.

Desinety (1877, quoted by Corner, 1915) believed that the corpus luteum of pregnancy and that of ovulation are structurally alike. Marshall (1910) supports this idea. Corner (1915) said the corpora lutea of ovulation have irregular luteal cells; whereas those of pregnancy have regular cells. Corner (1921) was not able to distinguish between the corpora lutea of pregnancy and those of ovulation during the first two weeks after ovulation in the sow. The

corpora lutea of ovulation are smaller than those of pregnancy in the mouse and in the stoat (Deanesly, 1930, 1935). In mink the corpora lutea of ovulation are smaller than those of pregnancy (Enders, 1952). Provost (1962) said that the corpora lutea of ovulation in the beaver are indistinguishable during the formative stages from the true corpora lutea of the pregnancy.

Loeb (1911) states that the corpus luteum of pregnancy differs from the ordinary corpus luteum and the presence of a well functioning corpus luteum inhibits ovulation in the guinea pig. The corpora lutea of ovulation markedly differs in size and histological appearances from those of the spring pregnancies in the stoat (Deanesly, 1943).

Corpus Luteum of Pregnancy

Corner (1915) noticed the regularity in the structure of the lutein cells. They are rounded and of uniform size having vesicular nuclei rich in chromatin. The luteal cells are large and functional during pregnancy in the ferret (Hammond and Marshall, 1930), Peromyscus (Brown and Conaway, 1964). These cells in the cat have fine and uniform vacuolation with eccentrically located nuclei (Dawson, 1941). The luteal cells in the weasel (Wright, 1942) are like those of the cat (Dawson, 1941). The vacuoles in the luteal cells have been described for Gulo and the weasel,

(Wright, 1942; Wright and Rausch, 1955) and for the fisher (Eadie and Hamilton, 1958). The vacuolations in the wolverine luteal cells disappear after implantation of the embryos (Wright and Rausch, 1955).

Deanesly (1935) said that corpora lutea resembling those of pregnancy are not formed until the last week of February. The corpora lutea in the stoat are small in size during the free vesicle stage and attain full development during implantation (Watzka, 1940, quoted by Hansson, 1947). In Peromyscus there is a gradual increase in the size of corpora lutea throughout most of gestation (Brown and Conaway, 1964).

The corpora lutea during delay have large central cavities and poor vascularization, whereas after implantation the gland is very hyporaemic (Mathews and Harrison, 1949). Hamlett (1935) observed large corpora lutea during the free vesicle stage, which did not undergo noticeable change at implantation. The weasels and martens with unimplanted blastocysts have small conspicuous corpora lutea, which appear inactive (Wright, 1942). Corpora lutea in the fisher (Eadie, Hamilton, 1958) and wolverine (Wright and Rausch, 1955) resembled those of the weasel. Neal and Harrison (1958) said that the corpora lutea were ill developed, poorly vascularized and showed little evidence of secretory activities during the delay in the badger. This has also been noted in seals (Harrison et al., 1952). The corpora

lutea of pregnancy are large, active, and well vascularized. The corpora lutea of otter with unimplanted blastocyst are small (Hamilton and Eadie, 1964) as in weasels (Wright, 1942). The corpora lutea of otter with implanted blastocysts have large secretory luteal cells.

Harrison (1948) said that the phases of the corpora lutea development in goat were related to the development and activities of the placenta.

Deanesly (1934) noticed that in hedgehogs the corpora lutea of pregnancy did not exceed in size those from sterile matings. The corpora lutea of the weasels show continuous growth up to their maximum size and regress very gradually until the end of the breeding season (Deanesly, 1944). They are well vascularized and reach full size when the foetus measured 2.5 to 4.0 mm. Pearson (1952) noticed a corpus luteum in pregnant sea otter, measuring 5.5 to 3 mm. in diameter. In dogs the corpora lutea of pregnancy are retained until the end of gestation (Mulligan, 1942).

The corpora lutea are generally fully developed at the time of implantation of blastocysts (Harrison, 1948). Mossman and Judas (1949) said that theaccessory corpora lutea were formed by the luteinization of the atretic follicles. They can be divided into three groups, i. e., the transitional, lutein and hyalinized.

Nelson and Greene(1958) said that in the human female

the corpora lutea of the pregnancy might be distinguished by their greater size and central fluid filled cavities.

Corpus Luteum of Pseudopregnancy

Deanesly (1935) said that the corpora lutea of pseudopregnancy in the stoat have small lutein cells, having an area of 80-120 sq. micra, while at implantation they are about 250 sq. micra and in the fully formed corpora lutea they measure about 300-400 sq. micra. The corpus luteum of pseudopregnancy is small like that of non-pregnancy. It is large in the hedgehog (Deanesly, 1934). In mink the corpora lutea of pseudopregnancy and pregnancy are alike (Enders, 1952).

Hammond and Marshall (1930) observed that the duration of corpora lutea in pseudopregnancy was approximately the same as that of pregnancy in the ferret. In the ferret, there is no post-partum estrus, so the corpora lutea are either of pseudopregnancy or pregnancy. This was later supported by Hammond and Walton (1934).

Corpus Luteum Following Parturition and In Lactation

Loeb (1911) said that degenerative changes set in the corpus luteum before the end of pregnancy in guinea pigs. The corpus luteum in the stoat begins to retrogress soon after mid-pregnancy (Deanesly, 1935). It may disappear

before parturition in Blarina (Pearson, 1944). Brambell (1956) noticed that corpora lutea in the shrew, stoat, cat (Dawson, 1941, 1946), and in man began regression during gestation, and exhibited marked shrinkage by parturition.

Mulligan (1942) states that corpora lutea in the dog are retained until the end of gestation and regress after parturition. Corpora lutea regress rapidly in the Rocky Mountain pika (Duke, 1952), and in the badger (Harrison and Neal, 1959; Canivec, 1957, quoted by Harrison, 1962) following parturition. Brambell (1956) said that the corpora lutea in rat, mouse, sow, cow, hedgehog, ferret, spotted hyena (Mathews, 1939) and cat did not regress appreciably before parturition. Regression did follow parturition. In Peromyscus the decrease in size of post-lactational corpus luteum was very gradual (Brown and Conaway, 1964).

Mathews and Harrison (1949) said that in seal the corpus luteum showed signs of regression when the embryo was 70 mm. long. The luteal cells were small and shrunken.

Neal (1957) observed large and highly vacuolated luteal cells (probably of the previous pregnancy) in lactating badgers without blastocysts. On the other hand, in badgers that had recently ovulated but were also lactating and had blastocysts, the luteal cells were finely vacuolated. The corpus luteum of the previous pregnancy retrogresses rapidly

after the post-partum ovulation. The corpora lutea during the delay also show retrogressive signs. In the female otter, two sets of corpora lutea are present, one set of the lactation, which is regressing and the other set of the freshly ovulated corpora lutea (Hamilton and Eadie, 1964). This indicates that the adult female exhibits a post-partum estrus. In the event of post-parturitional pregnancy, corpora lutea of the preceding gestation period degenerate rapidly to corpora albicantia in Peromyscus (Brown and Conaway, 1964).

Hammond and Marshall (1930) noticed that corpora lutea of ferrets did not remain large during suckling, as had been described for the rat (Long and Evans, 1922), but regressed rapidly like those of the rabbit (Hammond and Marshall, 1925). In Peromyscus the lutein cells in early lactation appear normal and secretory (Brown and Conaway, 1964). In badgers, corpora lutea degenerate rapidly during lactation (Harrison and Neal, 1956). After parturition the luteal cells loss is rapid and the connective tissues soon predominate the corpus of the fur seal (Craig, 1964).

Dawson (1941) states that in the cat during post-partum involution, there is a fatty infiltration of the luteal cells, which marks the end of the functional activity period of the gland. The vacuoles in the luteal cells mostly disappear in the corpora lutea of lactation (Dawson,

1946). There is a considerable reconstitution of the cytoplasm, with no reduction in the cell size; whereas in corpora lutea of lactation a gradual reduction in cell size is noticed. Neal and Harrison (1958) said that involutionary changes in the corpora of the badger involved shrinkage, fragmentation and heavy vacuolation of the luteal cells. Nuclei become pycnotic and this is followed by invasion with connective tissue and small round leucocytes.

Brambell (1956) concludes that in many species the retrogressive processes are essentially similar, whether the corpora lutea are of ovulation, pseudopregnancy, pregnancy or lactation. The first stage of retrogression is marked by fatty degeneration in the luteal cells, followed by vacuolation, shrinkage and the disappearance of luteal cells.

Corpus Albicans

The formation of corpus albicans from the corpus luteum has been mentioned by many workers, especially those who have studied the structure of the latter. The systematic studies have been taken up recently by Joel and Foraker (1959, 1960).

Loeb (1911) said that the degeneration of the corpus luteum starts in its periphery and proceeds to the center. The connective tissue in the cortex and the periphery becomes hyaline and forms a relatively prominent part enclosing a small number of vacuolar cells. Long and Evans

(1922) observed an abundance of macrophages in regressing corpora lutea. The breakdown of the corpus luteum in the sow is rapid (Corner, 1921) and as retrogression proceeds the reticulum gradually becomes more dense and thick until it gains an appearance like that of the collagenous tissue, which is characteristic of the corpus albicans. In the opossum the corpus is recognized as a small fibrosed remnant for three months (Martinez, 1942).

Deanesly (1935) said that the corpora lutea of ovulation or pseudopregnancy underwent early regression and had disappeared by the time of the succeeding ovulation in the stoat. The corpora lutea of gestation begin to retrogress soon after mid-pregnancy. The corpora lutea regress very rapidly in the weasels (Deanesly, 1944). Leach (1960) said that the three days post partum ovary showed luteal degeneration in the striped skunk. In the 56 days post partum ovary the scar tissue of the corpus albicans was well developed and was being incorporated into the stroma of the ovary. In beavers, the shape and size of the corpora albicantia vary greatly; however, they persist at least until the next breeding season (Provost, 1962).

Eckstein (1962) concludes that as a rule corpora albicantia persist histologically for only a few cycles, but in whales they remain recognizable for many years (Mackintosh, 1946; Harrison, 1949).

Dawson (1946) said that the luteal cells are reduced by a diffuse but progressive cytolysis, which is usually not accompanied by an invasion of the leucocytes or tissue macrophages. The advential cells associated with the luteal blood vessels, gradually infiltrate the luteal parenchyma to replace the degenerating luteal cells.

Brambell (1956) observed that the disappearance of the luteal cells took place gradually. Polymorphonuclear leucocytes are abundant in the regressing corpora lutea. The corpus fibrosum gradually becomes merged with the surrounding tissues of the stroma, but it remains distinct for a very long time before it finally disappears.

Craig (1964) said that the luteal degeneration in the fur seal takes two forms; namely, vacuolization and pyknosis. Vacuolization is a characteristic sign of luteal degeneration and the nuclei in the luteal cells may remain healthy for some time. Pyknosis is characterized by degeneration of the nuclei to a hyperchromatic mass. The final degeneration of the luteal cells result in their resorption and their replacement by proliferation of vascular and connective tissue net work surrounding each cell. The connective tissue elements of the peripheral margin proliferate and penetrate into the body of the corpus luteum. The corpus luteum is now termed an "amorphous" corpus albicans. The fibroblastic tissue eventually consolidates around the core

to form the characteristic "stellate" or "straight scar" corpus albicans. This can be seen in the ovary for two to four years.

Mossman and Judas (1949) said that the corpora albicantia are usually located deep in the ovary with yellow to brown pigments, fibrous connective tissue, a glandular adreno-cortex-like interstitial tissue with reticulum, hyalinized vessels and connective tissue. Fibroblasts are more numerous in the younger corpora albicantia.

Joel and Foraker (1959) observed the same number of corpora albicantia in the pre and post menopausal ovaries, where no quantitative difference was found. During the ovulatory period of 25 years in humans the ovary should be packed with corpora albicantia. Since this does not occur it appears that there are two possibilities concerning the apparent disappearance of many corpora albicantia during the productive life span of the ovary in average women, i.e. each corpus luteum does not form a corpus albicans or the corpus albicans undergo a form of resorption. The available literature contains no reference to the disappearance of the corpus luteum without the formation of corpus albicans. It seems that the resorption of the corpus albicans somehow stops at or near the menopause period.

Joel and Foraker (1960) sometimes had difficulty in differentiating between the corpus albicans and the hyalinized

form of the atretic follicle and the corpus fibrosum. In the pre-menopausal ovary, fragmentation of the corpus albicans, fibroblastic activity and the blending of the corpus at the periphery were observed. There is complete replacement of the corpus albicans by the fibroblasts. They do not appear to be a static structure and hence are assimilated in the stroma. In the post menopausal ovary the corpus albicans is well circumscribed in relation to the surrounding stroma, although fragmented and hyalinized. The absence of fibroblastic activity has been noticed in the corpus albicans and hence they are not assimilated.

It seems that at or near the menopause, the stimulus for the corpus albicans replacement by fibroblasts begin to decrease, and eventually ceases. Ringrose (1962) said that the abilities of the ovary to resorb corpora albicantia decrease with age.

The Polyovular Follicles

Leach (1960) in his thesis entitled "Origin and Fate of the Polyovular Follicles in the Striped Skunk (*Memphitis memphitis*)" extensively reviewed the literature on this topic. In this chapter, it is intended to review the abnormalities in the follicles.

Arnold (1912), Mainland (1928), Hartman (1926), Brambell (1956) and Leach (1960) reviewed the literature on

the polynuclear oocytes and the polyovular follicles. Since the first report on the abnormalities in ova by Von Baer (1827, quoted by Mainland, 1928) and the latest by Leach and Conaway (1963), these abnormalities fall into one of the three categories as mentioned below.

The Polynuclear Oocytes

The polynuclear oocytes have been described in man, monkey, lemur, dog, cat, pig, rabbit, mouse, guinea pig, and opossum (Hartman, 1926; Engle, 1927; Harrison, 1948, 1949). Brambell (1956) said that the binuclear oocytes are more frequent than those with more nuclei, yet numbers up to 16 have been recorded for an ovum.

The Polyovular Follicles

The polyovular follicles have been described in man, monkey, lemur, dog, ferret, pig, rabbit, mouse, opossum and mink (Hartman, 1926; Engle, 1927; Mainland, 1928; Evans and Swezy, 1931; Ota, 1934; Dawson, 1951; Harrison, 1948, 1949; and Enders, 1952).

The occurrence of biovular follicle is by far the most common in mammals (Hartman, 1926; Mainland, 1928; Enders, 1952). O'Donoghue (1912) observed a ripe follicle containing more than one ovum. As many as 7 eggs arranged in a row occur in opossum, which is unique in the abundance of

polynuclear ova and polynuclear follicles. Bouin and Bouin (1900) observed as many as 10 eggs in a single follicle of the dog's ovary. Mainland (1928) said that in ferrets the pluriovular follicles were much more frequent in immature animals than in adults. He found 14 eggs in a pluriovular follicle of a ferret, but did not find mature pluriovular follicles.

The Anovular Follicles

The anovular follicles have been described in the ovaries of bats (Van Beneden, 1880, quoted by Mainland, 1928), opossum, dog, monkey (League and Hartman, 1925), and skunk (Leach and Conaway, 1963). League and Hartman (1925) said that these always degenerate. Leach and Conaway (1963) state that the ova may degenerate without the degeneration of the follicular granulosa cells, which remain as anovular medullary cords. When the entire follicle degenerates which is the usual case in skunk, the thecal interstitial gland is formed and it contributes to the mass of the medullary interstitial tissue.

Interstitial Tissue

The literature on the interstitial tissue in mammalia is extensive and has been reviewed several times (Popoff, 1911; Rasmussen, 1918; Kingsbury, 1914; Athias, 1920;

Wilkerson, 1926; Corner, 1932; Kingsbury, 1939 and Brambell, 1956).

Plufger (1863, quoted by Wilkerson, 1926) said that these cells in the cat ovary contained fine lipoid granules. His (1865, quoted by Wilkerson, 1926) applied the term 'Kornzellen' to the lipoid cells. Tourneaux (1879, quoted by Wilkerson, 1926) compared these cells with the interstitial cells of the testis. MacLeod (1880, quoted by Wilkerson, 1926) used the term interstitial cells for the lipoid cells. Bouin (1900, quoted by Kingsbury, 1939) introduced the term 'interstitial gland' finally embracing under that term the large cells which are characterized by the rich lipoid contents.

Morphology of the Interstitial Tissue

Wilkerson (1926) states that in rabbit, mouse, and rat the interstitial cells are polyhedral with clear vesicular nuclei and they are not believed to be the gland of internal secretion. Hamilton and Gould (1940) noticed that the resting ovary of ferret contains many interstitial cells, which are polygonal and finely vacuolated with moderately large nuclei and prominent nucleoli. The interstitial cells in the guinea pig are polyhedral, but sometimes spindle shaped with large vesicular nuclei (Stafford and Mossman, 1945). The interstitial cells in the dog are spindle shaped

(Mulligan, 1942). Athias (1920) described the structure of interstitial cells of a bat. Neal and Harrison (1958) said that in the badger the interstitial cells are polyhedral.

Brambell (1956) said that mammalian ovarian interstitial cells are large polyhedral or globular elements which possess the cytological characteristics of glandular cells. There is a relationship between the theca interna of the atretic follicles and interstitial cells. Both systems to some extent are functionally interchangeable.

The location and the arrangement of the interstitial cells vary greatly in different animals. Robinson (1918) states that the interstitial cells form the main mass of the cortex of the ferret and ferret-polecat's ovary. They are divided into columns and nests by the lamellae of spindle cells of the tunica albuginea. Hammond and Marshall (1930) noticed large amounts of interstitial cells in the ferret, but not as much as in the rabbit. The mink has a large amount of interstitial cells in the cortex of the ovary (Hansson, 1947), which is also true in the weasel (Deanesly, 1944), fisher (Eadie and Hamilton, 1958), and badger (Hamlett, 1932; Harrison and Neal, 1956). The badger's cortical region is invaded by numerous communicating epithelial tubes. The fetuses of elephant seals have large amounts of interstitial cells. The interstitial cells are well developed in the wolverine cortical region (Wright and

Rausch, 1955).

Mossman (1937) described four types of the interstitial tissues in the pocket gopher's ovary.

1. Granular type, where the interstitial cells are small and irregular.

2. Large irregular vacuolated cells, often shrunken and have pycnotic nuclei, probably degenerating cells.

3. Mature cells, which are large vesicular with definite cell membrane and large vacuoles.

4. Orange 'G' cells which are of uncertain relation to the interstitial cells, less than 1% in amount. They are most numerous near the estrus and pregnancy of the animal.

Stafford and Mossman (1945) state that the interstitial cells in the guinea pig are grouped into discrete clumps or masses in the cortical region of the ovary, usually surrounding ova and the remnants of the involuting follicles. They are most numerous around the smaller arterioles and capillaries.

Cyclic Variations in the Interstitial Cells

Asami (1920) said that the ovaries of 8-week-old rabbits contained medium sized follicles, but no interstitial glands. The primary interstitial tissue occupies most of the interfollicular areas in the juvenile rat (Dawson and McCabe, 1951).

Rasmussen (1918) reviewed the cyclic changes of the interstitial cells of the ovary and testis. In the woodchuck, which is a seasonal breeder, the interstitial cells are maximum at the time of ovulation and beginning of the pregnancy. This is also true in the European mole, certain bats, hedgehog, badger, long tailed weasel, Canada porcupine, grey squirrel, fox squirrel, and pocket gopher (Stafford and Mossman, 1945). Brambell (1956) said that the increase in number and the hypertrophy of the interstitial cells during the latter part of the pregnancy has been described frequently such as in bats (Athias, 1920) and water shrew (Price, 1953). This increase is associated with the increased follicular atresia, which commonly occur during the second half of the gestation. These cells may assume the appearance of the luteal cells. Wilkerson (1926) states that the interstitial cells in the woodchuck disappear annually. Interstitial cells are abundant at the beginning of sexual maturity in rat and mouse.

Kingsbury (1914) said that in cat the development of the interstitial tissue was correlated with the activity of the follicle cells in the absence of germ cells. Cole et al. (1933) observed that the interstitial cell mass reached its maximum size after the gonad stimulating hormone is no longer demonstrable in the maternal bloodstream of the horse. The theca interna and interstitial

cells may constitute the 'Oestrogen producing cell system of the ovary', (Falack, 1959, quoted by Eckstein, 1962). Falack also indicated that the cholesterol content of both the theca interna and interstitial cells is gonadotrophically regulated and appears to be correlated with the secretion of the estrogen. Leach (1960) said that in the striped skunk the interstitial tissue development showed a cyclic response, which is positively correlated with the follicle growth. Mossman (1937) said that the interstitial tissue has unknown function. Claesson and Hillarp (1947) said that the interstitial cells are concerned with estrogen secretion. The interstitial cells and the theca interna may function independently.

Mulligan (1942) said that the interstitial cells in the dog are prominent during anestrus. The resting ovary of the ferret has well developed interstitial cells (Hamilton and Gould, 1940). Deanesly (1935) states that the interstitial cells in stoat reach their maximum growth in the post-lactation period. It seems that they shrink and degenerate in large numbers just before the breeding season and during the pregnancy. During lactation in wolverines the interstitial cells form the main mass in the ovaries, otherwise they are considerably regressed (Wright and Rausch, 1955).

Robinson (1918) states that the animals which ovulate

following insemination such as cat and ferret, the interstitial tissue forms a preponderant part of the cortex. In the spontaneous ovulators such as human, monkeys, mares, asses, pigs and Dasyurus, the interstitial tissue is either absent or present in small quantities and is not glandular. Patzelt (1955, quoted by Harrison, 1962) maintains that wild carnivores (wild cat, fox) exhibit more extensive interstitial cell development than do the domestic animals. Holmstrom (1920, quoted by Hansson, 1947) said that the interstitial tissue is present chiefly in the lower forms of mammals, which have non-periodic ovulation. Rasmussen (1918) indicated that the review of earlier literature showed that hypertrophy of the interstitial cells during rut and pregnancy was common.

Rasmussen (1918) said that regression of interstitial cells was effected by a decrease in size and number of the cells. The interstitial cells undergo changes in size and in cytological characteristics during the estrous cycle and pregnancy (Harrison, 1962). These changes are such as to suggest that they are at least, at some time, secretory elements. Harrison and Neal (1956) found that the cortical interstitial tissues pass through a distinct series of cytological changes during the unimplanted period in the badger. Histochemical techniques suggest that they are active during this period.

Mossman (1937) said that the interstitial cells have no obvious cyclic variation in the pocket gopher. There is no degenerative phenomenon seen in the interstitial cells, during any phase of the ovarian cycle of the guinea pig (Stafford and Mossman, 1945). Mossman and Judas (1949) observed no cyclic variation in the interstitial cells of the porcupine. Harrison (1962) said that the cyclic nature of the interstitial cells is not yet fully known.

Epithelial Origin of the Ovarian Interstitial Cells

Among the many workers, who have supported the epithelial origin of the interstitial cells are Schron, Janosik, Popoff, Kohn (1862, 1887, 1911, 1926, all quoted by Brambell, 1956) and Rasmussen (1918). Brambell (1956) has given a detailed review of the literature on this aspect. Rasmussen (1918) states that in the woodchuck the interstitial cells, which disappear annually, were replaced from the germinal epithelium as well as from the stroma (directly or indirectly from the theca interna).

Medullary Cord Origin of the Interstitial Cells

There are relatively few, who have supported the medullary cord origin of the interstitial cells (Popoff, 1911, quoted from Brambell, 1956; Kingsbury, 1914; Vellose de Pinho, 1925). Kingsbury (1914) states that in the immature and the newborn cat, the interstitial cells appear

to be associated with medullary cords. In the 11-month-old badger the medullary interstitial cells contain numerous vacuoles (Neal and Harrison, 1958).

Stafford and Mossman (1945) state that the medullary interstitial cells are derived from the cortical interstitial masses, which migrate to the medulla. In the wolverine the medullary interstitial cells are well developed in October (Wright and Rausch, 1955). Leach and Conaway (1963) said that the theca interna from atretic medullary polyovular follicles and the theca interstitial gland of medullary and cortical follicles eventually contributed to the medullary interstitial tissue in the striped skunk.

Dual Origin of the Interstitial Cells

Rasmussen (1918) said that the interstitial cells have a dual origin, i.e., the germinal epithelium and the stroma (theca interna). Dawson and McCabe (1951) found that the primary interstitial tissue in neonatal and juvenile animals was germinal epithelial in origin and it was replaced by the secondary interstitial cells derived from the theca interna. Rennels (1951) concluded on the basis of histochemical characters that in rat the interstitial cells have a dual origin. The primary interstitial cells of the juvenile ovary are formed from the epithelial cells of the cortical ingrowths or from the granulosa cells of the follicles.

The secondary interstitial cells formed later are derived from the theca interna cells of the atretic follicles.

The Connective Tissue Origin of the Interstitial Cells

This theory has received wider support both from the earlier and recent workers (Pflugor, 1863; His, 1863; Van Beneden, 1880; Rabl, 1898; Limon, 1902; Bouin and Ancel, 1909, all quoted by Brambell, 1956; O'Donoghue, 1916; Athias, 1920; Long and Evans, 1922; Deanesly, 1935; Mossman, 1946; and Wright and Rausch, 1955).

Athias (1920) studied the interstitial cells in several species of bats of the age ranging from embryos to parous adults killed at all seasons of the year. He concluded that in the young animal the interstitial cells were derived entirely from the connective tissue elements of the stroma, but in the older animals they were derived mainly from the theca interna cells of the atretic follicles, although some were derived from the stromal elements. The interstitial cells are modified stroma cells and hence are of the connective tissue origin (Kingsbury, 1914, 1939).

Mossman (1937) said that the interstitial cells originated from the theca interna of the atretic and ruptured follicles. In the pocket gopher some of thecal cells of normal follicles transform into interstitial cells. Wilkerson (1926) noticed that the spindle shaped cells of theca in the rabbit, mouse

and rat transformed into interstitial cells. Most of the interstitial gland tissues are derived from the degenerating follicle of the theca interna of the porcupine ovary (Mossman and Judas, 1949). The interstitial tissues in the stoat (Deanesly, 1935) and wolverine (Wright and Rausch, 1955) are developed from the theca of the atretic follicles. Mossman (1946) concluded that the interstitial gland develops in most mammals from the theca interna of atretic follicles.

Wilkerson (1926) agreed with Saintmont (1905) and Kingsbury (1914) that the interstitial cells revert to their original stroma cells apparently by the process of dedifferentiation. They also noticed intermediate stages of dedifferentiation. Kingsbury (1939) suggested that there was a phenomenon of degeneration and regeneration in the interstitial cells. Stafford and Mossman (1945) said that occasionally the interstitial cells may revert to fibroblasts. They state that the cells forming theca interna of developing follicles arise from the adjacent stroma and they might transform into interstitial cells. The interstitial cells might possess the capacity of reverting again to the connective tissue type. The process is apparently not of degeneration or regeneration as suggested by Kingsbury (1939).

Thecal Gland

Robinson (1918) said that the theca interna in the ferret was interspersed with blood vessels and interstitial cells. The theca externa was less vascular and composed of dense compact connective tissue and fewer interstitial cells. The normal theca is formed from the stromal cells of the rat, mouse, and rabbit (Wilkerson, 1926). The theca and membrana granulosa cells are separated by a basement membrane, which can be seen distinctly in some cases. The theca mostly forms the interstitial cells. Warbritton (1934) states that the theca interna cells in the ewe temporarily take part in the formation of the corpus luteum and later it degenerates. Corner (1932) said that the theca lutein cells could be found first around the periphery and later in the corpus luteum, where they could be distinguished by their smaller size from the true luteal cells. They persist in the regressing corpus luteum after the true luteal cells have disappeared. He found no evidence that the cells of the theca interna are ever converted into fibroblasts or that they lay down the fibrils of the connective tissue reticulum.

Mossman (1937) considered that the hypertrophied theca interna in the pocket gopher at ovulation resembled an endocrine gland. He called it "thecal gland." It begins to degenerate rapidly following ovulation and by the time

the corpus luteum is formed, it is almost gone. Some of its cells differentiate into the interstitial cell clumps of the stroma. By the time the embryos begin to implant in the uterus only nonfunctional thecal gland is left in the ovary. The fully developed theca interna or thecal gland of both the large normal and atretic follicles is highly vascular. The thick zone of the epitheloid polyhedral gland cells is separated from the granulosa cells by a delicate membrane. At the time of estrus the thecal gland tissues surround both large normal and atretic follicles. This suggests that the estrous hormone is produced here, rather than in a non-glandular follicular epithelium.. The thecal gland never remains in the confines of the corpus luteum proper or form any of its elements.

Harrison (1948) observed the thecal gland in the goat as described by Mossman (1937). He could not differentiate it from the corpus luteum after 15 days of the ovulation. It probably degenerates. The ripe follicles of the porcupine possess a thin but definite thecal gland (Mossman and Judas, 1949). Dawson and Friedgood (1940) said that the theca interna cells are hypertrophied and may be in patches around the periphery of the follicle.

Duke (1952) said that the theca interna is poorly developed in lagomorpha. He also noted the presence of adreno-cortex-like tissue in association with the parts

of the old mesonephric duct. Stafford *et al.* (1942) state that there is no evidence of thecal gland formation around the atretic follicles of the guinea pig.

White *et al.* (1951) distinguished in the human theca interna, a second type of cell in the maturing follicles. This cell has a small, dense, irregular, hyperchromatic nucleus and strikingly eosinophilic cytoplasm. They called it the "K" cell and thought that it secretes progestins.

Strassman, (1941) and Harrison (1948) state that the theca interna shows a hypertrophied region in the form of a cone projecting towards the ovarian surface. The cone may play a part as a 'pathmaker' for the ascent of the growing follicle to the surface of the ovary. Brambell (1956) said that the theca interna also thins or disappears at the site of impending rupture in mature follicles and may also be hypertrophied in the region of cumulus.

Harrison (1962) concludes that in late maturing follicles the theca interna cells rapidly enlarge, become highly vascularized and assume a polygonal shape with vacuolated cytoplasm and vesicular nuclei. The degree of development of the theca interna varies in different placental mammals, but it is always maximal just before ovulation.

The role of the theca interna in the formation of various components of the corpus luteum has been described in the chapter of the corpus luteum. The recent works

indicate that the theca interna cells of the maturing follicles are glandular tissues and they are involved in the production of estrogen (Mossman, 1937; Corner, 1938; Dempsey and Bassett, 1943; Deane, 1952; and Nishizuka, 1954, quoted by Harrison, 1962).

Granulosa Cells

The membrana granulosa cells of the multilayered follicles are small, polygonal or cuboidal with granular cytoplasm and densely stained nuclei (Harrison, 1962). Robinson (1918) observed mitosis frequently during follicular growth. Lane and Davis (1939) supported Robinson.

Pearson and Enders (1943), Evans and Cole (1931), and Mulligan (1942) observed marked foldings in the granulosa layer of the mature follicles. The folds of granulosa invaginate into the antrum and contain connective tissue and blood vessels from the thecal plexus. Deane (1952) described the growth changes in the granulosa of rats. He also gave the cytological details of the granulosa cells. Knigge and Loathem (1956) described the growth changes in hamsters.

The Bursa Ovarii

Agduhr (1927), Alden (1942), Enders (1952), and Franchi et al. (1962) reviewed the literature on the bursa ovarii.

In some mammals ovaries may be enclosed in a peritoneal capsule (the ovarian bursa) which may or may not communicate with the coelomic cavity, through a slit.

Trevernius (1824, quoted by Enders, 1952), Weber (1826, quoted by Enders, 1952) and Hartman (1932) said that the ovaries in Mustelidae are enclosed in an ovarian bursa. Weber (1826, quoted from Robinson, 1887) described the ovarian bursa completely shut off from the abdominal cavity in Lutra vulgaris and Mustela putorius. The tube and the cornu uteri did not open directly into the bursa as shown by Treviranus (1824, quoted by Enders, 1952) in Mustela foina. The ovarian bursa when it completely encloses the ovary prevents loss of ova into the peritoneal cavity and insures their rapid passage through the oviduct (Robinson, 1887). Sobotta (quoted by Hartman, 1939) said that in laboratory rodents the fluid in the ovarian capsule served to carry the ova into the infundibulum. Fischel (1914, quoted by Alden, 1942) states that the contraction of the smooth muscle present in the capsule compresses the periovarial fluid and thus aid in the transfer of the ova to the oviduct. Westman (1926) concluded that the presence of the bursa enclosing the ovary was not essential for the transit of the ovum in rabbit.

Gerhardt (1904, quoted by Kellogg, 1941) said that the bursa ovarica in weasels, wolves and shrews are incomplete.

Robinson (1918) noted the presence of an ostium in the bursa ovarii of the ferret. Hansson (1947) and Enders (1952) observed the ostium in mink. Alden (1942) said that the rat has an incompletely closed bursa. The slit-like opening in bursa is located antimesometrially near the uterine cornua. This slit is closed periodically during the estrous cycle by the fimbrae of the oviduct. Kellogg (1941) states that in dog, bear, sea lion, and raccoon a small opening exists in the wall of the sac. In a large number of animals such as the cat, hyena, guinea pig, mole, pig, a wide open capsule is present. It is totally absent in whales. Porcupine has no ovarian bursa (Mossman and Judas, 1949).

The periovarian space is closed in species like the weasel, otter, and shrew, but communicates with the peritoneal cavity by a narrow slit or ostium (Harrison, 1962).

Hammond and Walton (1934) said that the amount of the fluid varied in the capsule of the ferret. This is also true in mink (Enders, 1952). Enders (1952) said that when fluid is injected into the bursa, it does not escape into the peritoneal cavity regardless of the stage of the cycle because the opening of the bursa is blocked by the long fimbriae. During or following the copulation the bursa fills slowly with fluid until the capsule is greatly distended.

Westman (1926) observed a negative correlation between

the development of the bursa ovarii and development of the fimbriae of the oviduct. The animals with well developed bursa ovarii, i.e., mink, mice, dogs, rats, guinea pigs, and rabbits, have slightly developed fimbriae, while animals with slightly developed bursa ovarii such as apes, cows, mares, and sows, have well developed fimbriae (Hansson, 1947).

Agduhr (1927) said that in mouse the thickness of the wall of bursa ovarii varied at different places. Dorsally and caudally it is thicker and muscular. The histological structure of the bursal wall shows changes connected with the activity of the genital apparatus. This has also been noticed in rat (Kellogg, 1941) and mink (Enders, 1952). Kellogg (1941) said that the periovarial sac is a double layer of mesothelium completely surrounding the ovary and contain within its cavity the opening of the oviduct. Enders (1952) said that the periovarial sac in mink consists of thicker inner and thinner outer layer of the mesothelium and a layer of connective tissue. Both layers are separated at every place except at the hilus. The adipose tissue around the hilus of the bursa is maximum in the fall and minimum during estrus.

The Oviduct

Snyder (1924), Agduhr (1927), Kellogg (1941), Alden (1942) and Brambell (1956) reviewed the literature. The

epithelial mucosa of the oviduct shows cyclic changes which are correlated with the sexual cycle (Schaffer, 1908; Katz, 1911; Troschner, 1917, all quoted by Snyder, 1924; Moreaux, 1909, 1913). These changes have been reported in a wide variety of animals such as the rabbit, guinea pig, mouse (Allen, 1922), mink (Hansson, 1947; Enders, 1952), rat (Long and Evans, 1922), mouse (Agduhr, 1927; Espinasse, 1935), sow (Snyder, 1923), man (Snyder, 1924), stoat (Deanesly, 1935), and weasel (Deanesly, 1944).

Snyder (1923) said that the periodic changes in the oviduct of the sow involve the height of the epithelium, the nucleus and the fluid content of the stroma. Pregnancy inhibits cyclic alternations in the tubes of sow and man (Snyder, 1924).

Active cilia were seen at all stages of the cycle. The cyclic variation of the oviducts in man is closely associated (during the menstrual cycle) with the alteration in the endometrium (Snyder, 1924). The fallopian tube enlarges in the weasels as estrus approaches and gradually regresses after ovulation (Deanesly, 1944). This is also true in the stoat (Deanesly, 1935). Hafez (1963) states that the mammalian uterine tube undergoes profound changes in histology and secretory activity during the different phases of the reproductive cycle.

Marshall (1904) said that the oviduct in the ferret

is a slender and much coiled structure which opens into the bursa by fimbriae. Long and Evans (1922) noticed 8 to 10 folds in the rat's oviduct. The oviduct enters the bursa near the caudal end, while the opening of the oviduct into the uterine cornu of the mouse corresponds to that of the cat, dog, and horse among the domestic animals (Agduhr, 1927). The oviduct is not coiled in the stoat (Deanesly, 1935).

Hansson (1947) described the oviduct in the mink. The oviduct runs around the ventral surface of the ovary in the cranio-medio-caudal direction and during tis course forms 10-11 regular coils. The infundibulum thus comes to lie in the immediate proximity of, but medial to, the utero-tubal junction. Enders (1952) said that the oviduct passes along the lateral aspect to the anterior end and then to the medial side around the periovarial sac and through the inner mesothelial layer. It opens into the bursa by an ostium at the posterior pole of the ovary. Fimbriae are well developed at estrus.

Sobotta (1891, quoted by Agduhr, 1927) divided the oviduct of the rat into 4 subdivisions, but Agduhr (1927) described 7 subdivisions on the basis of the lumen, folds of the mucous membrane and the condition of the epithelium. Variations of the oviduct are partly due to variations in the structure and partly due to the functional condition of the oviduct.

Espinasse (1935) subdivided the oviduct of the mouse into three parts, i.e., the fimbriated funnel, the ampulla with ciliated epithelial cells (which show nuclear displacement), and the isthmus, which has non-ciliated secretory cells without any sign of the nuclear displacement. Besides the ciliated and non-ciliated cells thin dense cells, so-called "Peg Cells" are found between the lighter ones. The non-ciliated cells probably form the majority of the so-called "Peg Cells" and show a phenomenon of nuclear displacement. Frommel (1896, quoted by Snyder, 1924) said that the fallopian tubes are lined with a single layered epithelium consisting of ciliated cells and non-ciliated cells. Cage (1904, quoted by Alden, 1942) reported that the ciliated cells are numerous in the infundibular and ampullar region of the oviducts, while in other parts there is columnar epithelium.

Alden (1942) subdivided the oviduct of rat into 4 parts such as the infundibulum with its fimbriae, the dilated ampulla, the narrow isthmus, and the intramural portion of the isthmus as described for larger mammals by Huber (1915). He could not demonstrate clearly cyclic changes in the histological picture of the oviduct. The histological structure of the oviduct is like that of the mouse (Agduhr, 1927).

Hansson (1947) noticed columnar cells of the oviduct

which have basophilic nuclei during the anestrus period of the mink. The lumen of the oviduct is completely filled with secretions during proestrus and estrus. Following ovulation decreased secretion was noticed.

Bloom and Fawcett (1962) described the structure of the mammalian oviduct. The wall of the oviduct consists of a mucous membrane, a muscular layer and external serous coat. The mucous membrane in the ampulla is thick and forms highly branched folds. The epithelium is a simple columnar type, which may appear pseudostratified. The lamina propria has thin fibers and numerous fusiform or angular cells. There is no true muscularis mucosa.

The Tubo-Uterine Junction

Anderson (1928) and Lee (1928) studied the tubo-uterine junction in mammals.

Bischoff (1852, quoted by Lee, 1928) described the gradual transition of tube and uterus in the guinea pig. He did not note any mucosal fold at this point. Valve-like folds guard the tubo-uterine junction in the mouse (Allen, 1922). Numerous folds were observed in the guinea pig by Kelly (1927). Lee (1928) observed numerous polyp-like folds at the tubal ostium of the rabbit. The tube joined the cornua of the uterus obliquely in the non-pregnant cat, dog, mouse and rabbit whereas in the rat and guinea pig the tube

joined at the apex of the cornua. The histological structure showed a transition from the tube to the uterine horn. Uterine glands are absent at the junction.

Anderson (1928) said that the tube in the cat opened into the uterus through a low papilla formed of mucosa, which protrudes into the uterine cavity. No villi are present. The presence of a special thickening at the junction was noted in the cat, like that of the non-pregnant guinea pig, cat, dog and lion (Lee, 1928). At this point the circular muscle layers of the tube and the uterus were continuous. In sheep and cows the tube opening is protected neither by the mucosal fold nor by the sphincter. It is protected in other animals either by the sphincter or by villi or by mucosal folds of some sort. The tube in the cat, hedgehog and mink (Hansson, 1947) opened at the end of a papilla.

Enders (1952) states that the tubal ostium in the mink is circumscribed by a simple fold, which projects into a pocket formed in the uterine wall. The tubal projection lies within this pocket so that no elevation within the uterus is formed. The muscles are not well developed at the junction and the opening is not guarded by the utero-tubal sphincter. Contrary to this Hansson (1947) stated that the tube opens on a papilla. Hafez (1963) said that the increase in the degree of the flexure of the utero-tubal

junction (during the follicular phase) is brought about by edema in the horn's wall.

Anderson (1928) recognised three distinct anatomical patterns in the utero-tubal junction of mammals.

1. The first is characterized by tortuous isthmus and wide lumen, found only in Marsupials.

2. All the Monodelphia with bicornuate uteri have an isthmus part either straight or tortuous with thick muscular walls and a narrow lumen.

3. In all mammals with the simplex type uterus the tube joins the uterine cavity.

The Cervix

The literature on the structure of the cervix and the corpus uterus of the family Mustelidae is scanty.

Tatarian (1962) traced the historical aspects of the cervix dating back to the 16th B.C. in the "Papyrus Ebers".

Hansson (1947) states that in mink the cervical canal opens into the vagina on a somewhat dorsally projecting papilla. Harrison et al. (1952) said that the cervix in the seal projects on a prominent dome. In the monkey the dorsal fornix of the cervix is deeper than the ventral while the external orifice of the cervix is oblique in relation to the axis of the vagina (Sandys and Zuckerman, 1938). Enders (1952) said that in mink the external orifice of the cervix is a transverse slit, where the ventral fornix is far deeper

than the dorsal fornix. A transverse fold (which is small in anestrus and enlarged in estrus) of the vaginal wall covers much of the dorsal lip. The cervical canal is straight and simple. During estrus and pregnancy both the dorsal and ventral fornices deepen and the infra-vaginal protruding segment of the cervix is greatly flexed, so that the canal is bent at right angles to the antero-posterior axis, and the opening of the cervix is directed ventrally instead of posteriorly. At parturition the cervical canal is once again straight.

Corner (1921) observed mitotic figures in the cervix during estrus of sows. Grant (1934) described the histological changes occurring in the epithelium of the ewe. Deanesly (1935) states that the cervical canal in the stoat is lined by the uterine endometrium almost to the vaginal opening. Hamilton and Gould (1940) noticed cyclic variation in the structure of the cervix. During anestrus the epithelium in the cervix is low cubical. This becomes columnar and later pseudo-stratified until it is about 6-8 layers in thickness during the estrus period of the ferret.

Hamilton and Gould (1940) state that the lumen of the fused horns is continued into the cervix, which is 'multi-diverticular' and invaginated into the upper part of the vagina. Pearson (1952) said that the lumina of the two horns of the sea otter are separated by a membrane, so that

there is a common uterine and cervical canal only for 20 mm. The fusion of the horns to the tip of the cervix measured about 85 mm.

The Uterus

Eckstein and Zuckerman (1956) said that the uterine changes which occur during the oestrous cycle vary from species to species. In general there is a phase of glandular proliferation during proestrus and estrus, when the Graafian follicles are maturing; and a phase of secretory differentiation during metestrus (or pseudopregnancy), when the corpora lutea are present.

The cyclic changes in the uterus have been observed in a wide variety of animals such as the dog (Friedlander, 1870 quoted by Eckstein and Zuckerman, 1956; Marshall and Jolly, 1906; Retterer, 1892; Evans and Cole, 1931 and Mulligan, 1942), in the stoat (Deanesly, 1935), in the weasel (Deanesly, 1944), in the ferret (Marshall, 1904; Hammond and Marshall, 1930; Hill and Parkes, 1933; and Hamilton and Gould, 1940), in the cat (Dawson and Kosters, 1944), in the mink (Hansson, 1947), in the sow (Corner, 1921), in the rat (Long and Evans, 1922), in the guinea pig (Stockard and Papanicolaou, 1917; Loeb, 1914), in whales (Mathews, 1948), in the seal (Harrison et al., 1952) and the badger (Harrison and Neal, 1956).

Structure of the Uterus During the Estrous Cycle

Marshall (1904) observed four phases in the uterus of the ferret such as the period of rest (anestrus), the period of growth (proestrus), the period of degeneration (proestrus) and the period of regeneration (estrus). The uterine structures during the above phases as well as following parturition and during lactation will be discussed in the present review with special reference to the Mustelidae.

The Period of Rest (Anestrus)

Marshall (1904) said that the cuboidal epithelium of the ferret's uterus during anestrus is composed of a single row with numerous glands. In dogs the immature and anestrus uteri have a similar structure (Evans and Cole, 1931). The epithelium is thin and almost devoid of glands (Hill and Parkes, 1933). This is also true in the ferret (Hammond and Marshall, 1930). Hamilton and Gould (1940) state that the epithelium in the ferret is low cuboidal with large nuclei and there are short glands which are widely separated. The submucosa is not oedematous and it is thin. In the cat (Dawson and Kosters, 1944) the anestrus uterus is like that of the ferret. Mulligan (1942) said that the endometrium in the dog is relatively conspicuous. There is a columnar epithelium and macrophages are present. The uterus in the badger (Neal and Harrison, 1958) is like that of the ferret

(Marshall, 1904). In the fur seal the muscle layers are thin and are composed of sparse cytoplasm (Craig, 1964). Vessels are regressed. The mucosal epithelium is inactive and glands are straight.

The Period of Growth (Proestrus)

Marshall (1904) observed mitotic figures in the nuclei of stromal cells of the ferret's uterus during proestrus yet it had no marked changes in the epithelial lining when compared to that of the anestrus animal. It is similar in the case of dogs (Evans and Cole, 1931) and cats (Dawson and Kosters, 1944). Hamilton and Gould (1940) noticed low columnar epithelium, but hypertrophied glands and thick oedematous submucosa in the ferret. In the period of degeneration (Proestrus) as reported by Marshall (1904) there is a slight breakdown of the epithelium and stroma of the ferret.

The Period of Regeneration (Estrus)

Marshall (1904) said that the uterine cavity during estrus is relatively large and has a complete uterine epithelium. Hammond and Marshall (1939) noticed columnar epithelium mucosal folds and an oedematous stroma in the ferret. Hill and Parkes (1933) reported about the well-developed uterine glands and the oedematous stroma of the

ferret. Hamilton and Gould (1940) agreed with Hill and Parkes (1933) and Hammond and Marshall (1940) in regards to the uterine structures.

Retterer (1892) observed the growth of the endometrium in dogs preceeding estrus (Evans and Cole, 1931). This has since been found to be a regular occurrence in all species. Evans and Cole (1931) said that in estrus the dog uterus is like that of the proestrus ferret (Marshall, 1904). They noted mitotic figures in the uterine gland during metestrus. In the cat, estrus is marked by hypertrophy of the epithelium, which may appear pseudostratified (Dawson and Kosters, 1944). The maximum growth of the corpus luteum coincides with the growth of the endometrium preceeding implantation. Neal and Harrison (1958) said that the mucosa in badger is thick during estrus. The uterus during pregnancy and pseudo-pregnancy are alike (Hammond and Marshall, 1930). The glands are straight. The columnar epithelial cells are 'palisade type', with centrally located nuclei. The glands become coiled and secretory at the time of pregnancy in the badger. Hamilton and Eadie (1964) state that the uterine horns in the river otter at the first estrus are 3 mm. in diameter and 50 mm. in length. In the multiparous female, which had recently ovulated, the horns measured 12 mm. in diameter and 90 mm. in length.

Hamlett (1932) said that the uterine mucosa in the badger showed no influence of the corpus luteum during the

unimplanted period. The lumen was expanded at the site of the blastocyst. The uterus has straight glands like that of anestrus during the delay in badgers (Harrison and Neal, 1959). The stroma is cellular and non-oedematous. There is a recession of glands during delayed implantation in seals (Harrison et al., 1952).

Hammond and Marshall (1930) state that the uterus of the lactating ferret is rather long and flattened in outline. In the dog macrophages are most evident during proestrus and following parturition, and least obvious in pregnancy (Mulligan, 1942). The glandular and submucosal changes are like those found in other animals. Neal and Harrison (1958) said that the horns in the post partum badger exhibit swellings at the placental sites.

Deanesly (1935) said that uterine changes in the stoat, during estrous, pregnancy, parous, nonparous, and post-partum stages are like those of the ferret. Generally 4-6 endometrial folds are present. The uterine epithelium in weasels (Deanesly, 1944) resembled that of the stoat and ferret. The glands increase enormously in size and form a labyrinthine complex during implantation in the fur seal (Craig, 1964). Deanesly (1944) divided the uteri in the weasel into parous and non parous groups on the basis of the presence or absence of the fibrosed blood vessels. She then

classified the uteri into anestrus, estrous, pregnant and post partum groups. The uterine changes in the mink (Hansson, 1947) are like those of other mammals. The uterus is true uterus bicornis and it is thread-like in the nonparous anestrus mink (Enders, 1952).

Corner (1921) states that the uterus of sows undergoes regular alternations in the structures. The changes are correlated with the ovarian cycle. This cycle is interrupted only during pregnancy. This phenomenon has also been observed in the badger (Harrison and Neal, 1956).

The Vagina

The cyclic changes in the vaginal epithelium have been observed during the estrous cycle in the guinea pig (Stockard and Papanicolaou, 1917), the rat (Friedlander, 1870, quoted by Eckstein and Zuckerman, 1956; Retterer, 1892; Marshall and Jolly, 1906; Long and Evans, 1922), the dog (Evans and Cole, 1931; Mulligan, 1942), the ferret (Marshall, 1933; Hamilton and Gould, 1940), the stoat (Deanesly, 1935), the mink (Hansson, 1947; Enders, 1952), the cat (Dawson and Kosters, 1944), the weasel (Deanesly, 1944), the badger (Neal and Harrison, 1958; Harrison and Neal, 1959) and the seal (Harrison et al., 1952). The vaginal smear techniques have also demonstrated these cyclic changes in the vaginal epithelium.

Evans and Cole (1931) said that the vaginal epithelium in the dog is low columnar during anestrus. It is high and stratified squamous during proestrus. The epithelium becomes squamous and superficially cornified in estrus. In the post estrus period it is low again like that of anestrus. Mulligan (1942) also observed these changes in the dog. He said that the outer columnar layer of cells during pregnancy contained mucin. The mucin disappears in the post-partum animal. Marshall (1933) also noted these changes in the ferret. The vagina and vulva undergo well marked cyclic changes in the ferret in correlation with those of the ovaries and the uterus. The cyclic changes in the stoat (Deanesly, 1944) and in the mink (Hansson, 1947; Enders, 1952) are like those of the ferret and the dog.

Deanesly (1935) states that the vaginal epithelium in the stoat shows a well defined cyclic change in growth, stratification and cornification before estrus. The vaginal epithelium (during the latter phases of pregnancy) consists of a single row of columnar cells with basal nuclei. The epithelium varies in thickness from the cervix to the vulva. The vagina in the stoat shows marked cornification at estrus (Deanesly, 1944).

Hamilton and Gould (1940) described the histology of the vagina of the ferret. The epithelium in anestrus is stratified and it has a basal zone of columnar cells and a luminal

zone of rounded cells (3-8 layers in thickness) with leucocytes, but no cornification. The submucosa does not show vascularized areolar connective tissue and smooth muscles. During proestrus, there is slight cornification of the epithelium and some increase in the vascularity of the submucosa. In estrus, there is distinct cornification of the epithelium with papilla, which are deep and tooth-like in appearance. The submucosa is oedamatous and contains abundant areolar tissue. They also noted that the ferret came into estrus slowly. Vaginal cornification is more marked at first in the upper part of the vagina and then in the lower part. During lactation and pseudopregnancy the epithelium consists of narrow and low columnar cells. Hansson (1947) and Enders (1952) said that the changes in the vaginal epithelium of mink were like those of the ferret. The proestrus period is long and the changes from anestrus to estrus are slow as in the ferret. Enders (1952) noticed that mink had long, distensible and muscular vaginae. The mucosa is thrown into longitudinal folds which disappear before the cervical segment is reached. Transverse folds become prominent as the longitudinal folds disappear near the cervix. The entire dorsal wall of the vagina is thickened at estrus, and the muscular bands at this time show hypertrophy. The vaginal wall of the river otter is very thick during estrus (Hamilton and Eadie, 1964).

Neal and Harrison (1958) state that the vaginal epithelium in the badger passes through a striking cycle. This is correlated with the ovarian events. The vaginal epithelium of the badger is more sensitive to the prevailing hormonal environment than the uterine mucosa (Harrison and Neal (1959).

The Blastocyst

Ballard (1964) states that in the placental mammals the hollow ball of cells show a thickening at one pole called the inner cell mass from which the later embryo arises. It could perhaps be compared with the blastodisc of the early reptile or bird embryo. The rest of the sphere of cells is a thin layer called trophoblast which soon serves to attach the little embryo to the wall of the uterus and initiates the formation of the placenta. This mammalian stage is called the blastocyst.

Mossman (1937) said that the cleavage up to and including the blastocyst stage is alike in all the Eutherian mammals. The trophoblast regardless of its possible phylogenetic origin, is classed as an extra-embryonic ectodermal layer and the inner cell mass is classed as undifferentiated tissue. The trophoblast is probably the most important single tissue in the placenta of higher mammals (Wimsatt, 1962). The blastocysts of the animals

belonging to the family Mustelidae are reviewed here.

Fries (1880, quoted by Neal and Harrison, 1958) was the first to notice the unimplanted blastocysts in the badger. Hamlett (1932) said that the blastocysts in badger are completely quiescent during the delay which was also supported by Neal and Harrison (1958). This is also true in mink (Enders and Pearson, 1946; Enders, 1952). Notini (1948, quoted by Harrison and Neal, 1956) said that the blastocysts show some evidence of slow but steady increase in size.

Robinson (1904) noticed that the blastocysts in the ferret differentiate into the inner cell mass and the trophoblastic ectoderm, which has polygonal cells with circular nuclei (Robinson, 1893). The zona pellucida is uniformly thin. In the germinal area the nuclei are large and closely packed. The trophoblastic cells are greatly attenuated in the badger (Hamlett, 1932). The inner cell mass is small and undifferentiated into the germ layers. Hamilton (1934) said that the trophoblast in the ferret has a single layer of flattened cells which cover the inner cell mass. The disappearance of the covering trophoblast takes place relatively early in the ferret. The blastocyst in the marten (Marshall and Enders, 1942) is like that of the ferret and badger, but the zona pellucida is conspicuous and collapsed possibly due to the preservation. The blastocysts of the long-tailed weasel, short-tailed weasel and

marten (Wright, 1942), fisher (Enders and Pearson, 1943), wolverine (Wright and Rausch, 1955) and stoat (Deanesly, 1943), are like that of the ferret and badger. Hamilton and Eadie (1964) said that the blastocyst in the otter was semi-transparent and had an inner cell mass and the outer trophoblast.

The size of the blastocysts varies in different animals. It has an average diameter of 378 micra in the long-tailed weasel, 318 micra in the short-tailed weasel and 409 micra in the marten. The zona pellucida is much thicker in the fisher (Enders and Pearson, 1943; Eadie and Hamilton, 1958) than in the weasel.

The Inner Cell Mass

The number of cells in the inner cell mass varies in different animals of the same species and in different species. Cell number depends on the developmental stages of the blastocyst. The cells in the inner cell mass have been reported as from 46 to 55 in the badger (Hamlett, 1932), 798 to 807 in the fisher (Enders and Pearson, 1943), 28 to 164 in the mink (Enders, 1952), 313 to 393 in the marten (Marshall and Enders, 1942) except a few which had 508 to 589 cells, possibly an indication of the active blastocyst, and 147 to 151 in weasels (Wright, 1942). The wolverine blastocyst has more cells in the inner cell mass than the marten.

The Fetus

The literature contains no detailed description of sea otter embryos. Brandt (1865, quoted by Barabash-Nikiforov, 1947) described the fur color of a 310 mm. long embryo. Allen (1910, quoted by Barabash-Nikiforov, 1947) said that a newborn sea otter is about 380 mm. long. Barabash-Nikiforov (1947) states that the anatomical characteristics of the sea otter can be described sketchily due to the absence of detailed data in the literature. He described the hair color, body curvatures, tail, eyes and ears of four embryos, measuring 45 mm., 90 mm., 130 mm. and 190 mm. in length. Apparently he described the internal anatomy of the largest fetus. The ovaries are lenticulate with oviduct at the posterior edges of the ovary. Normally the sea otter has one cub, although two embryos have been found in the uterus (Barabash-Nikiforov, 1947). Snow (1910, quoted by Barabash-Nikiforov, 1947) observed a female with two cubs. Fisher (1940) said that the young sea otter birth is about 380 mm. in length and thus supported Allen (1910, quoted by Barabash-Nikiforov, 1947). Pearson (1952) noticed a 464 mm. long fetus of the sea otter which had 82 mm. long tail and it was near birth. Sea otters usually bear but one young at a time, a characteristic in common with nearly all other marine mammals rather than other Mustelids (Asdell, 1946).

Gestation

The gestation period in some carnivores has been greatly complicated by the delay in the implantation; it could be divided into two distinct periods, i.e., one of true gestation and growth of the implanted blastocyst and the other of the delay in the unimplanted stage of the blastocyst, where the latter remains quiescent. It seems that the actual period of growth of the blastocyst in most of the carnivores, is rather short.

Gestation in the Mustelids have been reported as 103 days in the pine marten (Cocks, 1900), 42 days in the ferret (Cocks, 1891, quoted by Deanesly, 1935), 40-42 days in the ferret-polecat hybrid (Harting, 1891; Cocks, 1891, both quoted by Deanesly, 1935), 40-75 days in the mink (Hansson, 1947; Pearson and Enders, 1944; and Enders, 1952), 40-55 days in the Meles (Fischer, 1931 and Hamlett, 1932), 61 days in the European otter (Cocks, 1881), 61-67 days in the striped skunk (Leach, 1960), 50-55 days in the stoat (Watzka, 1940, quoted by Hansson, 1947; Deanesly, 1935).

Enders and Pearson (1943) said that the fisher has fifty-one weeks total gestation period. This has also been supported by Laberee (1941) and Hall (1942). Barabash-Nikiferov (1947) states that the gestation period in the sea otter is about eight to nine months. Observations in captivity supported this. Lensink (1960) states that the

gestation period may normally be from eight to nine months. He concludes that the delayed implantation, which seems to occur in sea otters, as it does in many other mustelids, does not completely regulate the period of birth. Hamilton and Eadie (1964) said that the embryos of the river otter implant in February, March and April. The implantation is delayed for eight months and the total gestation period is approximately twelve months. Wilson (1959) said that the North Carolina otters have gestation periods of sixty-one days. The period of delayed implantation in the fur seal is three and one half to four months, extending from July to November (Craig, 1964).

The Breeding Season of the Sea Otter

Barabash-Nikiforov (1947) observed copulation of sea otters in January, March, May, November; Jones (1951) in February; Kenyon and Wilke (1956) in May, August and September, and Lensink (1960) in February, March, April, June, July and August. It seems that coitus takes place throughout the year. Malkovich (1937, quoted by Barabash-Nikiforov, 1947) and Shidlovskaya (1938, quoted by Barabash-Nikiforov, 1947) and Lensink (1960) observed normal active spermatogenesis throughout the year. There was no sign of degenerative changes. Kenyon (1964) has observed mating behavior in all months except October and November. Barabash-Nikiforov (1947)

did not observe copulation during October and December. Kenyon (1964) observed the mating cycle of several days duration. He concluded from his observations that the females enter in estrus only when they are not accompanied by a pup.

Kenyon and Wilke (1956) and Lensink (1960) said that the peak of breeding is in August or September. More recently, however, Kenyon (1964) concludes from behavioral observations that the peak of breeding activity is in late spring and early summer. He also points out that breeding and birth may occur at any season.

The breeding seems to occur throughout the year and the pups are born in every month (Steller, 1751, quoted by Barabash-Nikiforov, 1947; Fisher, 1940; Murie, 1940; Lensink, 1960; and Kenyon, 1964). The peak of pupping seems to occur during March and April (Lensink, 1960). The pups when born are completely formed, but dependent on their mother (Barabash-Nikiforov, 1947, and Lensink, 1960).

CHAPTER III

MATERIALS AND METHODS

Over a period of ten years the reproductive tracts of the sea otter (Enhydra lutris L.) were collected around Amchitka, Sand, Sameonof, Little Koniuji, Amak, Shumagin and Aleutian Islands. One hundred and forty female tracts were made available to me by Dr. C. H. Conaway, University of Missouri. Dr. C. M. Kirkpatrick, University of Purdue made available 1953-54 collections of the sea otter to Dr. Conaway. Karl W. Kenyon, Biologist, United States Fish and Wildlife Service, Seattle, Washington, collected all the tracts from 1955 to 1962.

Kenyon recorded the animals as "immature" or "sub-adult", adult", and "old adult". Using the field notes and the morphology of the ovary and uterus, these animals were grouped as anestrus, proestrus, estrus, pregnant and post partum anestrus (Appendix Tables I, II and III). The collections include 4 sub-adult females, 20 anestrus females, 6 proestrus females, 4 estrous females, 39 pregnant females, 44 assumed pregnant females and 23 post partum anestrus lactating females. Most of the animals were killed by Fish and Wildlife Service personnel, but a few were found dead on the beaches. The tracts were fixed in AFA (alcohol, formalin and acetic acid) or ten per cent formalin. They

were later stored in 70% ethyl alcohol or 70% isopropyl alcohol.

Both ovaries of 140 sea otters were sectioned at eight or ten micra. Sample sections were mounted and stained in a modified Schorr stain. Sample tissues from uteri, oviducts, bursa, corpora uteri, cervices and vaginas of the representative groups were sectioned and stained in a modified Schorr stain. The ovaries, corpora lutea, uterine horns and fetuses were measured with a millimeter scale. The microscopic structures of the ovaries, horns and oviducts were measured with an ocular micrometer. All the measurements were taken at the greatest diameter.

CHAPTER IV

RESULTS

I. SUB-ADULT SEA OTTER

Ovary

The ovary of the sub-adult sea otter is either smooth or lobulated. It is covered by a single layer of cuboidal or low columnar germinal epithelium. The covering epithelium invaginates into the subjacent tunica albuginea to form shallow crypts. The crypts are unbranched and simple. The tunica albuginea is a narrow band of collagenous fibers situated under the germinal epithelium. Connective tissue lamellae leave the tunica at right angles and divide the cortex into irregular columns and nests giving a labyrinthine appearance. The lamellae converge in the medulla where they blend with the medullary connective tissues (Plate I, Figure 1).

The immature ovary has large numbers of undifferentiated oocytes and small follicles in the cortex. Small medullary follicles occasionally are seen. Many of the small follicles are atretic. There is a small amount of interstitial cells, usually around atretic follicles and sometimes in the stroma. The ovary is relatively avascular and the stroma is abundant.

Uterus

The uterus in the sea otter is bipartite. In the immature animal the horn is short, slender and thread-like. The two horns fuse to form the corpus uterus which is approximately one third the length of the horn (Plate VII, Figure 51). The cornua are round in cross section whereas the body is dorso-ventrally compressed. The two horns, in the body are separated by a muscular membrane and they later unite to form the common uterine and cervical canal.

The myometrium is composed of inner circular and outer longitudinal smooth muscles. The endometrium is thin and has vary shallow rugae. The mucosa contains simple straight tubular glands, which are very few in number. Occasionally a few glands open on the mucosal epithelium. The mucosal epithelium is squamous or low cuboidal, whereas the glands are formed of cuboidal cells (Plate I, Figure 2). The uterus is non-oedamatous.

II. ADULT ANESTRUS SEA OTTER

Ovary

The ovary of the sea otter is roughly lenticulate, compressed oval or bean shaped. The craniocaudal length of the ovary usually exceeds other measurements. The size of the left and right ovaries do not differ significantly

throughout the year. The smallest ovary was eleven millimeters long while the largest was twenty-three millimeters. The size of the ovary in adult animals does not vary with the different periods of the cycle. Large and small ovaries have been seen in the anestrus and pregnant animals (Plate I, Figures 3, 4 and 5; Plate III, Figure 23).

The surface of the ovary is either smooth or lobulated. Smooth and lobulated surfaces have been seen in the anestrus, estrous and pregnant animals. Occasionally a lobule may contain a large follicle or a corpus luteum giving the appearance of the projecting lobe. The ovarian surface elevations are frequent in estrous and pregnant animals.

Germinal Epithelium

The germinal epithelium is supported on a distinct basement membrane and invests the contours of the ovary, whether smooth or lobulated. The cuboidal or low columnar cells have large elongated vesicular nuclei and distinct nucleoli.

A multilayered germinal epithelium has been seen in animals of different periods. The occurrence of the multilayered epithelium is common in anestrus, estrous, pregnant and post partum anestrus animals. These multilayered epithelial thickenings are not continuous in a given ovary. The thickenings project either into the cortex or on the outer

surface of the ovary. Many ovaries were examined for the outer surface thickenings. Mitosis is common at the thickenings and the resulting daughter cells are small and cuboidal. These cells have vesicular rounded nuclei instead of the elongated type as seen in the single layer germinal epithelium (Plate II, Figure 16). The germinal epithelium at the thickenings are a proliferating cell layer. Usually the tunica albuginea is disrupted at such thickenings. Proliferation of the germinal epithelium at the site of crypt formation has also been seen. One ovary (62-135) shows streaming of the epithelial cells into the cortex, where the staining reaction of these cells is different from that of the adjacent stromal cells.

Tunica Albuginea

The tunica albuginea is a narrow band composed of collagenous fibers and connective tissues like that of the sub-adult ovary. The tunica is occasionally disrupted in the ovary. In the regions of multilayered germinal epithelium the loss of the tunica is very apparent (Plate II, Figure 16). The tunica at this point cannot be distinguished from the adjacent stroma. Like the sub-adult ovary, the cortex is divided into irregular columns and nests giving the ovary a labyrinthine appearance (Plate I, Figure 3). The formation of the columns and nests have been seen in the estrous,

pregnant and post partum anestrus animals.

Sub-surface Crypts

Sub-surface crypts have been seen in all periods of the estrous cycle. There is no pattern in the complexity of these crypts. The simple unbranched and complex branched types of crypts have been observed in anestrus, estrous and pregnant animals (Plate I, Figures 3, 5 and 6; Plate III, Figures 21 and 24; Plate IV, Figure 40). These crypts leave the ovarian surface and usually extend up to the outer margin of the medulla.

Crypt Formation

The ovary is more or less smooth in young animals. At first a shallow invagination is seen on the ovarian surface involving the germinal epithelium. The germinal epithelium usually has mitotic figures. Occasionally multilayered thickenings of the germinal epithelium are seen at the site of the crypt formation. The surface invagination continues to grow into the cortex. The crypt is lined by low columnar cells, which are continuous with the germinal epithelium. Crypts are simple and unbranched at the beginning of their formation. The simple crypt terminates into a rounded bulb or remains pointed at the terminal tip. A crypt has a bulb or terminal tip and neck through which

it is connected to the surface (Plate I, Figures 3, 5 and 6). The crypt usually extends to the medulla. Branching of the crypts results in the formation of complex crypts, which may have a single layer or multilayered lining. Occasionally these complex crypts extend into the medulla. One ovary (62-154) is of special interest in showing the process of budding of the crypt (Plate II, Figure 19). These budded crypts are located in the cortex, and others are in the process of budding.

The epithelial cells in another ovary (59-86) are seen leaving the crypt and migrating into the stroma of the cortex (Plate II, Figure 15). The staining reaction in the epithelial cells and the adjacent stromal cells is different. Usually primary oocytes are seen adjacent to the crypts. Large numbers of atretic and small normal follicles are also present around these crypts. Occasionally the crypt encloses within its lumen oocytes and stromal cells which may be normal or degenerating.

Atresia

The anestrus ovaries have primary follicles and some small and medium sized Graafian follicles. These follicles are either normal or atretic. Graafian follicles which do not reach ovulation size become atretic. Atresia in the sea otter ovary is characterized by the formation of a

"glassy membrane" (Plate II, Figure 14), located between the theca interna and the membrana granulosa. With the resorption of the follicular elements the "glassy membrane" thickens and folds back like knotted ribbon. No luteinized atretic follicles have been observed.

In types I and III follicles of Pincus (1936) the ova show degeneration of the nuclear membrane. Sometimes it is difficult to distinguish early atretic follicles from normal follicles. Signs of atresia in small Graafian follicles have been seen in the granulosa and theca cells, where the nuclei are pycnotic. One ovary (62-134) shows the invasion of the ovum by the granulosa cells. Occasionally precocious antrum formation is seen in small follicles. The old atretic follicles usually have large amounts of connective tissue differentiated around them. The zona pellucida is usually seen shrunken in them.

Polyovular Follicle

The occurrence of polyovular follicles seems infrequent. Some biovular, triovular and tetra-ovular primary follicles have been seen.

Corpus Albicans

Six ovaries in this group have old corpora albicantia. Each has dense collagenous fibers, some fibroblasts and

advential vessels on the periphery. Three animals have two corpora albicantia in the same side of the ovary. One is larger than the other (Plate I, Figure 5). The large one is of the later pregnancy, whereas the smaller is apparently of the previous pregnancy. Since no pups were seen with these females and they were not lactating, and the ovaries were inactive, it is assumed that the large corpus albicans was at least a year old. The old corpus albicans is gradually reduced and incorporated in the ovary and becomes indistinguishable.

Interstitial Tissues

The location and the arrangement of the interstitial tissues vary greatly in different animals. In the anestrus animal the interstitial cells are usually located in the cortical region and form around the involuting follicles. Often the interstitial cells are grouped into discrete clumps or masses in the cortex. Animals in this group have large amounts of interstitial cells.

Mossman (1937) distinguished four types of interstitial cells in the pocket gopher's ovary: (1) the 'granular type', where the interstitial cells are small and irregular, (2) the 'large irregular type', with vacuolated cells which have shrunken and pycnotic nuclei, (3) the 'mature type', where the cells are large polyhedral having vesicular nuclei

and (4) the 'orange G type' interstitial cells. The interstitial tissues in the present study have been grouped into the first three types omiting the orange 'G' type.

Granular Type Interstitial Cells

The anestrus ovaries have large amounts of the 'granular type' interstitial cells. They are abundant in the cortex and around the atretic follicles. The cells are small, irregular having small and rounded nuclei. These cells often form discrete clumps around the atretic follicles and in the stroma of the cortex (Plate II, Figures 12 and 14).

Mature Type Interstitial Cells

Anestrus ovaries have small amounts of the 'mature type' interstitial cells. Only six of the twenty anestrus ovaries have relatively more of the mature type cells than the granular type (Appendix Table I). These cells are located in the cortex or around the involuting follicles. The 'granular type cells', differentiate into the 'mature type cells' (Plate II, Figure 12). An atretic follicle of one ovary (62-134) shows both granular and mature cells. The mature cells differentiate mostly from the thecal elements of the atretic follicles and partly from the granulosa cells (Plate II, Figure 13). The amount of connective tissues and fibroblasts is less around the mature interstitial

cells. These large, polyhedral cells have sinusoids around them. In appearance they are like miniature luteal cells. There is a definite cell membrane and a large vesicular nucleus with a prominent nucleolus. These cells appear secretory.

Large Irregular Type Interstitial Cells

The anestrus ovaries have very small amounts of the 'large irregular type' interstitial cells. Usually these cells are seen when the other two types are also present in the ovary. The interstitial cells classified as the large irregular type are in fact the degenerating mature interstitial cells. These degenerating mature interstitial cells are large irregular, highly vacuolated cells with pycnotic nuclei. Degeneration of the mature interstitial cells begins in the nuclei. The nucleoli cannot be distinguished in the large irregular interstitial cells. Vacuolation of the cell follows. Finally there is shrinking of the cell and differentiation of the 'granular type' interstitial cell (Plate III, Figure 20).

Medulla

The medulla extends from the hilus into the body of the ovary forming only a small portion of the organ. Usually the medulla is sharply distinguished from the cortex. The

medulla is smaller in estrous and pregnant animals, and larger in anestrus and post partum anestrus animals. It contains most of the vessels and connective tissues of the ovary (Plate I, Figure 4).

Ovarian Bursa

Each ovary is enclosed in an ovarian bursa. The periovarian space between the ovary and the peritoneal lining of the bursa is distended. The periovarian sac consists essentially of an inner and an outer layer of mesothelium and a central layer of dense connective tissues and vessels. The thickness of the wall of the ovarian bursa varies at the different places. The bursa is dorsally and caudally thicker. The oviduct passes between the mesothelial layers of the bursa and penetrates the inner layer to open into the periovarial cavity.

Uterus

The uterus of the sea otter is bipartite (Plate VIII, Figure 52). The horns are thin and slender in the anestrus animals. The uterus is composed of five layers: the outermost serosa layer, the longitudinal smooth muscle layer, the vascular layer (stratum vasculare), the circular smooth muscle layer, and the mucosa. In a quiescent horn the muscle layers are thin and composed of cells sparse in

cytoplasm. The vascular layer is located between the two muscle layers and contains advential vessels. It is a thin layer in the anestrus horn (Plate V, Figure 41).

The endometrium usually has four to six rugae. The stroma is abundant and non oedematous. The mucosal epithelium consists of cuboidal cells which have large central nuclei. The cells are uniform in height. The endometrium has straight tubular uncoiled glands which are apparently non secretory. There are no differences in structure of the two horns.

Oviduct

The oviduct in the sea otter is firmly attached to the ovarian capsule. It loops back and forth over the periphery of the capsule between the outer and inner layers of the ovarian bursa. It extends from the uterus along the lateral aspect of the ovary curving around the anterior end to pass along the median side. At the posterior pole of the ovary, the oviduct penetrates the inner layer of the ovarian bursa and opens in the periovarial cavity by a tubal ostium. The tubal ostium is surrounded by the long fimbriae. The fimbriae are slender and free in the anestrus animals. Occasionally one or two of them swell to form a cyst like structure (Plate I, Figure 10). These cystic structures compress the ovary. The oviduct is not free anywhere, but

lies between the two layers of the fascia, which form the ovarian bursa.

The oviduct has muscular and mucosal layers as seen in other carnivores. The muscular layer consists of outer longitudinal and inner circular smooth muscles. The mucosa is compact and thrown into many folds. The stroma is non oedamatous. The mucosal epithelium consists of cuboidal cells, having basal elongated nuclei.

Corpus Uterus and Cervix

The uterine horns are long and join caudally to form the body of the uterus (corpus uterus). Both horns are separated for roughly two thirds of the entire length of the body (Plate VIII, Figure 52). The common uterine and cervical canal is usually between 15 to 25 millimeters long.

The cervix is about 20 millimeters long. It is a muscular organ attached dorsally to the wall of the vagina and the corpus uterus, leaving a short unattached portion of cervix caudally. On the ventral side the cervix is free and it is enclosed by the cranial portion of the vagina. The vagina is smooth and devoid of muscular rugae. The common cervical canal opens ventrally by a slit-like opening in the cervix. The slit is usually guarded by five to eight

papillae. The opening is usually in the middle of the cervix.

The horns in the corpus uterus are structurally similar to the horns already described for the anestrus animals. The glands in the horns are persistant at the cranial end of the common uterine canal but absent caudally.

Vagina

The vagina in the sea otter is conspicuously long and muscular. The mucosa is thrown into longitudinal folds, which are less at the cervical region. The vaginal epithelium has stratified cuboidal cells in the anestrus animals.

III. PROESTRUS SEA OTTER

Ovary

The ovaries with Graafian follicles measuring between 1500 and 4000 micra are considered to be in the proestrus stage (Appendix Table I). The relative amount of interstitial cells and the structures of the horns have also been taken into account in grouping proestrus animals. The germinal epithelium, tunica albuginea and medulla in these ovaries compare with thoseof the anestrus ovaries. Both the simple unbranched and the complex branched crypts have been observed. Some of the ovaries have old corpora albicantia. The ovaries have medium sized Graafian follicles. Some of these

are normal, whereas others are atretic.

Interstitial Cells

The total amount of the interstitial cells in these ovaries compare with that of the anestrus ovaries. The granular type interstitial cells are in abundance, usually located around the atretic follicles. All the ovaries in this group have small amounts of the mature type interstitial cells.

Uterus

The uteri of these animals are not yet stimulated and are essentially like those of anestrus uteri.

IV. ESTROUS SEA OTTER

Ovary

Ovaries with the Graafian follicles larger than 4000 micra are considered to be in the estrous stage. The relative amounts of interstitial cells and the structure of the horns have been taken into account in grouping the estrous animals. The germinal epithelium, tunica albuginea, and crypts are like those of anestrus ovaries. Old corpora albicantia were also seen in this group.

Interstitial Cells

The total amount of the interstitial cells do not vary greatly from those of the anestrus ovaries. The mature type interstitial cells predominate in this group, instead of the granular type as observed in the anestrus and proestrus ovaries. The increase of the mature type interstitial cells correlates with the increase in the size of the Graafian follicle (Appendix Table I). Small amount of the large irregular interstitial cells are also present in these ovaries.

One ovary (62-325) has roughly equal amounts of granular and mature interstitial cells (Plate I, Figure 6). The Graafian follicle shows the formation of the cone. The interstitial cells occur around the atretic follicles and in the stroma. Both types of interstitial cells are found between the atretic follicles. Another ovary (E. 1290) has a large amount of the mature interstitial cells. The numbers of granular interstitial cells are reduced. The ovary has a very large pre-ovulatory follicle with a cone. The entire ovary is dominated by the mature interstitial cells (Plate II, Figure 11; Plate IV, Figure 40). Differentiation of these cells around the atretic follicles and in the stroma has greatly reduced the other types of ovarian cells and connective tissues. The ovary has an abundance of sinusoids, especially between the mature interstitial cells. These cells are large polyhedral with

vesicular nuclei and granular cytoplasm. The large quantity of mature interstitial cells indicates the increased requirements of the secretory products for the preovulatory follicle. The estrous animals show the inverse relationship in the amount of the 'granular type' and 'mature type' interstitial cells. This also indicates the differentiation of the mature interstitial cells from those of the granular interstitial cells.

Uterus

The horns in the estrous animals do not differ in length from those of the anestrus animals. The mucosal rugae are well developed and the uterine lumen is small. The mucosal epithelium and the glands have columnar cells and basal nuclei. The uterine glands show coiling near the myometrial region of the mucosa (Plate V, Figure 42). The stroma is oedamatous.

In the estrous animals the oviduct folds become prominent. The fimbriae are greatly enlarged and swollen. The epithelium of fimbriae is of the cuboidal type. The mucosal folds are prominent and complex. The mucosal epithelium has mostly columnar cells and occasionally pseudostratified cells (Plate V, Figure 43). The epithelial cells are usually ciliated in the first half of the oviduct. The cilia greatly decrease in number at the posterior portion

of the oviduct or may be absent. The oviduct shows the stimulation comparable to the horn.

V. PREGNANT SEA OTTER

Ovary

The germinal epithelium, tunica albuginea and the crypts are as in anestrus ovaries. Both atretic and healthy Graafian follicles occur in pregnant animals. The medulla is smaller in the implanted stage than in ovaries from anestrus animals.

Corpus Luteum of Ovulation

Five ovaries have corpora lutea of recent ovulations. Each corpus luteum has a large antrum with fluid in it. The granulosa cells show mitotic activity and some luteinization. The luteal cells are small and fewer in number. Each luteal cell has a vesicular nucleus and a distinct nucleolus. The cytoplasm in these cells is scanty and occasionally shows vacuolation. The luteal cells stream on the connective tissues network from the periphery to the center of the corpus luteum. The luteal cells in the corpus luteum are dispersed and are irregularly arranged (Plate III, Figure 20).

Interstitial Cells in Recently Ovulated Animals

The total amount of the interstitial cells compares with the amount present in the estrous ovaries. The ovaries in the recently ovulated animals have large amounts of mature interstitial cells. A typical ovary (62-51) shows differentiation of the mature interstitial cells from the granulosa cells of the atretic follicle as well as from the thecal elements (Plate II, Figure 13). Granulosa cell luteinization has started and the antrum of the corpus luteum is very large. The mature cells do not show degeneration as yet and appear healthy as seen in the estrous ovary (Plate IV, Figure 40). Another ovary (62-224) shows more luteinization of the granulosa cells than in the ovary number 62-51. In the former ovary a large percentage of the mature interstitial cells show vacuolation in the cytoplasm and pycnotic nuclei, thus resulting in the formation of the 'large irregular type' interstitial cells (Plate III, Figure 20). Granular and mature interstitial cells are also present. The degeneration of the mature cells is a rapid process and is greatly accelerated after the granulosa cells have partially luteinized the follicle.

The large amount of mature interstitial cells in the ovaries (E1290 and 62-51) suggests a role of these cells. Prior to ovulation estrogen production by the ovary is high. Following ovulation estrogen production is greatly reduced

and this corresponds with the rapid degeneration of the mature interstitial cells.

Corpus Luteum in Animals with Blastocysts

The corpora lutea in the animals with blastocysts are smaller than the ones with the implanted embryos. The number of the trabeculae in corpora lutea varies between ten and twenty-eight. These trabeculae carry the connective tissues, the thecal elements and the vessels into the corpus luteum. Each trabecula grows from the periphery of the corpus to the central portion of the antrum. The collagenous fibers and the fibroblasts rapidly lay down the network of the connective tissues in the antrum. There is an initial proliferation of the vessels in the early corpus luteum (Plate III, Figure 21). The size of antrum varies in different corpora lutea and may be absent (Plate III, Figure 22).

The luteinized granulosa cells stream toward the center of the corpus luteum. This streaming takes place along the network of connective tissues. The luteal cells are arranged in the 'epithelial cords'. These cords are very distinct near the trabeculae. The luteal cells around the trabeculae are regular in arrangement, but become irregular between the trabeculae (Plate III, Figure 25). The epithelial cords of the luteal cells occasionally extend from the peripery of the corpus luteum to the center. Usually these

cords are branched and rebranched.

The luteal cells in the animals with blastocysts vary in size. They are small in animals with recent blastocysts and hypertrophied at the time of implantation. The luteal cell is polyhedral and has a large basophilic vesicular nucleus and a central nucleolus. The cytoplasm in these cells is uniform and usually nonvacuolated. The vascularity is usually low in the corpora lutea with small blastocysts, whereas it is greatly increased when the blastocysts are near implantation. The luteal cells in the latter case are hypertrophied (Plate III, Figure 28). These cells appear to be secretory. The intermediate size of luteal cells (Plate III, Figure 27) indicates progressive hypertrophy of the small cells to large luteal cells at the time of implantation. There is no difference in the structure of the luteal cells of the blastocysts except in their size.

Corpus Luteum in Animals With Assumed Blastocysts

Since the recovery of unimplanted blastocysts is extremely laborious, no attempt was made to confirm the presence of unimplanted blastocysts in most reproductive tracts. Based upon the morphology of the corpus luteum and other aspects of the reproductive tracts, forty-four additional animals were classed as in unimplanted stages even

though blastocysts were not demonstrated. The luteal cells in these animals are comparable to those already described in unimplanted stages (Plate III, Figures 25 and 26). One animal (62-268) has two corpora lutea, one in each ovary. The luteal cells are like the others described in this group.

Two of the forty-four animals were described as lactating in the field notes. The reproductive tracts of these two conform to the unimplanted tracts in all respects. It may be that these represent instances of a post partum estrus.

Interstitial Cells in Animals with Blastocysts

The total amount of the interstitial cells is less than in the estrous and in the anestrus ovaries. The granular interstitial cells predominate. The mature cells are usually localized around the periphery of the corpora lutea. Many multinucleate interstitial cells form discrete masses in the connective tissues. The multinucleate cells usually have two to twelve nuclei. The interstitial cells in the recovered and the unrecovered blastocyst tracts are alike.

Uterus in Animals with Blastocysts

The uteri in the animals with recovered blastocysts and in the animals with assumed blastocysts are alike in structure (Plate V, Figures 44, 45 and 46). This indicates

the presence of the blastocysts in those tracts in which no attempt was made to recover blastocysts.

The muscle layers in these animals are thicker than those of the anestrus horns. The stratum vasculare occupies the major portion of the myometrium. The mucosa usually has five to eight well developed rugae. The endometrium is congested with coiled glands. The stroma is greatly reduced, and is oedematous. Usually the glands have all columnar cells where the basal nuclei are rounded or oval. The uterine glands on the side of the blastocyst have slightly taller columnar cells than in the side without the blastocyst (Appendix Table II). In both the horns occasionally the glands open on the mucosal epithelium. The surface mucosal epithelial cells have cuboidal or low columnar cells and elongated nuclei. The epithelial cells of the horn containing the blastocyst are slightly taller than those of the other horn. Two animals (79-56 and 62-41) have blastocysts ready to implant. The endometrium can be sub-divided into an inner complex zone and an outer spongy zone (Plate V, Figure 47). The glands are greatly coiled and have an enlarged lumen. The stroma is greatly reduced. The horn is highly vascularized.

Blastocyst

Eleven unimplanted blastocysts were recovered between

January and September. Nine blastocysts have a thick zona pellucida, whereas two are ready to implant. Most of the blastocysts have shrunken and broken zona pellucida (Appendix Table V). Three of the blastocysts are oval and have intact zona pellucida. The size of these three blastocysts varies from 97 micra to 202 micra. It suggests an increase in the size of the blastocyst during the quiescent period.

The blastocyst in the sea otter differentiates into the inner cell mass and the trophoblastic ectoderm. The trophoblast layer is composed of flattened cells, closely attached with the zona pellucida. The inner cell mass is undifferentiated in the blastocyst not ready for the implantation. The cells in the inner mass are polygonal. The zona pellucida is thick and usually differentiated into two distinct layers. The outer layer is dense and thin, whereas the inner layer is homogeneous and thick (Plate V, Figure 45 and 46). The blastocyst in the horn of 59-14 shows cell division in the inner cell mass. The blastocysts near implantation have approximately 500 cells in the inner cell mass.

Corpus Luteum in the Implanted Animal

Two animals with blastocysts ready to implant have compact corpora lutea (Plate III, Figure 22). The ovary of an animal (62-40) with a recently implanted embryo of about

fifteen millimeters has a compact corpus luteum (Plate III, Figure 23). All other animals having implanted embryos have loosely packed luteal cells. The corpora lutea in the implanted animals are roughly two times larger than the corpora lutea in the unimplanted ones. The animals with the blastocysts ready to implant have compact corpora lutea. Once the embryo has implanted and reached about ten millimeters in size, the corpus luteum as a whole hypertrophy. The increased size of the corpus luteum results in the formation of secondary cavities. The secondary cavitations in the corpus luteum are filled with fluid (Plate III, Figure 24). The size of these cavities varies in different animals. These cavities are present in the animals having fetuses measuring from 45 to 440 millimeters long. The extent of the cavitation in the corpora lutea varies from animal to animal. Occasionally the secondary cavities or the antra have networks of connective tissues. These cavities seem to increase in the late pregnant animals.

There is no difference in the luteal cells of the early and the late pregnancy (Plate III, Figures 29, 30 and 31). The corpora lutea in the implanted animals are highly vascularized.

The luteal cells are large and polygonal in shape. Each cell has a vesicular nucleus and a prominent nucleolus. The cytoplasm is uniform and granular. Very few vacuolated

cells are noticed. The luteal cells are arranged as epithelial cords, which have at least one surface in contact with capillaries. These epithelial cords are regular at the trabeculae. The luteal cells remain hypertrophied until parturition. Following parturition the luteal cells degenerate rapidly.

Interstitial Cells in Implanted Animals

The ovaries in this group have small amounts of interstitial cells (Appendix Table I). All the ovaries have mature interstitial cells, usually located close to the corpora lutea. The multinucleate mature cells are dispersed in the connective tissues. The granular and mature interstitial cells are roughly equally abundant. The granular cells are usually located around the atretic follicles. Small quantities of the large irregular degenerating interstitial cells are also present in these ovaries.

Uterus in Implanted Animals

Structurally both the horns are alike in the regions away from the placenta (Plate IX, Figure 53). The endometrium has an inner complex folded zone and an outer spongy zone. Usually the horns are similar to the animals having blastocysts ready to implant (Plate V, Figure 47). The stroma is

highly oedamatous. The glands are coiled and complex. The height of the glandular columnar cells of these animals compares with the height of the gland cells in the unimplanted animals. The same is true for the mucosal epithelial cells.

Fetus

The fetuses of the sea otter are similar in the morphological characters, such as the hair color, shape and size to those described by Barabash-Nikiforov (1947).

VI. POSTPARTUM SEA OTTER

Ovary

The ovaries in this group are similar to the anestrus ovaries yet all the animals are either lactating or have pups or both (Appendix Table I). The estimated age of the pups varies from about a week to ten months. The germinal epithelium, tunica albuginea, sub-surface crypts and the medulla are as in anestrus ovaries. None of these animals show ovarian activity.

Interstitial Cells

The total amount of the interstitial cells in these ovaries corresponds closely to the amount of the interstitial cells in the anestrus ovaries. The granular type interstitial cells are abundant. These cells are located around

the atretic follicles, which usually form discrete clumps or masses. Almost all the ovaries have some large irregular vacuolated and mature interstitial cells (Appendix Table I). The ovary (Plate I, Figure 9) has roughly equal amounts of the granular and the mature interstitial cells, located around the atretic follicles.

Corpus Albicans

The old corpora albicantia are similar to those described in anestrus ovaries. This group has two animals (60-16 and 60-19) with the pups estimated as less than a week old. These ovaries have large corpora albicantia (Plate I, Figures 8 and 9).

At the time of killing one of these animals (60-16), a female pup was recovered, which was about two to four days old. The umbilical scar was still fresh and claws were similar to the large unborn fetus. The ovary has a large corpus albicans with the degenerating luteal cells and a central core of the connective tissues. The degeneration of the corpus seems to proceed from the center to the periphery. More than eighty per cent of the luteal cells have already degenerated (Plate II, Figure 17). The remaining luteal cells have greatly shrunken, and have pycnotic nuclei. Very few luteal cells have vesicular nuclei. The luteal cells show vacuolation followed by resorption and replacement

by connective tissues. As the luteal cells degenerate the connective tissues and fibroblasts replace them. The presence of the large corpus albicans with degenerated luteal cells in an animal accompanied by a very young pup indicate the rapid resorption of the corpus luteum.

Another animal (60-19) had a pup less than a week old. The corpus albicans is large and similar to that already described above. The central core of the corpus albicans has a large amount of the collagenous fibers. Luteal degeneration and the replacement of the cells have occurred (Plate II, Figure 18). The advential vessels are located around the periphery of the corpora albicantia. Some capillaries are also present around the degenerating luteal cells. This ovary has another 'stellate scar' of a corpus albicans. The peripheral arrangement of the vessels and the central collagenous fibers in the recent as well as the old corpora albicantia in this group corresponds to the corpora albicantia already described in the earlier section. The two recent corpora albicantia show the rapid luteal degeneration and their subsequent transformation into the 'stellate scar'. These 'stellate scars' gradually blend with the connective tissues of the ovary.

Uterus

The recent postpartum uteri are thicker than those

classed as anestrus. The stratum vasculare is well marked and has many advential vessels. In the recently parturated animal the mucosal rugae are prominent. The horn (Plate V, Figure 48) has coiled glands and highly oedamatous stroma. The gland and the epithelial cells show decrease in the height of the cells. The horn in the older post partum animal is like that of the anestrus horn.

VII. THE GROWTH OF THE OVUM

In forty randomly selected ovaries, the diameter of one hundred follicles and ova were measured (Appendix Table IV). The relationship of ovum growth to follicle growth was plotted on millimeter graph paper (Plate VI, Figure 50).

The growth of the ovum and the follicle is divided into two phases as suggested by Brambell (1928). In the first phase the ovum grows rapidly until almost the adult size, while the follicle increases slowly. The full growth of the ovum is attained before the follicle reaches 200 micra in diameter (Plate VI, Figure 50). The size of the ovum in the follicle larger than 200 micra remains relatively stationary. The ovum is approximately 123 micra in diameter. In the second phase the follicle grows rapidly, chiefly by the enlargement of the antrum and by the differentiation of the thecal elements. The primary follicles are located in the cortex, whereas the growth in the secondary follicles

tends to bring them closer to the medulla. The presence of a large Graafian follicle or a corpus luteum inhibits the differentiation and the growth of the primary and the secondary follicles. Many of these follicles become atretic in the anestrus, estrus and the pregnant animals.

The follicles in the present study have been classified into nine types as suggested by Pincus and Enzmann (1937). The follicular growth during the vesicular stages and the antrum formation have also been studied as indicated by Robinson (1918) in the ferret.

Type One Follicle

The ovum is surrounded by a single layer of flattened disconnected epithelial cells. The ova are located in the cortex closer to the tunica albuginea (Plate IV, Figure 32).

Type Two Follicle

The ovum has a single layer of follicular cells. These cells are connected to form a definite cell layer around the ovum (Plate IV, Figure 33).

Type Three Follicle

A single layer of the cuboidal cells surrounds the ovum. These cells are compact. The zona pellucida is observed for the first time. The connective tissues and stromal cells

also show some differentiation from the adjacent stroma. It marks the beginning of the thecal differentiation. The ovum has a large vesicular eccentrically placed nucleus and a prominent nucleolus (Plate IV, Figure 34).

Type Four Follicle

Two rows of cuboidal cells are present around the ovum. The inner row may be completely or incompletely formed. It indicates the proliferation of the inner cells from the outer row cells. The follicle is slightly oblong in shape, instead of the oval shape as observed in the earlier types. The ovum shows an increase in size and volume. The thecal cells have differentiated from the surrounding stroma. The theca interna and the theca externa could be roughly demarcated (Plate IV, Figure 35).

Type Five Follicle

The follicle has a multilayered layer of cuboidal cells around the ovum. The oblong follicle shows two poles, where the cuboidal cells are actively proliferating. The cells are small and have large nuclei. The ovum is surrounded by a well demarcated zona pellucida. The theca shows further differentiation of its elements (Plate IV, Figure 36).

Type Six Follicle

The follicle in this stage shows the formation of antra in the poles. The antra are small in the beginning and eventually two of them coalesce to form a larger antrum (Plate IV, Figure 37). The zona pellucida is thick and distinct around the ovum. The cuboidal cells have increased in number. The antrum has follicular fluid of uniform texture. This follicular fluid may be called 'primary liquor folliculi', as indicated by Robinson (1918). The granulosa and thecal cells are well marked in the follicle.

Type Seven Follicle

The seventh stage is usually designated as the beginning of the vesicular phase, which coincides with the cessation in the increase of the ovum size. The ovum has a distinct layer of the corona radiata cells. Many small antra coalesce to form a large antrum (Plate IV, Figure 38), in the follicle except at the cumulus oophorus region. A secondary vacuolation can be noticed in the cumulus oophorus. These cavities have follicular fluid, designated as the 'secondary liquor folliculi'.

Type Eight Follicle

The ovum is suspended in the follicle giving an appearance of the 'spider web'. The corona radiata cells have further

differentiated (Plate IV, Figure 39). The zona pellucida is thick and the ovum has a vesicular nucleus. The corona cells are of the columnar type. The membrana granulosa layer shows some mitotic activities, usually two to four layers of the granulosa cells are observed in this type of follicle. The theca interna shows vascularization.

Type Nine or Preovulatory Follicle

The follicles show formation of the ovulation cone. The ovum is detached from the follicle and floats in the antrum. The granulosa cells have increased mitotic activity (Plate I, Figure 6 and Plate IV, Figure 40). The ovum in this follicle was not recovered. The theca interna is highly vascularized with the advential vessels and the capillaries. The membrana granulosa layers in the types eight and nine follicles are occasionly detached from their anchorage. At the time of ovulation, such detached granulosa would facilitate the collapse of the follicle. It would not be extruded out of the follicle because of its attachment with the basement membrane and theca interna layer. Some granulosa cells might be lost with the collapse of the follicle. These granulosa cells apparently drag with them some filamentous fibers, and thus result in the formation of the 'tertiary liquor folliculi'. The preovulatory follicles in the sea otter are about eight millimeters in diameter. The size of the ovum is approximately 123 micra in diameter.

CHAPTER V

DISCUSSION

The ovaries of one hundred and forty animals were studied to investigate the reproductive cycle of the sea otter (Enhydra lutris). Uteri and oviducts were studied from representative specimens in different stages of the reproductive cycle. The reproductive tracts were collected throughout the year and thus form an adequate sample to study the gross and microscopic structures.

The sea otter ovaries are roughly lenticulate or compressed ovals. They usually have lobulated surfaces. The size of the sea otter ovary does not vary greatly during the estrous cycle. This is contrary to the findings in mink (Hansson, 1947; Enders, 1952), in the ferret (Robinson, 1918; Hamilton and Gould, 1940), and in the weasel (Deanesly, 1944), where the variations in the size of the ovaries are greater.

The adult ovary is covered by the germinal epithelium, as seen in other mammals (Harrison, 1962). The germinal epithelium consists of a single layer of the cuboidal or low columnar cells. Occasionally, in local areas multilayered germinal epithelial cells project into the cortex or out from the ovarian surface. Ferrets also have multilayered germinal epithelial cells (Hamilton and Gould, 1940).

The germinal epithelium shows active mitosis in these areas and the tunica albuginea is usually disorganized or disrupted. Occasionally the sub-surface crypts are lined with multi-layered epithelial cells. The proliferation of the germinal epithelium indicates the addition of epithelial cells to the cortex. In the sea otter the formation of the multilayered cells apparently is not in response to the estrogen stimulation as in some other mammals (Bullough, 1946), since it occurs throughout all phases of the cycle.

The tunica albuginea in the sea otter is composed of collagenous fibers like that of the ferret (Robinson, 1918), and the mink (Hansson, 1947). The cortex is divided into irregular columns and nests, giving the ovary a labyrinthine appearance similar to that of the mink (Hansson, 1947). The cortex and the medulla of the sea otter do not differ from other mustelids (Robinson, 1918; Hansson, 1947; Wright and Rausch, 1955).

Sub-surface crypts of the simple and the complex types do not show cyclic patterns of development in the ovaries of the sea otter in contrast to the findings in the Pinnipedia (Harrison and Mathews, 1951). Pearson (1952) called these sub-surface crypts the 'fissures'. Sometimes the sea otter crypts show budding of 'epithelial tubes' into the deeper cortex. The budding shows the addition of the epithelial elements into the cortex, either by the streaming of cells

from the crypt or by detaching the 'epithelial tubes' from the crypt. The budding of the crypts has not been reported. This shows the functional utility of the crypts, which has also been suggested by Harrison and Mathews (1951).

The oocytes have been seen along the crypts or occasionally in the lumen. Craig (1964) showed the presence of oocytes in the cryptic lumen of the fur seal. Mossman (1938) said that the surface epithelium encloses surface oocytes of Erethizon dorsatum. Harrison and Mathews (1951) did not report the association of the oocytes with the crypts.

The sea otter ovaries show atresia both in the primary and the Graafian follicles, like those of other mammals (Asami, 1920; Clark, 1923; Kingsbury, 1939; Brambell, 1956; Ingram, 1962: and Craig, 1964). Atresia in the sea otter is characterized by the formation of the 'glassy membrane' as seen in the fur seal (Craig, 1964). The atretic follicles do not luteinize, as do those of the badger (Weal and Harrison, 1958). The signs of the atresia are seen in the granulosa and theca cells, besides the ova themselves, as reported earlier by Asami (1920), Mossman (1937), and Clark (1923).

The luteal cells in the sea otter's corpus luteum degenerate very rapidly after parturition like those of the sow (Corner, 1921) and the striped skunk (Leach, 1960).

They degenerate by vacuolation and fragmentation as seen in the badger (Neal and Harrison, 1958). The nuclei become pycnotic and the cells finally shrink. Degeneration of the luteal cells seems to begin in the center of the corpus luteum and then extend to the periphery, contrary to the guinea pig (Loeb, 1911). After the luteal cells have completely degenerated and been replaced by collagenous fibers and fibroblasts, the corpus albicans gives the appearance of a 'stellate scar'. This has also been reported in the fur seal (Craig, 1964). These corpora albicantia have peripheral hyalinized vessels and the dense irregular collagenous fibers similar to the one reported by Mossman and Judas (1949), and Corner (1921). The old corpus albicans with its peripheral vessels can be distinguished in the ovaries for about two years. Later it blends with the ovarian tissues and becomes indistinguishable as indicated by Brambell (1956), and Joel and Foraker (1960).

The luteal cells in the sea otter are derived from the granulosa cells as in the rabbit (Deanesly, 1930), and the pocket gopher (Mossman, 1937). The granulosa origin of luteal cells has been supported by Corner (1948) and Harrison (1962). The connective tissue elements of the corpus luteum are supplied by the theca interna as seen in the pocket gopher (Mossman,1937). The connective tissues lay down a network in the ovulated follicle, along which the luteal cells

stream to the center of the corpus. This network of connective tissues possibly keeps the ovulated follicle in a distended condition until the luteal cells fill the antra. Robinson (1918) said that the ruptured follicles redistend, which is the beginning of the corpus luteum formation.

The corpora lutea in the sea otters can be sub-divided into three groups: the corpora of ovulation, corpora of the free blastocyst stage and corpora of implanted pregnancies.

The corpora lutea of ovulation are smaller than those of the implanted pregnancies. These corpora have a very large antrum and partially luteinized granulosa cells. The luteal cells are small and have vesicular nuclei. The luteal cells in the corpus luteum lack regularity in their arrangement as in the pig (Corner, 1915) and the dog (Evans and Cole, 1931). The granulosa cells in the pre-ovulatory follicle of the sea otter show mitotic activities as noticed in the mink (Hansson, 1947).

The corpora lutea in animals with recovered and unrecovered blastocysts are structurally alike, and are smaller than the corpora lutea of the implanted animals. The luteal cells are polyhedral and have uniform granular cytoplasm with vesicular nuclei, as seen in the river otter (Hamilton and Eadie, 1964). The corpora lutea in the blastocysts ready to implant have no antra but they show hypertrophied luteal cells. Deanesly (1944) reported continuous growth of the

corpus luteum in the weasel, which is also true for the fur seal (Craig, 1964). The luteal cells are regular at the trabeculae and irregular in the intertrabecular spaces. These luteal cells are arranged in the form of cords which show branchings and rebranchings in the corpus luteum.

The corpora lutea in the implanted animals are approximately twice as large as the corpora lutea of ovulation or of blastocyst stages. The corpora lutea in the early implanted animals have no cavities. The cavitation in the corpora lutea increases in the animals with the larger fetuses. These cavities have some fluid precipitate and a connective tissue meshwork. In the pregnant sea otter the cycle of the corpus luteum as a whole appears like that of the human corpus luteum (Nelson and Greene, 1958). The cavitation of the corpus luteum is associated with the increase in size of the corpus. The corpus luteum of pregnancy is a highly vascular structure. The luteal cells have a granular and uniform cytoplasm with vesicular nuclei as seen in the ferret (Hammond and Marshall, 1930) and the river otter (Hamilton and Eadie, 1964). These cells apparently remain secretory until parturition. After parturition the luteal cells regress rapidly like those of the Rocky Mountain pika (Duke, 1952) and the badger (Harrison and Neal, 1959).

The structural similarities of the luteal cells in

animals with blastocysts and implanted stages suggest their functional relations. The corpus luteum with the unimplanted blastocysts may secrete some progesterone, which maintains the uterine glands and the mucosal epithelial cells in the differentiated form. The amount of the secretion increases rapidly in the hypertrophied luteal cells at implantation. The sea otter has no accessory corpus luteum.

The interstitial cells form the main mass of the cortex and are usually arranged in discrete clumps as reported by Stafford and Mossman (1945) in the guinea pig. The type and the relative amount of the interstitial cells indicate a distinct cycle in the sea otter. The interstitial cells were grouped as suggested by Mossman (1937). The granular type interstitial cells were more abundant in anestrus and post partum animals than in the estrous and pregnant ones. Interstitial cells are abundant in the anestrus ovaries of the dog (Mulligan, 1942) and the ferret (Hamilton and Gould, 1940). There is a distinct increase of interstitial cells of the mature type in the estrous ovaries. Graafian follicles in the estrous ovaries grow and reach the pre-ovulatory stage during the follicular phase, when the production of estrogen is at its peak. Leach (1960) said that the interstitial cells in the striped skunk show cyclic response, which is positively correlated with the growth of the follicle. The mature cells

have uniform cytoplasm and vesicular nuclei, giving them the appearance of miniature luteal cells. The structure of these cells, and their abundance correlated with the estrous ovaries, and the preovulatory follicles, suggests a secretory function. These cells may secrete estrogen or allied products. Harrison (1962) said that the interstitial cells undergo changes in the size and the cytological characteristics during estrus and pregnancy. Falack (1959, quoted by Eckstein, 1962) said that the theca interna and the interstitial cells may constitute the estrogen producing cell system of the ovary. Recent studies have indicated the close histogenetic relationship between the theca interna of the atretic follicles and the interstitial cells (Brambell, 1956). It is also considered that both of the systems are to some extent functionally interchangeable. Cleasson and Hillarp (1947) showed that the interstitial cells are concerned with estrogen secretion. The interstitial cells and the theca interna may also function independently.

Most of the mature interstitial cells differentiate from the theca interna of the atretic follicles (Athias, 1920; and Mossman, 1937), and occasionally from the granulosa cells of the atretic follicles (as shown in Plate II, Figure 13). These cells differentiate from the granular type interstitial cells located around the atretic follicles. The granulosa and the thecal origin of the interstitial cells

have been shown by Dawson and McCabe (1951) and Rennels (1951). The differentiation of the large amount of the mature cells from the granular cells in the estrous ovaries suggests the functional need for these cells.

After ovulation and the partial luteinization of the follicle, the mature interstitial cells degenerate rapidly (Appendix Table I) resulting in the formation of the large irregular vacuolated type interstitial cells. These cells show cytoplasmic vacuolation and pycnotic nuclei as in the pocket gopher (Mossman, 1937). The large vacuolated cells shrink and form the granular type interstitial cells. The rapid degeneration of the mature cells after ovulation would indicate the sharp reduction of the estrogen requirement by the animals. This fact is consistent with the suggestion that the mature interstitial cells of the sea otter are involved in secretion of estrogen or allied products. The sharp decline in the estrogen might be due to decline in the follicle stimulation hormone from the anterior pituitary in the post ovulatory phase. The interstitial cells in the woodchuck are maximum at the time of ovulation and early pregnancy (Rasmussen, 1918). This is also true in the badger, weasel and the pocket gopher (Stafford and Mossman, 1945).

The ovaries with uterine blastocysts have more interstitial cells than those from implanted animals. The

ovaries from animals with implanted fetuses have a small amount of the mature interstitial cells, suggesting a source of estrogen during the pregnancy. These cells are usually around the corpora lutea and also among the connective tissues of the stroma. Multinucleate mature interstitial cells have been noticed in the ovaries of pregnant animals.

The uterus of the sea otter shows structural changes correlated with the ovarian structures as seen in other mammals (Evans and Cole, 1931; Mulligan, 1942; Deanesly, 1935; Hamilton and Gould, 1940; Hansson, 1947; and Neal and Harrison, 1958). The anestrus uterus has simple tubular glands and non-oedamatous stroma, whereas the estrous uterus has coiled tubular glands and oedamatous stroma. The endometrium in the pregnant animal has an inner complex zone and an outer spongy zone. The height of the mucosal epithelium and of the uterine glands in both the horns is essentially alike, contrary to the condition shown by Craig (1964) in the microphoto of the fur seal horns, which he did not discuss in the text.

The relationship of ovum growth to the follicle growth in the sea otter is like that of other mammals (Pincus and Enzmann, 1937; Brambell, 1928; Parkes, 1931; Mathews, 1939; and Harrison, 1962). The ovum of the sea otter attains a mature size of approximately 123 micra in diameter, when the follicle is less than 200 micra in diameter. The pre-

ovulatory follicle of the sea otter is approximately eight millimeters in diameter. The primary, secondary and the tertiary liquor folliculi are also present in the sea otter similar to the ferret (Robinson, 1918). The sea otters have blastocysts throughout the year, which are at the different stages of their growth. The smallest blastocyst is 97 micra in diameter with 40 cells, whereas the largest blastocyst is approximately 472 micra in diameter with an estimated 500 cells. This indicates the growth of the blastocyst during the unimplanted stage, also observed by Notini (1948, quoted by Harrison and Neal, 1956). Completely quiescent blastocysts during delay have been seen in the mustelids (Hamlett, 1932; Enders, 1952; and Neal and Harrison, 1958). The blastocysts differentiate into the inner cell mass and the trophoblastic layer as in all Eutherian mammals (Mossman, 1937). Most of the blastocysts have collapsed and have broken zona pellucida, possibly due to the poor preservation.

The presence of the blastocysts and the corpora lutea throughout the year, indicates the absence of a distinct breeding season in the sea otters. This has also been reported by the earlier workers (Fisher, 1940; Murie, 1949; Barabash-Nikiforov, 1947; and Lensink, 1960). The unimplanted blastocysts indicate that delayed implantation occurs in the sea otter, but the delay does not seem to synchronize the time of birth in the animals. This is also supported by

Lensink (1960). Usually one follicle ovulates at a given time, which is followed by the delay, implantation and the parturition. Only two animals show the presence of one corpus luteum in each ovary.

CHAPTER VI

SUMMARY

The reproductive tracts from 140 female sea otters were studied. Histological structures of the ovaries and the uteri are described and related to the estrous cycle of the animal.

The sea otter ovaries are roughly lenticulate or compressed ovals, having lobulated surfaces. The size of the ovary does not vary greatly during the estrous cycle.

The multilayered germinal epithelial cells project both into the cortex or upon the ovarian surface. The tunica albuginea is usually disorganized or disrupted at these thickenings. The cortex is divided into irregular columns and nests, giving the ovary a labyrinthine appearance.

There is no cyclic pattern in the formation of the simple or complex sub-surface crypts. These crypts are formed by the invaginations and branchings. Occasionally buddings of the crypts are seen to form epithelial tubes projecting into the cortex. The crypts may have single or multilayered epithelial cells. The streaming of the epithelial elements from the crypts, the budding of the epithelial tubes and the proliferations of the germinal epithelium indicate the addition of epithelial elements to the cortex.

Follicular atresia in the sea otter is like that of

other mammals. Atresia is characterized by the formation of the 'glassy membrane'. Atretic follicles in the sea otter do not luteinize.

The luteal cells in the sea otter are derived from the granulosa. The theca interna supplies the connective tissues to the corpus luteum. The formation of the connective tissues network in the recently ovulated follicle possibly keeps the ovulated follicle distended.

The corpora lutea of unimplanted pregnancies are smaller than those of implanted pregnancies. There is secondary cavitation in the corpora lutea during mid and late pregnancy. These cavities may be formed by the hypertrophy of the corpus luteum. The cavities have fluid precipitate.

The luteal cells immediately after ovulation and during unimplanted stages are either polygonal or cylindrical in shape and smaller in size than those of the implanted pregnancies. The luteal cells in implanted stages are greatly hypertrophied and highly secretory. Cytoplasm is granular in the luteal cells of corpora lutea in both implanted and unimplanted pregnancies. Since the luteal cells of unimplanted pregnancies are similar histologically to those of implanted pregnancies, it may be that progesterone is produced during both periods.

The luteal cells do not degenerate until parturition. The degeneration of the cells is rapid and extends from the

center of the corpus to the periphery. A completely retrogressed corpus luteum or corpus albicans gives the appearance of the 'stellate scar'. The latter is mostly composed of central collagenous fibers and peripheral hyalinized advential vessels. The corpus albicans in the sea otters ovary is probably distinguishable for about two years.

The interstitial cells of the sea otter show a distinct cycle. Three types of interstitial cells have been observed.

1) The granular type interstitial cells are small and irregular. They are found mostly around the atretic follicles, and are thecal in origin. These cells are abundant during the anestrus period.

2) The mature type interstitial cells are large polyhedral cells with vesicular nuclei. They are abundant during estrus and in recently ovulated animals. They are mostly thecal and partly granulosa in origin.

3) The large irregular type interstitial cells are vacuolated, shrunken and have pycnotic nuclei. These are degenerating mature type cells, and are abundant in ovulated animals.

The anestrus animals have more of the granular type interstitial cells than the pregnant animals. The amount of the granular cells decreases as the animal approaches estrus and at the same time the mature cells increase in the ovaries having pre-ovulatory follicles. The sharp increase

in the amount of mature interstitial cells in the estrous animals and the decrease in the granular interstitial cells suggest their possible functions during the follicular phase. The mature interstitial cells may secrete estrogen or allied products required by the large Graafian follicles.

The mature interstitial cells are polygonal in shape, with uniformly granular cytoplasm and vesicular nuclei, giving them the appearance of miniature luteal cells. These cells are mostly differentiated from the granular interstitial cells, which are located around the atretic follicles and partly from the granulosa cells of the atretic follicles. Small amounts of the mature interstitial cells are present in pregnant animals.

The mature interstitial cells become vacuolated and have pycnotic nuclei in the recently ovulated animals. These cells form the large irregular vacuolated type interstitial cells and are therefore probably non-secretory. This may correlate with the decrease of the estrogen in the ovulated animals. The large vacuolated cells shrink and redifferentiate into the granular type interstitial cells.

The uterus in the sea otter is bipartite. The changes in the mucosal epithelium and the uterine glands correlate with the changes in the ovarian structures. The endometrium has a complex inner zone and a spongy outer zone in the pregnant animals. The cyclic changes in the oviduct corres-

pond with the changes in the horns and in the ovaries.

Both the horns in the corpus uterus are separated by a muscular membrane or septum. They later unite to form the common uterine and the cervical canal, which opens ventrally on the cervix.

The relationship of the ovum growth and the follicle growth in the sea otter is like that of other mammals. The ovum reaches the mature size of approximately 123 micra before the Graafian follicle reaches the size of 200 micra. The preovulatory follicle in the sea otter is about eight millimeters in diameter.

The sea otters have blastocysts throughout the year, the smallest recovered blastocyst being 97 micra and the largest being 472 micra. The presence of many uteri with unimplanted blastocyst indicates a delay in implantation.

Pups of all sizes have been reported throughout the year. This indicates that the delay in the implantation of the blastocyst does not regulate the birth time. The sea otters do not show distinct breeding seasons.

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APPENDIX

APPENDIX TABLE I

ANESTRUS OVARIES

Animal Number	Date	Wt. in Lbs.	Ovary Size in Millimeters L	R	Interstitial Cells: Total Amount	Granular Type	Large Irregular Vacuolated Type	Mature Type	Remarks
62-6	Jan. 22	45	15	14	+++++	+++	++	++++	
62-36	Jan. 25	48	19	21	+++++	+++++	++	+	
62-52	Jan. 29	51	20	20	++++	++++	++	-	
62-65	Jan. 29	38	15	16	++++	++++	-	+	
62-62	Jan. 29	35	16	20	++++	+++	-	++	Two corpora albicantia
62-113	Feb. 5	41	19	18	++++	+++	+	+	
62-130	Feb. 9	36	14	14	++++	++++	-	-	
62-134	Feb. 9	45	17	15	++++	++	-	+++	
62-151	Feb. 10	42	17	17	+++++	+++	++	++++	Two corpora albicantia
62-156	Feb. 13	41	18	17	+++	+++	-	-	
62-159	Feb. 13	42	20	15	+++	+++	-	-	
59-16	Feb. 17	44	18	15	++	++	-	-	Two corpora albicantia
62-225	Mar. 9	34	15	15	++++	++++	++	-	One corpus albicans
8-54	Mar. 15	44	--	--	++	++	-	-	Two corpora albicantia
59-77	Mar. 21	40	17	16	++	++	-	-	
59-86	Mar. 27	29	20	16	+++++	++++	+	++++	
59-94	Mar. 29	32	18	21	+++++	+++	+	+++	Two corpora albicantia
62-310	Oct. 26	52	16	17	++++	+++	++	++	
62-316	Oct. 27	46	11	12	+++	++	-	+++	
62-322	Oct. 29	35	12	12	++	++	-	-	

POSTPARTUM OVARIES

Animal Number	Date	Wt. in Lbs.	Ovary Size in Millimeters L	Ovary Size in Millimeters R	Interstitial Cells: Total Amount	Interstitial Cells: Granular Type	Interstitial Cells: Large Irregular Vacuolated Type	Interstitial Cells: Mature Type	Remarks
62-12	Jan. 23	51	17	22	+++++	++++	++	++	One corpus albicans and lactating
62-21	Jan. 25	43	14	17	+++++	++++	++	+	Lactating
62-24	Jan. 25	35	20	18	++++	+++	+	+	Pup
62-38	Jan. 26	44	15	17	+++++	++++	++	-	Two corpora albicantia and lactating
62-50	Jan. 29	49	20	19	++	++	+	-	Lactating
62-54	Jan. 29	45	16	18	+++++	++++	++	-	lactating and 1 month pup
62-87	Feb. 3	49	20	20	++++	++	++	+++	Lactating
62-113	Feb. 6	40	19	15	+++	++	+	+	Lactating and large pup
62-143	Feb. 10	50	22	22	+++	+++	+	+	Lactating
59-60	Mar. 12	46	17	55	++++	++++	+	-	One month pup
60-16	Jul. 10	44	17	10	++++	+++	++	+++	2-4 days old pup
60-19	Jul. 10	55	18	19	++++	+++	++	++++	Less than week old pup
62-308	Oct. 26	47	16	13	++++	++++	++	-	Lactating
62-3099	Oct. 26	47	15	14	+++++	+++	++	+++	Lactating and pup
62-313	Oct. 26	46	20	20	++	++	+	-	Lactating and pup
62-315	Oct. 27	48	16	16	++++	+++	++	-	Lactating and pup
62-317	Oct. 27	45	18	17	+++	+++	+	-	Lactating and pup
62-321	Oct. 27	46	15	15	+++	+++	+	-	Lactating and pup
62-328	Oct. 30	50	20	16	+++	+++	+	-	Lactating and pup
D11-57	Nov. 3	--	15	15	++++	++++	+	+	1-2 week old pup
D14-57	Nov. 3	--	12	11	++++	++++	+	+	Young pup
D19-57	Dec. 11	--	19	18	++++	++++	++	++	Young pup
D17-57	Dec. 12	--	20	20	+++++	++	++	++++	Old pup

PROESTRUS OVARIES

Animal Number	Date	Wt. in Lbs.	Ovary Size in Millimeters L	R	Total Amount	Granular Type	Large Irregular Vacuolated Type	Mature Type	Remarks Follicle Size in Micra
62-61	Jan. 29	49	15	20	++++	+++	+	+++	1981.35
59-17	Feb. 2	--	20	17	++++	+++	+	+++	2163.7 1792.8
62-106	Feb. 5	45	18	15	+++++	+++	+	++	2967.4
62-112	Feb. 5	40	16	17	+++++	++++	++	+	2380.07
62-114	Feb. 5	46	20	20	+++++	+++++	++	+	1780.05
62-121	Feb. 6	47	18	16	++++	++++	++	+	2163.70

ESTROUS OVARIES

216	Apr. 19	--	16	21	++++	+	+	++++	7109.3
E1290	--	--	20	--	++++++	+	+++	++++++	7727.5
62-325	Oct. 29	55	16	15	++++	+++	-	++++	5872.9
D-23-57	Dec. 12	--	18	19	++++	+++	+	+++	4791.05

ANIMALS WITH BLASTOCYSTS

Animal Number	Date	Wt. in Lbs.	Ovary Size in Milli-meters	Corpus Luteum Size in Milli-meters	Interstitial Cells				Remarks
					Total Amount	Granular Type	Large Irregular Type	Mature Type	
62-2	Jan. 22	48	22(L)	6	+++	+++	-	+	
62-11	Jan. 23	45	16(R)	5	+++	++	++	-	
62-15	Jan. 24	47	21(L)	5	+++	+++	-	+	1 corpus albicans
62-19	Jan. 24	42	15(R)	5	+++	+++	-	+	
62-20	Jan. 24	39	19(R)	8	++	++	-	+	
62-22	Jan. 25	46	20(L)	10	++	++	-	+	1 corpus albicans
62-32	Jan. 25	58	15(R)	6	+	++	+	+	
62-34	Jan. 25	47	20(L)	5	+++	+++	-	-	
62-41	Jan. 26	55	18(R)	11	++++	+++++	+	+	Blastocyst ready to implant
62-42	Jan. 26	38	15(R)	7	++	++	-	+	
62-44	Jan. 26	48	18(R)	7	++	++	-	-	
62-46	Jan. 28	40	22(L)	10	++	++	-	+	
62-51	Jan. 29	37	19(L)	6	+++++	+	++	++++	Recent ovulation and lactating
62-60	Jan. 29	45	15(L)	5	+++	++	+	-	
62-64	Jan. 29	46	18(R)	14	++	++	+	+	
62-68	Jan. 31	49	17(R)	8	++	+	-	+	

ANIMALS WITH BLASTOCYSTS (continued)

Animal Number	Date	Wt. in Lbs.	Ovary Size in Milli-meters	Corpus Luteum Size in Milli-meters	Interstitial Cells				Remarks
					Total Amount	Granular Type	Large Irregular Type	Mature Type	
62-78	Feb. 1	38	16(R)	5	++++	++	+	++	
62-105	Feb. 5	52	19(L)	6	++	++	-	-	
62-111	Feb. 5	39	20(L)	7	++	+	-	+	
62-124	Feb. 6	39	12(L)	4	++	+	-	+	One corpus albicans
62-131	Feb. 9	45	16(R)	6	++	++	-	-	
59-13	Feb. 9	34	18(L)	8	++	++	+	++	Blastocyst recovered
62-133	Feb. 9	36	17(R)	6	++	+	+	-	
62-137	Feb. 9	41	18(R)	9	++	++	+	+	
62-140	Feb. 10	44	15(R)	7	++	+	+	+	
62-144	Feb. 10	49	19(L)	7	++	+	+	+	
62-146	Feb. 10	39	17(L)	8	++++	++	++	+++	Recent ovulation
62-154	Feb. 13	41	15(R)	5	++	++	-	-	Crypt budding
62-155	Feb. 13	40	20(L)	5	++	+	++	-	
62-160	Feb. 13	39	15(L)	9	++	+	-	+	
62-163	Feb. 13	50	16(R)	7	+++	++	+	+	
62-164	Feb. 13	51	18(L)	9	++	++	-	-	One corpus albicans
62-169	Feb. 13	48	12(L)	11	++	++	+	+	

ANIMALS WITH BLASTOCYSTS (continued)

Animal Number	Date	Wt. in Lbs.	Ovary Size in Milli-meters	Corpus Luteum Size in Milli-meters	Interstitial Cells				Remarks
					Total Amount	Granular Type	Large Irregular Type	Mature Type	
62-170	Feb. 13	45	19(L)	5	++	++	-	-	
59-14	Feb. 16	40	16(R)	6	+++	++	+	+	Blastocyst recovered
59-15	Feb. 16	52	13(R)	5	+++	++	+	+	"
59-48	Mar. 4	62	16(L)	6	+++	++	+	+	
62-224	Mar. 9	43	16(L)	5	++++	++	++++	+++	Recent ovulation
62-268	Mar. 9	--	20(L)	5	++	++	+	-	Two corpora lutea
59-73	Mar. 15	--	12(L)	5	++	+	+	-	
59-69	Mar. 15	--	19(L)	6	++	+	-	+	Blastocyst recovered
22-54	Mar. 15	43	16(L)	6	+++	++	++	+	
29-54	Mar. 20	39	15(L)	5	++	+	-	+	Blastocyst recovered
59-85	Mar. 27	37.5	16(L)	6	++	+	+	+	"
59-103	Apr. 21	61	18(R)	7	++	++	-	+	"
59-130	Apr. 21	39	18(R)	10	++	++	+	+	
59-160	May 9	56	14(R)	10	++	++	-	+	
60-9	June 11	--	18(R)	8	+++++	++	++	++++	Recent ovulation
79-56	July 22	37.5	17(R)	7	+++	++	+	++	Blastocyst ready to implant
107-55	Sept.	--	14(R)	6	++	++	-	+	Blastocyst recovered
B-12-57	Oct. 12	--	14(L)	5	++	++	-	+	"

ANIMALS WITH BLASTOCYSTS (continued)

Animal Number	Date	Wt. in Lbs.	Ovary Size in Milli-meters	Corpus Luteum Size in Milli-meters	Interstitial Cells				Remarks
					Total Amount	Granular Type	Large Irregular Type	Mature Type	
62-307	Oct. 26	50	18(R)	5	+++	+	++	+	One corpus albicans
62-326	Oct. 29	46	18(R)	8	+	+	-	+	Lactating
62-330	Oct. 30	47	12(L)	4	++	++	+	-	
D-7-57	Dec. 6	--	15(L)	6	+++	++	++	++	Recent ovulation

ANIMALS WITH IMPLANTED EMBRYOS

Animal Number	Date	Wt. in Lbs.	Ovary Size in Milli-meters	Corpus Luteum Size in Milli-meters	Total Body Length of Fetus	Interstitial Cells			
						Total Amount	Granular Type	Large Irregular Type	Mature Type
62-8	Jan. 23	44	17(L)	9	-				
			16(R)	14	225	++	+	+	+
62-23	Jan. 25	49	22(L)	14	45	++	++	+	+
62-27	"	47	18(L)	15	55	++	++	-	++
62-29	"	51	16(L)	11	190	+++	++	+	++
62-30	"	50	21(L)	12	--	+++	++	+	++
62-31	"	51	18(R)	12	142	+++	++	-	++
62-33	"	52	15(L)	13	320	++	+	-	++
62-35	"	56	15(R)	12	360	++	++	-	+
62-37	"	45	19(L)	17	370	++	+	+	+
62-39	Jan. 26	51	20(L)	15	370	+	+	-	+
62-40	"	47	22(R)	15	--	++	+	+	+
62-43	"	45	22(L)	12	--	++	+	+	+
62-47	Jan. 28	59	22(R)	17	240	++	++	+	+
62-48	"	39	17(R)	10	--	++	++	-	+
62-53	Jan. 29	44	16(R)	13	--	+++	+	+	++
62-90	Feb. 3	49	18(R)	12	--	+++++	++	++	++++
62-108	Feb. 5	47	16(R)	12	280	++	++	-	++
62-109	"	50	20(L)	14	--	++++	++++	+	+
62-135	Feb. 9	42	19(R)	14	190	++	+	-	++
62-136	"	51	18(R)	12	50	++	++	+	+
62-141	Feb. 10	54	20(L)	9	440	++	++	+	++
62-147	"	54	17(L)	70	--	++	-	+	+
62-149	"	54	23(R)	14	440	+++	++	+	++
62-150	"	53	16(L)	14	--	++	+	-	++
62-168	Feb. 13	35	22(L)	12	--	++	++	-	++
62-175	"	32	18(L)	15	--	++	+	+	+
62-176	Feb. 18	35	18(L)	15	280	++	+	+	+
60-4	June 6	65	19(L)	10	384	+	+	-	-

APPENDIX TABLE II

UTERINE HORNS

Animal Number	Estrous Condition	Rugae Lining With Cell Type		Height of the Surface Epithelial Cells	Uterine Glands, Cell Type	Height of Gland Cells	Secretion	Vascularity	Stroma	Remarks
59-93	Sub-adult	Squamous Low Cuboidal	L	4.9	Cuboidal	7.29	-	Very low	Large	Few straight glands
			R	5.0		7.29	-			
62-225	Anestrus	Cuboidal, central Large nuclei	L	9.7	Low columar Central or based nuclei	12.15	-	Low	Large	Few straight glands
			R	9.7		12.15	-			
60-16	Postpartum anestrus	Large nuclei Low columnar	L	9.72	"	12.15	-	Medium	"	coiled glands
			R	12.15	Columnar	14.58	-	"	Medium	Recent corpus albicans
62-24	Postpartum anestrus	Low columnar, central nuclei	L	9.72	Columnar central nuclei	12.15	-	Low	Large	Straight gland
		Cuboidal	R	7.29	Low Columnar	9.72	-	"	"	"
62-38	Postpartum Anestrus	"	L	9.72	"	12.15	-	"	"	"
		-	R	--	--	--	--	--	--	--

UTERINE HORNS (continued)

Animal Number	Estrous Condition	Rugae Lining With Cell Type		Height of the Surface Epithelial Cells	Uterine Glands, Cell Type	Height or Gland Cells	Secretion	Vascularity	Stroma	Remarks
62-121	Early Proestrus	Cuboidal	L	7.29	Low columnar	12.15	-	Low	Large	Not stimulated. Straight glands
		"	R	9.72	"	12.15	-	"	"	
59-14	--	Low columnar with elongated nuclei	L	17.01	Columnar with basal nuclei	19.44	--	Medium	Small	Compact and coiled glands
	Blastocyst recovered	Columnar with Basal nuclei	R	21.87	"	24.3	+	"	"	"
59-69	Blastocyst recovered	Columnar basal nuclei	L	19.44	"	26.73	+	"	"	"
	--	"	R	12.15	"	19.44	+	"	"	"
59-85	Blastocyst recovered	Columnar with elongated nuclei	L	26.73	Columnar cell with rounded nuclei	29.16	+	Medium	Small	Compact and coiled glands
	--	"	R	19.44	"	21.87	+	"	"	"

UTERINE HORNS (continued)

Animal Number	Estrous Condition	Rugae Lining With Cell Type		Height of the Surface Epithelial Cells	Uterine Glands, Cell Type	Height or Gland Cells	Secretion	Vascularity	Stroma	Remarks
79-56	--	Low columnar	L	17.01	"	24.3	+	Very High	Small	Compact and very coiled glands
	Blastocyst ready to implant	Columnar	R	24.3	"	24.3	+	"	"	"
107-55	--	"	L	17.01	"	19.44	+	Medium	"	Compact and coiled glands
	Blastocyst recovered	"	R	24.3		26.73	+	"	"	"
B-12-57	Blastocyst recovered	"	L	19.44	"	19.44	+	"	"	"
	--	Cuboidal or low columnar	R	9.72	"	14.58	+	"	"	"
60-9	--	"	L	12.15	"	19.44	-	"	"	"
	Blastocyst assumed	-	R	-	-	-	-	-	-	-
59-160	--	"	L	17.01	"	24.3	+	"	"	"
	Blastocyst assumed	-	R	-	-	-	-	-	-	-

UTERINE HORNS (continued)

Animal Number	Estrous Condition	Rugae Lining With Cell Type		Height of the Surface Epithelial Cells	Uterine Glands, Cell Type	Height of Gland Cells	Secretion	Vascularity	Stroma	Remarks
62-163	--	"	L	14.58	"	24.3	-	"	"	"
	Blastocyst assumed	"	R	17.01	"	24.3	-	"	"	"
62-41	--	"	L	17.01	"	24.3	+	"	"	Compact and very coiled glands
	Blastocyst ready to implant	"	R	19.48	"	24.3	-	"	"	"
62-146	Blastocyst assumed	Columnar cells	L	14.58	Columnar cells	17.01	+	Medium	Medium	Coiled glands
	--	"	R	14.58	"	17.01	+	"	"	"
62-140	--	"	L	19.44	"	24.3	+	"	small	Compact and coiled glands
	Blastocyst assumed	"	R	21.87	"	26.73	+	"	"	"
62-224	Blastocyst assumed	"	L	17.01	"	24.3	-	"	"	"
	--	"	R	17.01	"	24.3	-	"	"	"
62-40	--	"	L	17.01	"	19.44	+	Large	"	Very coiled, complex glands
	Fetus	"	R	21.87	"	19.44	++	"	"	"

UTERINE HORNS (continued)

Animal Number	Estrous Condition	Rugae Lining With Cell Type		Height of the Surface Epithelial Cells	Uterine Glands, Cell Type	Height or Gland Cells	Secretion	Vascularity	Stroma	Remarks
62-108	--	"	L	17.01	"	19.44	+	"	"	"
	Fetus	"	R	17.01	"	19.44	++	"	"	"
62-175	--	"	L	17.01	"	17.01	+	"	"	"
	Fetus	"	R	19.44	"	17.01	++	"	"	"
62-325	Estrus	Columnar cells with elongated nuclei	L	17.01	Cuboidal or low columnar cells	14.58	-	Medium	Medium	Beginning of coiling in the glands
D-23-57	"	"		14.58	"	17.01	-	"	"	"
62-326	Blastocyst assumed	Columnar cells with basal nuclei		21.87	Columnar	24.3	+	"	"	Compact and coiled glands

APPENDIX TABLE III

SUB-ADULT ANIMALS

Animal Number	Date	Weight in Pounds	Ovary Size in Millimeters L	Ovary Size in Millimeters R	Interstitial Cells: Total Amount	Interstitial Cells: Granular Type	Interstitial Cells: Large Irregular Vacuolated Type	Interstitial Cells: Mature Type	Remarks
59-93	Mar. 29	20	7	7	++	++	-	+	
62-157	--	--	10	11	++	++	-	-	
62-166	--	--	10	10	+	+	-	-	
62-320	Oct. 27	25	10	9	+	+	-	-	

APPENDIX TABLE IV

Animal Number	Follicle Size in Micra	Ovum Size in Micra
62-12	38.9	26.7
	36.45	26.7
62-15	51.03	31.6
62-21	43.7	34.0
	1143.7	99.7
62-36	19.44	17.0
	26.73	21.8
62-38	75.33	38.9
62-54	291.6	130.7
	38.9	34.02
62-61	1981.3	125.5
62-62	43.74	31.6
	121.5	97.2
	243.0	126.6
59-17	2163.7	123.7
	80.19	58.3
	121.5	85.0
	157.8	97.0
	1792.8	112.7
62-87	72.90	36.45
	29.16	21.9
	26.7	21.9
	60.75	34.02
	145.8	85.05
62-106	2967.4	123.7
62-112	2380.07	123.7
	53.5	31.6
62-121	4263.7	123.7
62-130	29.16	21.3
	29.2	24.3
	55.9	34.02
	65.61	34.02
	157.95	102.06
	36.45	29.16
60-16	80.2	41.3
	150.7	99.6
60-19	374.85	96.4
D-23-51	4791.05	--
62-2	1854.6	123.7
	51.03	38.9
62-11	1391.0	123.7
62-19	170.10	97.2
	121.5	60.75
	60.75	38.9
	888.9	128.5
	41.31	24.3
	36.45	29.16
	31.6	26.7
62-42	123.9	97.20
	60.75	31.6
62-51	111.8	91.4
	642.6	125.5
62-57	2009.2	123.6
	29.16	26.73
	55.9	31.6
	31.6	24.3
	36.45	26.7
	63.18	34.02

APPENDIX TABLE IV (continued)

Animal Number	Follicle Size in Micra	Ovum Size in Micra
62-124	85.0	36.4
	157.95	85.0
62-105	133.65	92.3
	170.1	85.0
62-131	34.0	24.3
	36.4	26.7
	41.3	31.6
	43.7	31.6
	29.16	24.3
62-140	85.0	46.2
	53.46	31.9
62-160	135.65	72.90
	206.6	85.0
62-31	31.6	26.73
	29.16	24.3
	41.31	26.73
59-85	535.5	117.8
60-4	1607.3	92.7
	1391.0	92.73
62-35	1298.2	123.64
62-37	36.45	29.16
	38.9	29.16
	53.96	36.45
	963.9	117.8
	803.2	128.5
62-39	696.15	74.97
62-40	36.45	26.73
	29.16	21.89
	155.5	102.0
	1700.0	123.64
62-90	206.6	110.64
	1483.68	92.74
	1124.55	149.94
	80.19	43.74
	89.91	43.74
62-176	910.35	126.4
	194.4	126.4
	803.2	123.6
	364.1	123.6
216	7109.3	--
E1290	7727.5	--
62-325	5875.0	--

APPENDIX TABLE V

BLASTOCYSTS RECOVERED

Animal Number	Size of the Blastocyst in Micra	Thickness of Zona Pellucida	Layers of Zona Pellucida		Number of Cells in the Inner Cell Mass	Trophoblast Cells	Remarks
			Thin Outer Layer	Dense Homogeneous Inner Layer			
59-13	97.2	4	-	-	-	Not distinct	Oval shape
59-14	153.0	5	+	+	40	Flattened	Cell division seen, oval shape
59-15	471.24	5	+	+	-	"	Broken Shrunken
59-69	471.3	5	+	+	250	"	"
59-85	353.0	6	+	+	cells lost	"	"
59-103	428.0	5	+	+	450	"	"
29-54	--	5	+	+	40	"	"
107-55	201.7	5	+	+	90	"	oval shape
B-12-57	--	4	+	+	250	"	Shrunken
62-41	425	5	-	-	500	-	Ready to implant
79-56	--	-	-	-	500	-	"

PLATES

PLATE I

Figure 1. Sub-adult ovary showing many oocytes and atresia. X 14.

Figure 2. Sub-adult horn is showing the mucosa, the uterine glands, the non-oedamatous stroma and the muscle layers. X 100.

Figure 3. Anestrus ovary. Large number of oocytes and small Graafian follicles are seen. Note the columns and nests in the cortex. X 12.

Figure 4. Inactive anestrus ovary showing large central medulla with vessels. X 6.5.

Figure 5. Anestrus ovary showing the deep sub-surface crypts. Note the old corpora albicantia with peripheral advential vessels. X 6.5.

Figure 6. Estrous ovary. A large pre-ovulatory follicle with the ovulation cone is seen. Note an old corpus albicans with peripheral advential vessels. X 8.

Figure 7. Post partum anestrus ovary. Many small and medium sized Graafian follicles are atretic. Also note an old corpus albicans with the advential vessels. X 8.

Figure 8. Post partum anestrus ovary. A recent corpus albicans showing the central core of collagenous fibers and the peripheral degenerating luteal cells. X 7.5.

Figure 9. Post partum anestrus ovary. A recent corpus albicans showing the central collagenous fibers and the peripheral degenerating luteal cells. Also note the peripheral advential vessels. X 8.

Figure 10. Anestrus ovary showing interstitial cells. Note the large cyst in the fimbria of the oviduct. X 8.

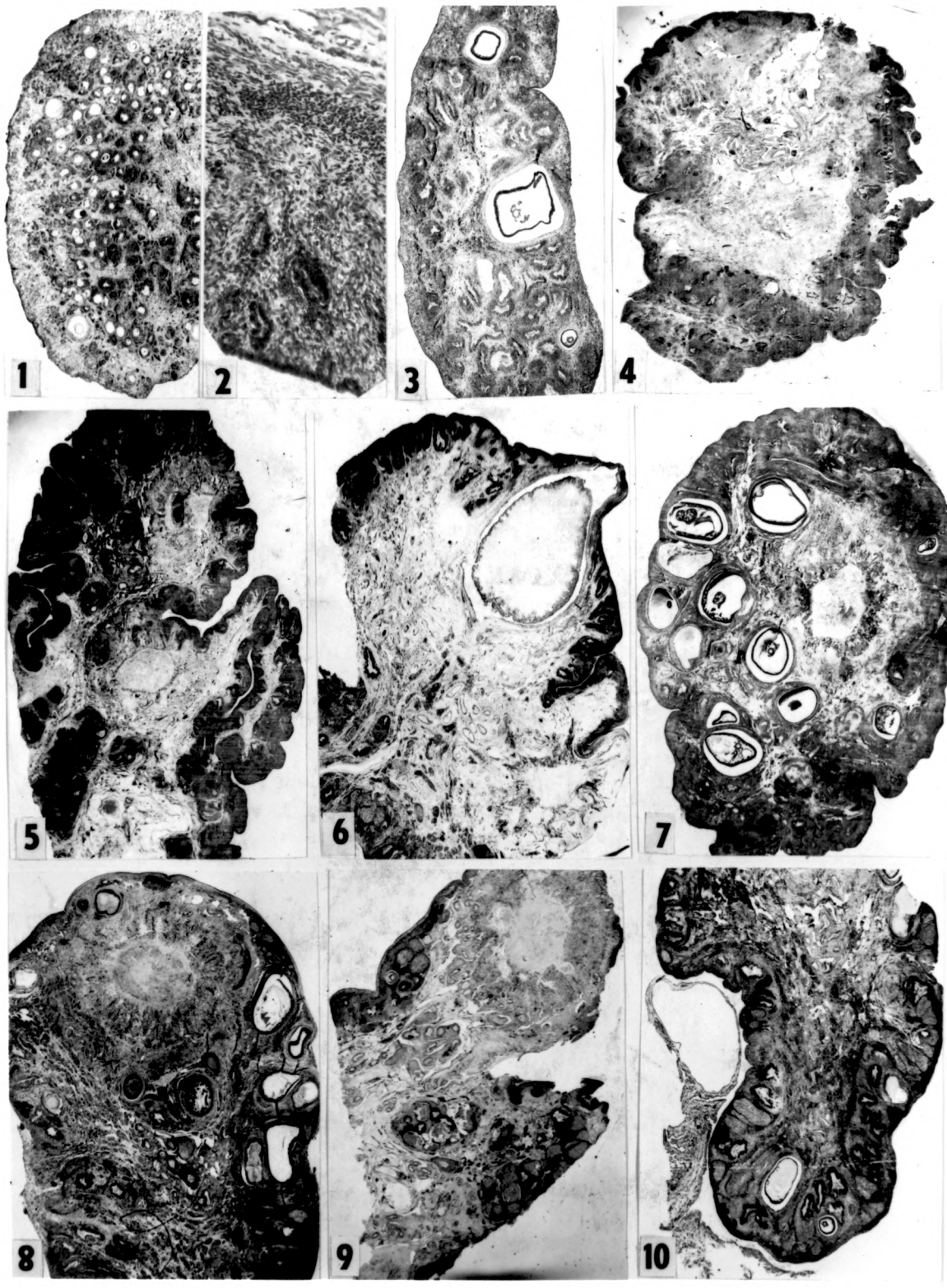
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PLATE II

Figure 11. Detail of the estrous ovary (Figure 40). Note the large amount of mature interstitial cells. X 100.

Figure 12. Both granular and mature interstitial cells are seen around an atretic follicle. X 250.

Figure 13. The mature interstitial cells are being differentiated from the thecal elements of the atretic follicle and from the granulosa cells of the atretic follicle. Note the polyhedral interstitial cells with the vesicular nuclei and the sinusoids. X 250.

Figure 14. The atretic follicle showing the glassy membrane, the zona pellucida and the degenerated ovum. Discrete clumps of the granular interstitial cells are seen. Note the mature interstitial cells. X 100.

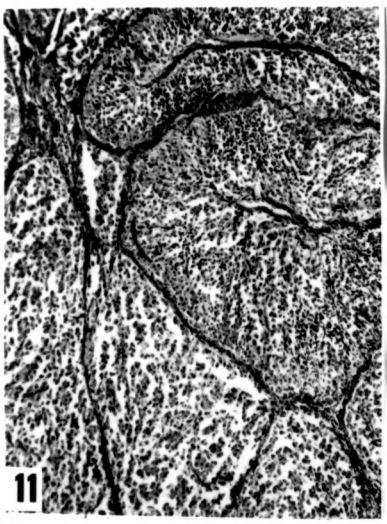
Figure 15. The epithelial cells are seen leaving the sub-surface crypt. X 250.

Figure 16. Ovary showing the multiple layered germinal epithelium. The epithelial cells are projecting into the cortex and on the ovarian surface. The tunica albuginea is disrupted and disorganized. The stromal cells are seen migrating to the epithelial cells at the disorganized tunica albuginea. Note mitotic figures in the epithelial cells. X 400.

Figure 17. Detail of the recent corpus albicans shown in Figure 8. The degenerating luteal cells show vacuolation, fragmentation and pycnotic nuclei. The luteal cells have greatly shrunken. X 400 oil.

Figure 18. Detail of the recent corpus albicans shown in Figure 9. The luteal cells are greatly shrunken and have pycnotic nuclei. X 400 oil.

Figure 19. A sub-surface crypt showing budding of the epithelial tubes into the cortex. X 100.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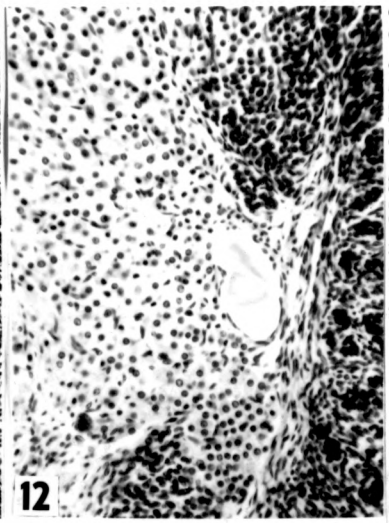
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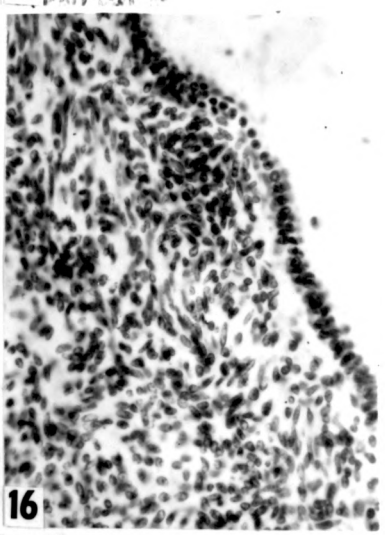
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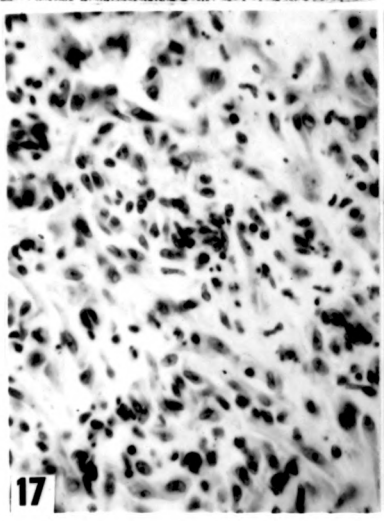
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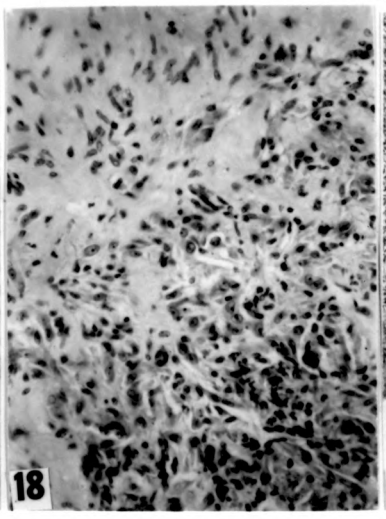
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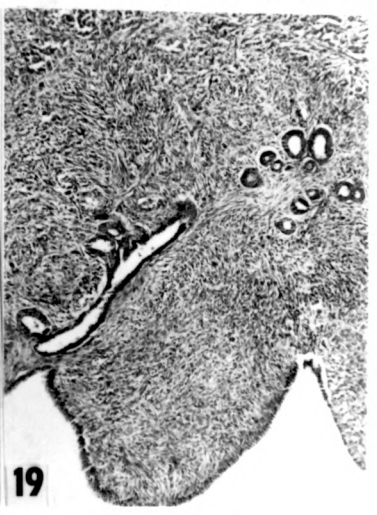
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PLATE III

Figure 20. Ovary with a recent corpus luteum in an animal with unrecovered blastocyst. Note the large irregular vacuolated and mature interstitial cells. X 8.

Figure 21. Corpus luteum with antrum in an animal with a recovered blastocyst. Also note the deep sub-surface crypts. X 8.

Figure 22. Ovary from an animal with a recovered blastocyst. The corpus luteum is compact. X 8.

Figure 23. Ovary of an early implanted pregnancy showing a compact corpus luteum. X 8.

Figure 24. Corpus luteum of mid-pregnancy showing cavitation. Also note the deep sub-surface crypt. X 8

Figure 25. Detail of the corpus luteum shown in Figure 21. Note the regularity in the cords of the luteal cells. The cells are polyhedral and elongated. X 400 oil.

Figure 26. Luteal cells in an animal with unrecovered blastocyst are similar to that of the luteal cells in Figure 25. X 400 oil.

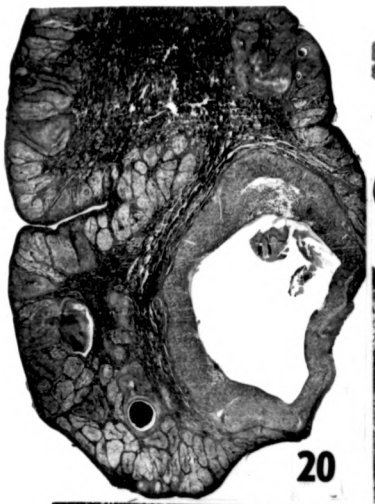
Figure 27. Ovary showing an intermediate size of the luteal cells in an animal with blastocyst. X 400 oil.

Figure 28. The animal ready to implant has greatly hypertrophied luteal cells. The corpus luteum is highly vascularized. X 400 oil.

Figure 29. Note the hypertrophied and polygonal luteal cells in the animal with about 20 millimeters long embryo. X 400 oil.

Figure 30. Note the luteal cells in the animal having 100 millimeters long fetus. X 400 oil.

Figure 31. Note the luteal cells in the animal having 440 millimeters long fetus. X 400 oil.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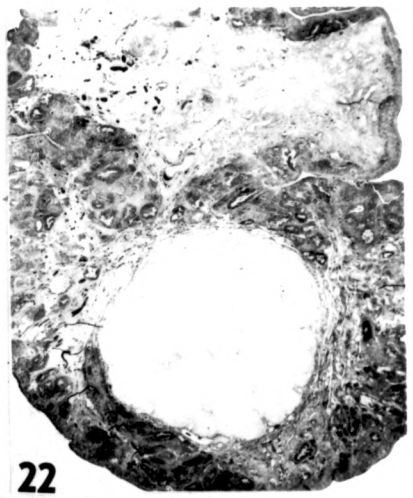
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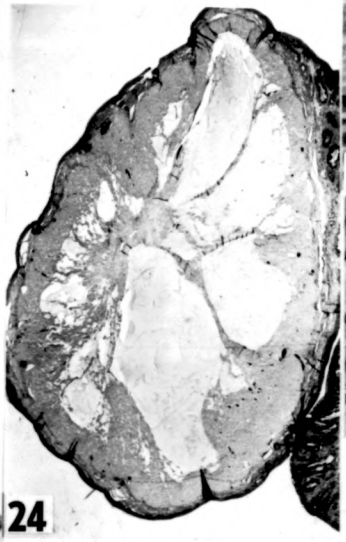
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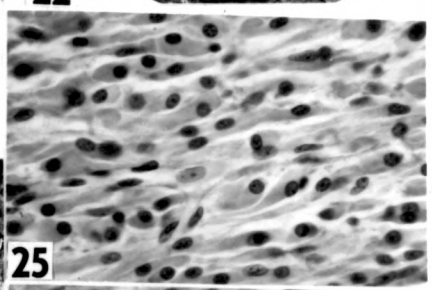
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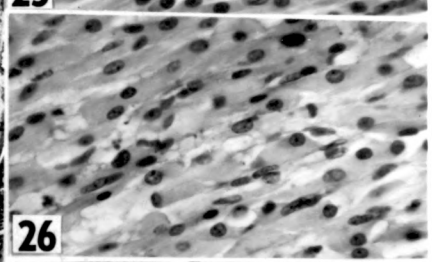
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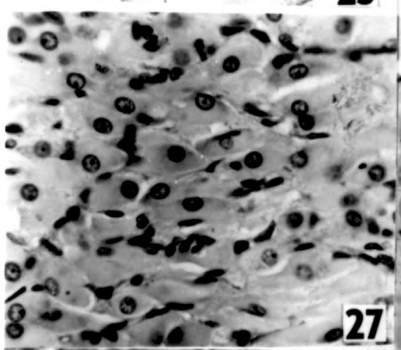
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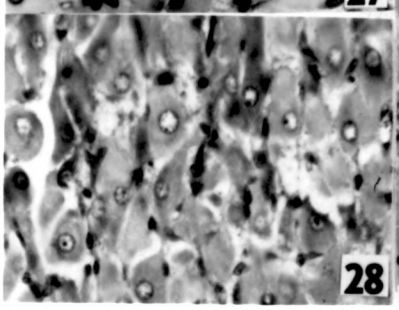
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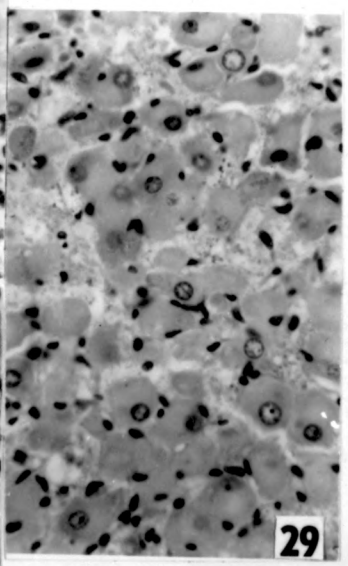
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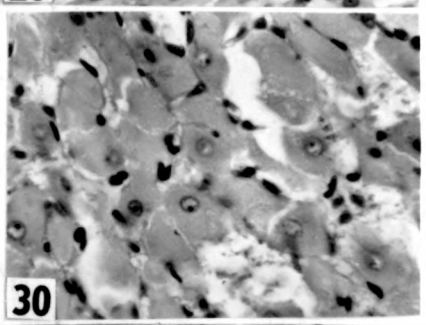
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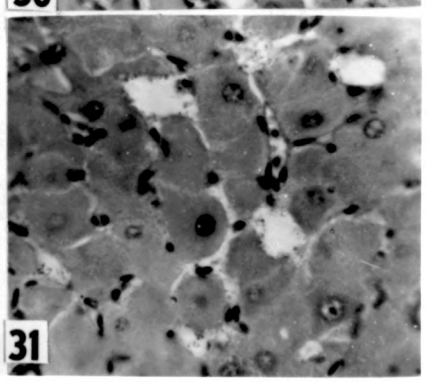
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PLATE IV

Figure 32. Ovary showing follicle type 1. X 400 oil.

Figure 33. Ovary showing follicle type 2. X 250

Figure 34. Ovary showing follicle type 3. X 400 oil.

Figure 35. Ovary showing follicle type 4. X 250

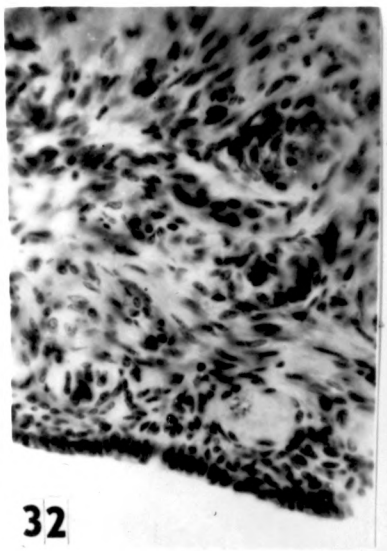
Figure 36. Ovary showing follicle type 5. X 250

Figure 37. Ovary showing follicle type 6. X 250

Figure 38. Ovary showing follicle type 7. X 100

Figure 39. Ovary showing late follicle type 8. Note the granular interstitial cells. X 40.

Figure 40. Estrus ovary. A pre-ovulatory follicle type 9, with the ovulation cone. Note the large amount of the mature interstitial cells and the deep sub-surface crypts. X 6.5



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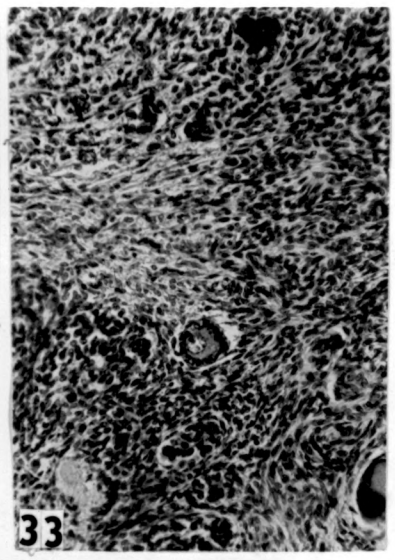

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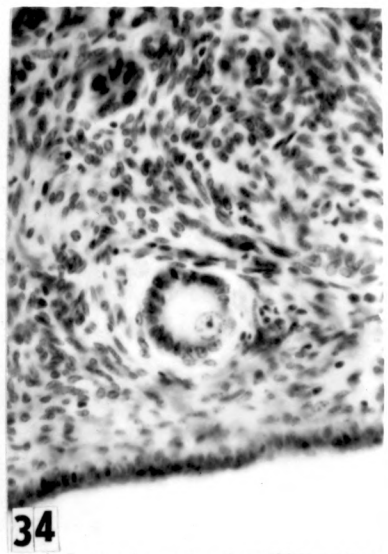

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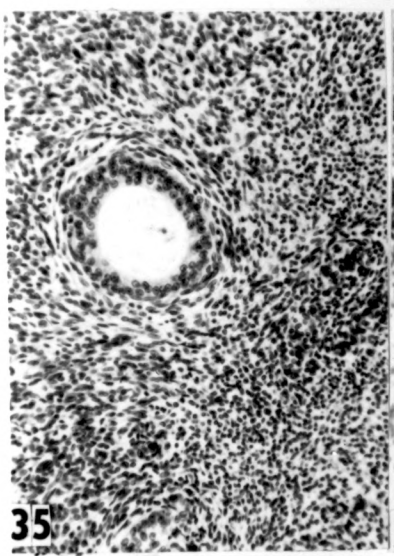

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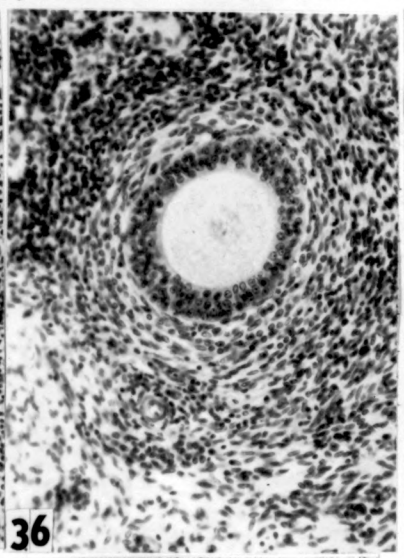

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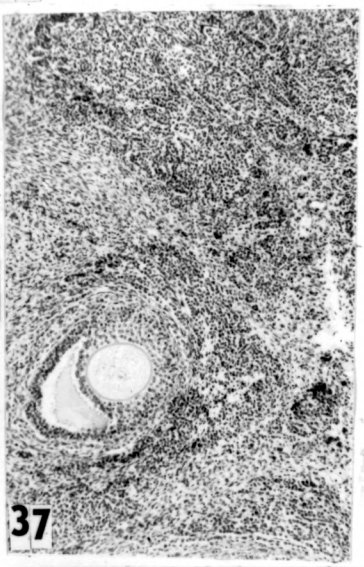

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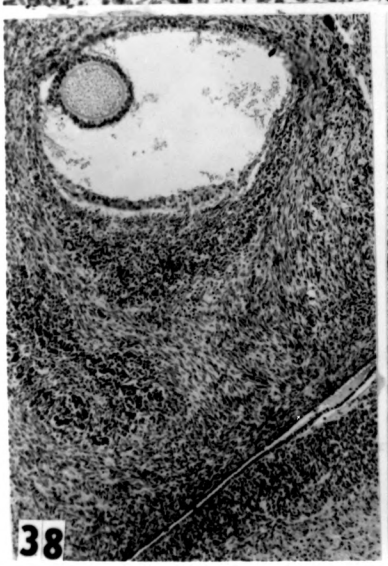

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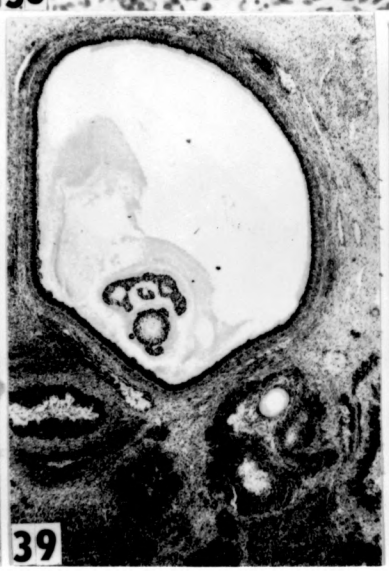

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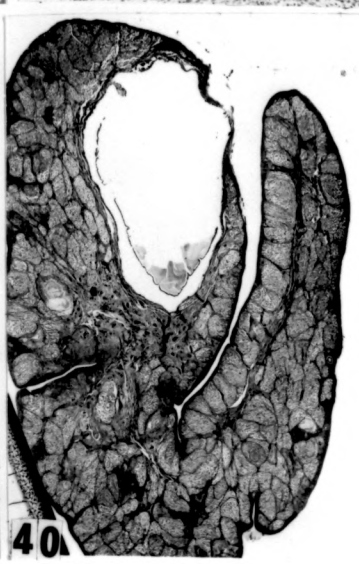

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PLATE V

Figure 41. An anestrus uterine horn showing surface epithelium, uterine glands and the stroma. X 100.

Figure 42. Estrous uterine horn showing the epithelium and the coiled uterine glands. The ovary of this animal is shown in Figure 6. X 100

Figure 43. Oviduct showing stimulation corresponding to the horn (Figure 42). X 100

Figure 44. Horn showing oedamatous stroma and compact uterine glands. The associated ovary is shown in Figure 20. X 100

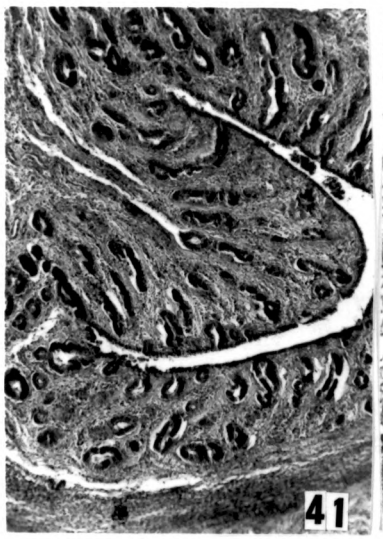
Figure 45. Uterine horn congested with uterine glands. Note the inner cell mass and the trophoblast cells in the unimplanted blastocyst. The zona pellucida has dense and thinner outer layer, and homogeneous and thicker inner layer. The ovary of this animal is shown in Figure 21. X 100

Figure 46. Horn congested with coiled uterine glands. Note the increase in cell number of the inner cell mass of the unimplanted blastocyst. X 100

Figure 47. The blastocyst in this animal is ready to implant. Note the increased complexity in the uterine glands. The endometrium is highly vascular. The associated luteal cells are shown in Figure 28. X 100

Figure 48. A recent post partum horn showing the complex inner zone of the uterine glands. The stroma is oedamatous. The ovary of this animal is shown in Figure 8. X 100

Figure 49. A post partum anestrus horn showing the reduced uterine glands and the epithelial cells. The stroma shows some advential vessels. X 100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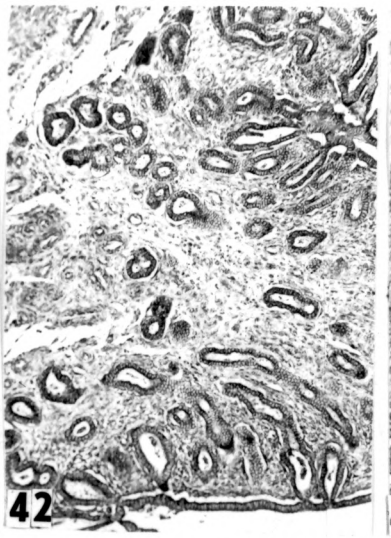
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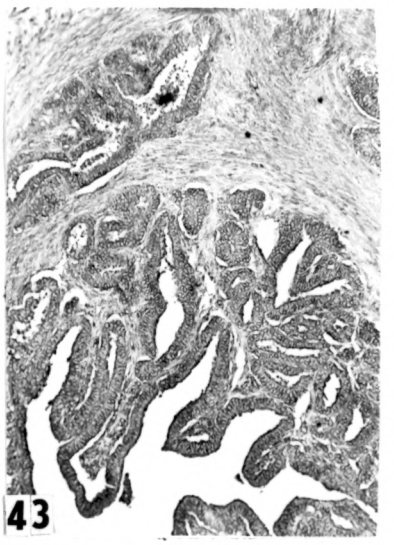
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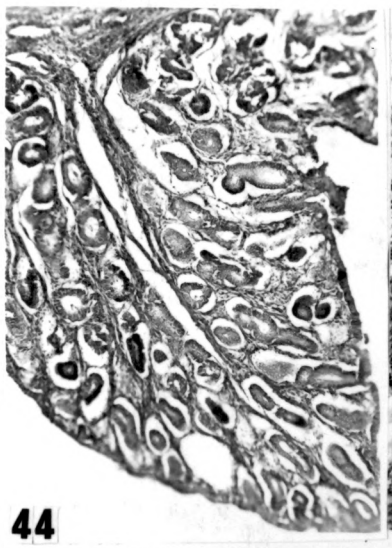
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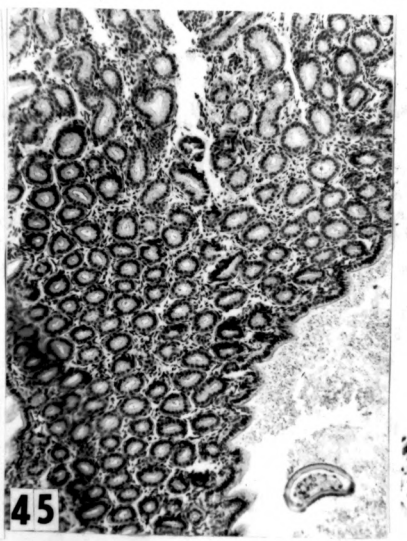
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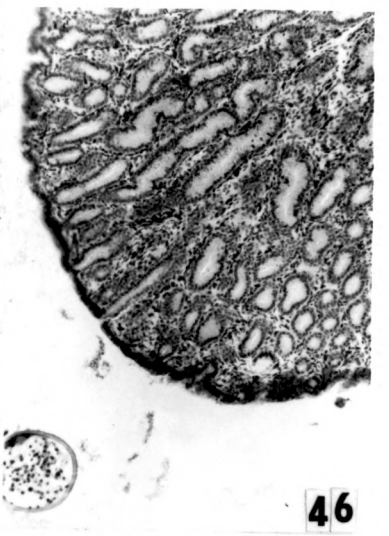
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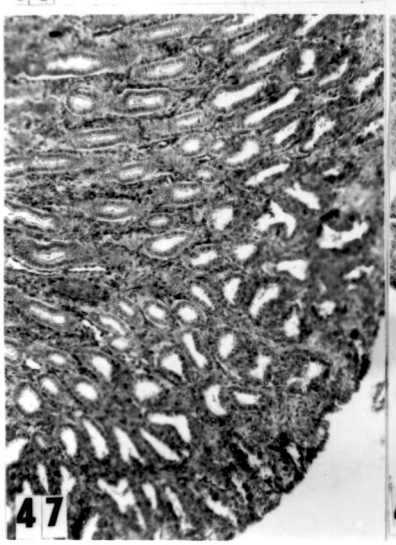
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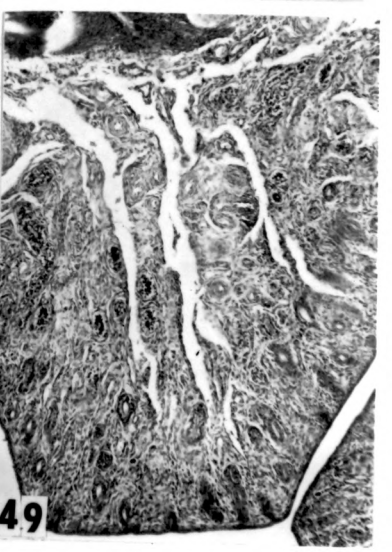
49

PLATE VI

Figure 50. Showing the relation of ovum growth to follicle growth. Data on the sea otter.

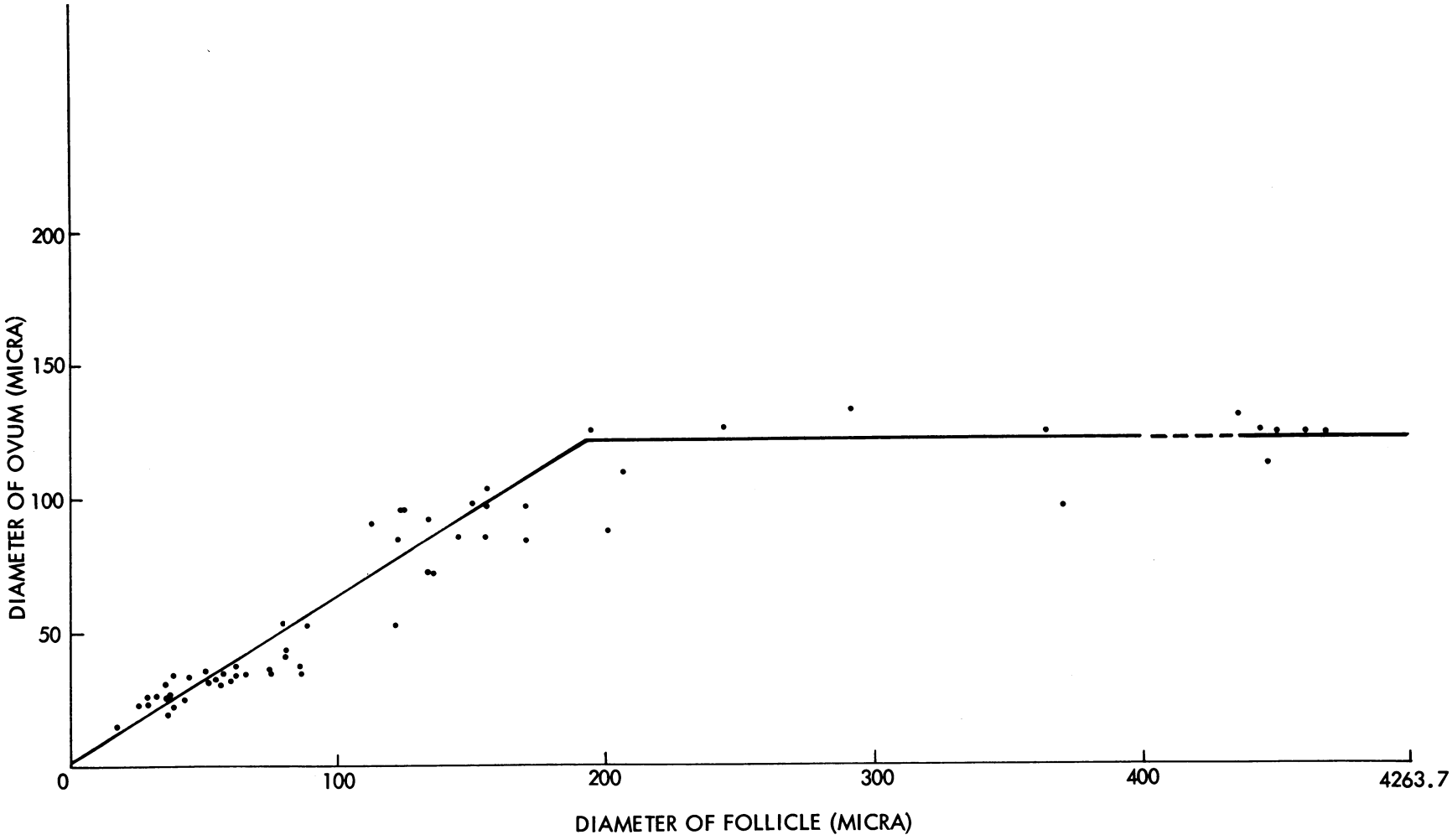
DIAMETER OF OVUM (MICRA)
200
150
100
50
0
100
200
300
400
4263.7
DIAMETER OF FOLLICLE (MICRA)

Figure 50

PLATE VII

Figure 51. Ovary, bursa, uterus and vagina of a sub-adult sea otter. Muscular membrane septum separating the two uterine horns in the corpus uterus. Note ventral slit-like opening of the cervix.

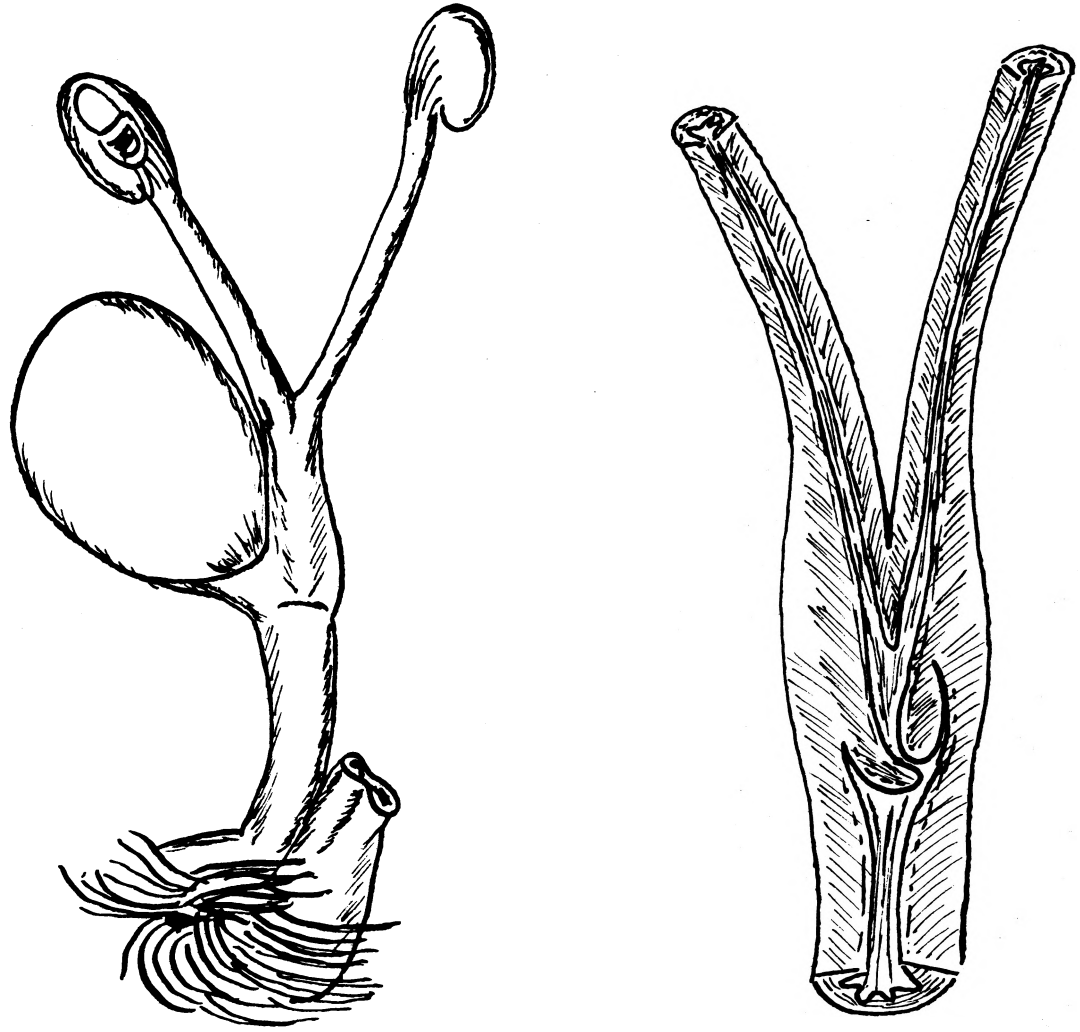

Figure 51

PLATE VIII

Figure 52. Ovary, bursa and uterus of an anestrus adult sea otter. Muscular membrane septum separating the two uterine horns in the corpus uterus. Note ventral slit-like opening of the cervix.

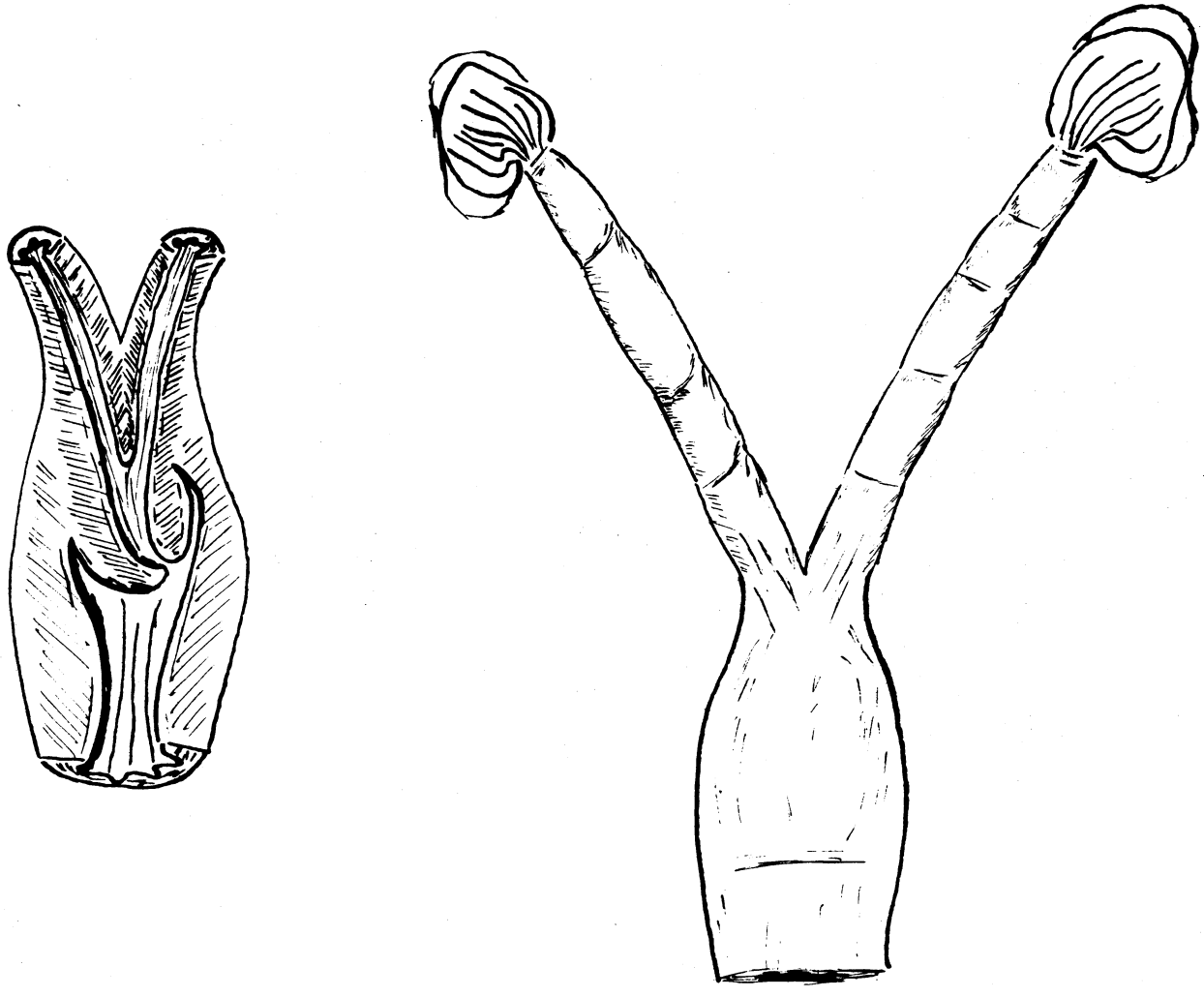

Figure 52

PLATE IX

Figure 53. Ovary, bursa, uterus and vagina of a pregnant sea otter. Uterus is showing fetus, zonary placenta, and the hemophagous organ.

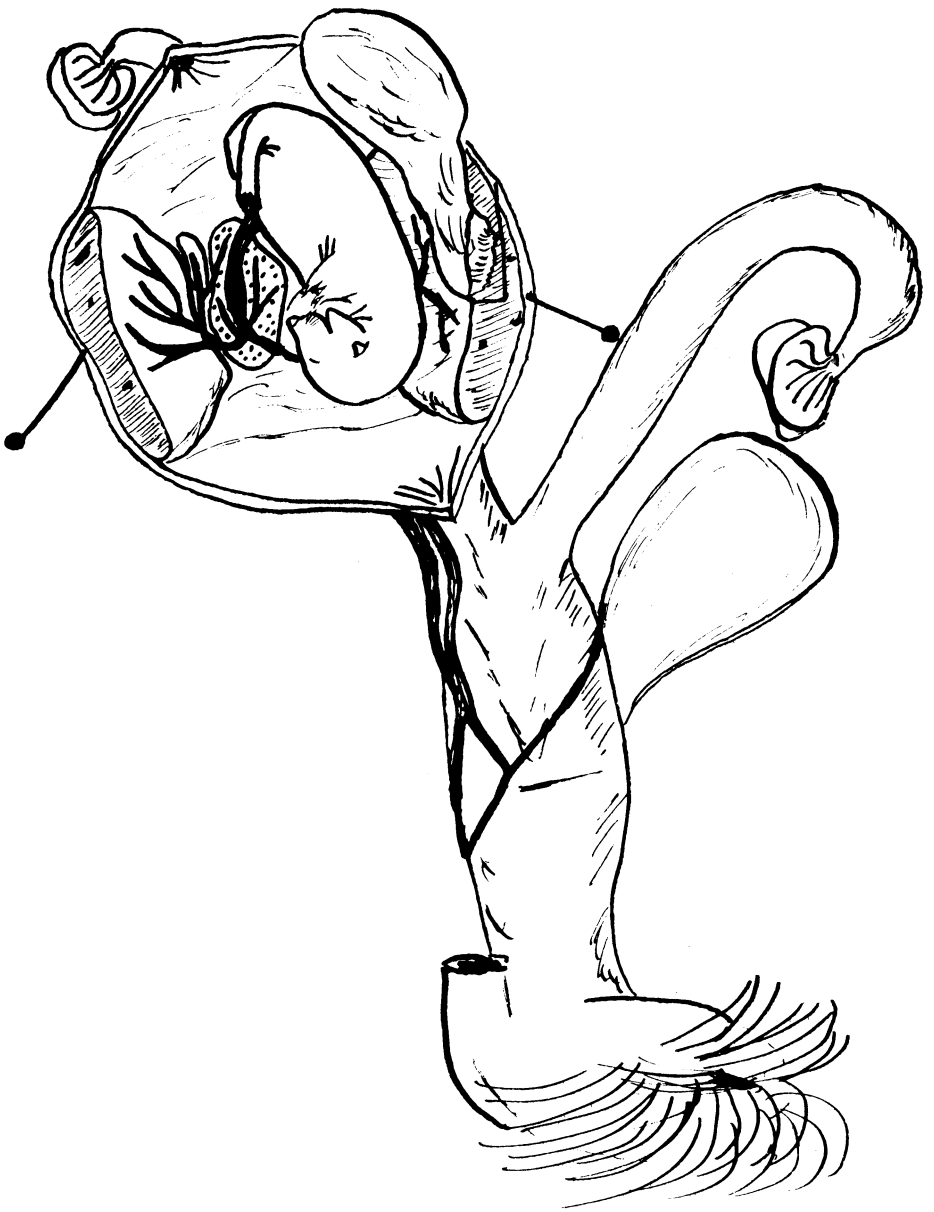

Figure 53

VITA

I, Akhouri Achyutanand Sinha, was born in Churamanpur, Bihar, India, on [redacted]. I attended high school at Arrah and graduated from Arrah Zila School, Arrah, Bihar, India, in June, 1950.

In August, 1950, I commenced undergraduate studies and received a B. S. degree in Zoology from Allahabad University, Allahabad, U.P. in June, 1954.

In September, 1954, I began graduate studies in the Department of Zoology, University of Patna, and graduated with a first class M. S. degree in Zoology in September, 1956.

From November, 1956, to August, 1961, I served with the Ranchi College, Ranchi (Bihar and Ranchi Universities) as a lecturer in Zoology.

Work toward a Ph. D. degree was started in September, 1961, under Dr. Clinton H. Conaway, Professor of Zoology, University of Missouri, Columbia, Missouri, U.S.A. During my doctorate work I was a Graduate and Research Assistant.

I have accepted a Post Doctoral Fellowship in the University of Wisconsin, Madison, Wisconsin, beginning February, 1965.

I owe a great deal of gratitude to my parents Mr. and Mrs. Akhouri Chandra Bhusan Sinha, who shaped my educational

career. I am also grateful to Mr. Gopaljee Prasad and many other relatives and friends who have been keenly interested in my education.

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