THE FOLLICULAR CYCLE IN THE DOG

by

KIT ELIZABETH POLING

B. A., Alaska Methodist University

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H T Gier

Major Professor

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INTRODUCTION

Several classical studies have been made on the follicular cycle in the dog (Evans and Cole, 1931; Evans and Swezy, 1931; Barton, 1945; Raps, 1948). Evans and Cole (1931), Evans and Swezy (1931) and Barton (1945) investigated the estrous cycle in the adult dog but paid little attention to follicular development in the fetus or the immature animal. Raps (1948) dealt solely with development and differentiation within the ovary from birth to six months of age. No comprehensive investigation of the total development and differentiation of the dog ovary has been made. Partial investigations, although helpful in defining the follicular cycle, do not afford an understanding of the total development within the ovary from fetal differentiation to the estrous activities in the mature adult.

The significance of accurate counts of the total number of follicles within the ovary has been ignored in previous studies; it was considered that such counts were tedious, time-consuming tasks without relevance to the follicular cycle (Evans and Swezy, 1931). The present study involves the history of ovarian development from migration of the primordial germ cells into the coelomic epithelium to form a germinal epithelium and subsequent differentiation of the female gonad, to activity during the adult estrous cycle. Fetal and immature ovarian changes were evaluated through histological examination and follicular counts. A pattern of activity in the estrous cycle was established through examination of the epithelial cord activity and computation of the follicular numbers in the ovary.

LITERATURE REVIEW

Many investigations have been made of various phases in the history of the development of the female gonad. One initial question is the origin of the primordial germ cells (PGC's). Basically, ideas on the origin of the PGC's can be summarized by two concepts: extragonadal origin, with accompanying migration and segregation, and gonadal origin with differentiation in situ.

Generally accepted is the concept of extragonadal origin in the yolk sac with subsequent migration from the yolk sac to the region which will differentiate into the gonad: human (Witschi, 1948; McKay et al., 1953); mouse (Mintz, 1957; Blandau, White and Rumery, 1963); rabbit (Chretien, 1966); dog and bovine (Gier and Marion, 1971; Everett, 1945; Fuss, 1912; Rubaschkin, 1912; and Vanneman, 1917.) In the 21 day dog embryo and the 26 day cow embryo, PGC's are present ventral to and between the early mesonephric tubules in the region of somites 16 through 24. PGC's are charcterized by cytoplasmic granules which are PAS positive. As the PGC's settle into the coelomic epithelium of the gonad, these granules disappear (Gier and Marion, 1971). Some consider that germ cells are not an integral part of the germinal epithelium of the gonad but are merely stored in the epithelium (Everett, 1945). Formation of the gonadal ridges occurs simultaneously with the arrival of the PGC's (Brambell, 1930).

By 22 days in the dog and 27 days in the cow, the PGC's are no longer detectable in the gonad but the epithelium where they were previously observed is thickened, indicating the formation of a germinal epithelium by the incorporation of the PGC's in the coelomic epithelium (Gier and Marion, 1971).

In contrast to the extragonadal origin of the PGC's, some attribute the origin to the peritoneal epithelium of the gonad (Hargitt, 1930d). Enlarged cells (generally accepted as PGC's) were observed in the splanchnopleure and dorsal mesentery, but were interpreted as somatic cells which had failed to divide (Hargitt, 1930d). Germ cells were described by Hargitt (1930b) as developing from the peritoneal epithelium of the rat gonad with subsequent migration into the gonadal stroma, thus supporting the concept of gonadal origin of the PGC's. Simkins (1923, 1928) arrived at similar conclusions regarding the origin of the PGC's in man, turtle, mouse and rat.

Following arrival or differentiation of the PGC's, gonadal ridges develop in the region destined to differentiate into the gonad by arrangement and proliferation of the PGC's within the germinal epithelium. Gonadal ridges are first visible in the dog at 23 days and in the cow at 28 days. At this time, there is a germinal epithelium and an increased mesenchyme layer between the thickened epithelium and the mesonephric tubules (Gier and Marion, 1971).

A set of primary epithelial cords develops in the undifferentiated gonad. No sexual distinction can be made at this time. The cords invade the gonadal mesenchyme, eventually breaking connection with the germinal epithelium (Jonckherre, 1930; Greunwald, 1942; Brambell, 1930; Marion and Gier, 1971.) Various origins of these cords have been reported: from the germinal epithelium (Brambell, 1930; Gier and Marion, 1971); mesenchymal, independent of the germinal epithelium (Greunwald, 1942); or a mixture of germinal epithelial and mesenchymal cells (Greunwald, 1942).

Retention of the primary cords to the surface epithelium at the time of secondary cord proliferation has been reported in humans and rodents (Greunwald, 1942). In the dog at 29 days the initial set of cords has separated from the germinal epithelium, completely at 30 days (Gier and Marion, 1971). After detachment, the primary cords in the ovary will define the medullary portion of the female gonad. Medullary cords are destined to degenerate (Brambell, 1930).

Differentiation of sex in the gonad occurs with the formation of a second proliferation of epithelial cords (Brambell, 1930; Gier and Marion, 1969). In the dog, the secondary cords are differentiated by 34 days. At this time, secondary cords define the ovarian cortex, with accompanying disorganization of the medulla (Gier and Marion, 1969). Greunwald (1942) reported that secondary cords in the cat and pig are separate from the germinal epithelium although both primary and secondary cords continue in some areas for a time.

Sexual differentiation of the dog fetal gonad has occurred by 34 days gestation (Gier and Marion, 1969). At birth, the ovary has a cortical rim, a medullary core, and a stalk (Andersen, 1970). The cortex contains oogonia and epithelial cells in clusters which are continuous with the surface epithelium. Raps (1948) reported the oogonia to be undergoing mitotic changes; Hargitt (1930b) and Andersen (1970) reported the oogonia to be undergoing meiotic changes. The medulla contains blood vessels, lymph vessels, rete, and degenerating medullary cords (Andersen, 1970). The germinal epithelium covering the ovary is generally described as being of variable type from cuboidal (Andersen, 1970) to squamous (Barton, 1945; Raps, 1948) varying with age and phase in the reproductive cycle.

Epithelial cords from the germinal epithelium pentrate the tunica albuginea and usually lose their connection to the epithelium (Jonckherre, 1930). Andersen (1970) considered that epithelial cords may penetrate the medulla but usually remain above the layer of primordial follicles. At birth, epithelial cords in the dog are continuous with the germinal epithelium and contain cells which have differentiated into oocytes (Gier and Marion, 1967). The germinal epithelial activity has been described as cyclic and not continuous, the activity being greatest at 13 days of age, then being inversely correlated to medullary activity (Raps, 1948). The rete, formed midsaggitally within the undifferentiated gonad, remains throughout the life of the ovary (Andersen, 1970). The rete originates from the lateral primary epithelial cords which do not contain enough germ cells to produce active cords (Marion and Gier, 1969).

Embryonic follicular development has been frequently noted but not adequately followed to give a complete picture. Large numbers of oocytes are produced during embryonic life, all of which degenerate after completing the normal maturation processes, meiosis occurring in embryonic life with completion by four days after birth in the rat (Hargitt, 1930d). Similar occurrences have been reported in the mouse (Lange, 1896; Kirkham, 1916; Kingery, 1917) and in the cat (Brambell, 1930; Kingery, 1917; Butcher, 1927.)

Massive atresia of the oocytes at and shortly after birth has been reported by many. In the dog, the vast majority of oocytes disappears through atresia in the first few weeks of life (Schotterer, 1928). Firkit (1920) reported a complete disappearance of the embryonic germ cells in mammals.

Counts of primordial follicles present in the fetal ovary indicates greatest numbers are present toward the end of gestation: cow, 113-130 days (Erickson, 1966); rat, 18.5-19.5 days (Beaumont and Handl, according to Mauleon, 1969); and guinea pig, 45 days (Ioannou, according to Mauleon, 1969). With age, the count decreases rapidly (Arai, 1920; Block, 1951; Zuckermann, 1951). The initial drop is greatest; in the rat and the mouse, there is 50-60% degeneration during the first week after birth (Jones and Krohn, according to Mauleon, 1969.) Degeneration continues with the proportion of oocytes lost per unit of time remaining constant (Krohn, according to Mauleon, 1969.)

The presence of large numbers of oocytes has been observed in the neonatal ovary, with massive degeneration of these embryonically derived oocytes reported in the cow, cat, and ewe (Mauleon, 1969; Winiwarter and Sainmount, 1908) with oogonia and oocytes disappearing during embryonic life or shortly after birth with only a small number remaining after. Some believe oocytes derived in the embryonic oogenetic period are the oocytes which are destined to ovulate at maturity (Mauleon, 1969; Zuckerman, 1962). In this situation, the number of oocytes declines with age, but all oocytes are considered to be present at birth that will ever mature; some oocytes will degenerate while others remain in a "resting" state until the animal reaches maturity (Zuckerman, 1962). Over 700,000 oocytes have been reported at birth in the ovary of the dog, with a reduction of about half by puberty; 500 are present at ten years of age (Schotterer, 1928).

Meiotic changes within the developing oocytes have been reported at various ages. According to Hargitt (1930d), leptotene, zygotene and pachytene have been observed in oogonia and oocytes in the rat from 18 days

gestation to three days after birth with meiotic changes completed by six days after birth. Three days after birth, the oocyte nests become surrounded by follicle cells, the nests break up and primordial follicles are formed. Maturation of these follicles is initiated at the same time followed by subsequent degeneration. None of these early oocytes survive. Degeneration of oocytes is accompanied by the production of new oocytes. At six days, oocyte masses and enlarged oogonial cells can be observed in the germinal epithelium (Hargitt, 1930d).

In the beagle, oogonia in meiotic prophase are considered by Andersen (1970) to indicate primordial follicle formation in the newborn pup. Oogonia mature to oocytes surrounded by a follicular layer. Three to five days after birth, the cortex reveals many meiotic changes, but no primordial follicles are observed until two weeks after birth (Andersen, 1970). Raps (1948) described the penetration of the connective tissue by "deeper cell groups" which maintain epithelial contact and contain "precursors of the follicle cells and ova", the nuclei of which are in mitosis.

Major controversy arises when consideration is made of whether there is a new generation of oocytes in each reproductive cycle or whether the entire supply of oocytes required for the entire reproductive lifetime is present at birth or shortly thereafter. Winiwarter and Sainmont (1908) proposed three proliferative cycles of the germinal epithelium with accompanying meiotic changes: (1) embryonically, (2) after birth to 35 days, and (3) beginning 36 days after birth. Oocytes from the first two cycles degenerated after undergoing maturation changes. Meiotic changes occur at least three times in the reproductive cycle: embryonically, shortly after birth and at puberty (Winiwater, 1908). Others contend that in the rat, meiotic changes can be found only in the embryo or shortly after birth (Harz, 1883; Lange, 1896; Kingery, 1917).

In the beagle, Andersen (1970) reported oogenesis up to 50 or 60 days after birth but generally ending near 38 days, assuming that oogonia undergo meiotic changes to form oocytes in primordial follicles, then some follicles degenerate and some enlarge to primary follicles. In the young ovary, degeneration is extensive, resulting in the presence of a narrow cortex with follicles at three to four months of age.

Several investigators have proposed neoformation of oocytes from the germinal epithelium after arrest of the classical oogenetic phenomena (Allen, 1923; Hargitt, 1930b; Evans and Swezy, 1931; Marx, 1941; Duke, 1941; Bullough, 1942; Burkl, 1954). Paladino (1887) reported that from birth to old age there is a continuous growth and degeneration of follicles with new formation of egg tubes and ova, the intensity varying with age, being maximal at sexual maturity; Evans and Swezy (1931) made the suggestion that there is a periodic formation of new ova, perhaps 400 to 500 new oocytes being produced at each estrus with eventual atresia of 99% of the oocytes from a previous cycle while Kingsbury (1913), Kirkham (1916) and Brambell (1930) proposed latency of oocytes in the ovary, considering that all oocytes for the reproductive lifetime of the animal are present at birth and some remain dormant until such time as they are needed.

Hargitt (1930d) and Papanicalaou (1924) contended that no oocyte can remain dormant; either growth or degeneration occurs, and no embryonic germ cells will last through sexual maturity. Either there is continuous activity throughout the reproductive life of the animal, the young animal merely producing more follicular or luteal cells (Papanicalaou, 1924), or there is new oocyte formation from the germinal epithelium at each estrous period (Allen, 1923). Papanicalaou (1924) and Butcher (1927)

proposed that there is accentuated activity in the germinal epithelium and high rate of oocyte formation, with accompanying degeneration at all stages, and a gradual slowdown at 65 days after birth.

Raps (1948) reported that in the 2-day dog, cuboidal epithelium covers the ovary, the ovary being penetrated by "deeper cell groups" which maintain epithelial contact and which contain precursors of the follicular cells and ova. The oocyte nuclei are in mitosis and remnants of the primary cords are retained. By six days, the connective tissue strands deep within the ovary retained a connection to the basement membrane, and were separated by cords of cells and cell nests. He described cells of two types: large spherical nucleated cells and small ovoid cells with flattened nuclei. The small ovoid cells he considered to constitute the primary cell type of the medullary parenchymatous tissue. Reports of the first occurrence of primordial follicles in the dog vary from four days (Raps, 1948) to 17 days (Andersen, 1970).

Raps (1948) described the ovary as being encased by a heavy tunica albuginea in the 17 day pup. The tunic is penetrated by groups of ingrowing germ cells extending from the now squamous germinal epithelium into the cortex. The nuclei of the germinal epithelium are undergoing mitosis at this time. Pfluger cords first appear at 15 days coincident with the initial formation of primary follicles. The dog ovary at 11 weeks is covered with cuboidal epithelium; the tunic is deep and the cortex is narrow. Connective tissue fills in between the tunica albuginea and the deeper ovarian stroma. Cords of primordial follicles with granulosa cells extend to the medulla.

From 15 to 20 weeks the canine ovary, according to Raps (1948), show development of secondary follicles; follicular atresia is evident

and primordial follicles are differentiating at the deep ends of the cords. At this time, the germinal epithelium is squamous (Raps, 1948) to a single cuboidal layer (Andersen, 1970). Follicles are separated from the epithelium, arranged in clusters, or isolated in the deeper portion of the tunica albuginea. Epithelial invaginations are a prominent feature, with cords losing continuity with the germinal epithelium and assuming a tortuous route through the periphery of the ovary. The periphery of the medulla is penetrated by some cords. Raps (1948) and Andersen (1970) described the dog ovary at five to six months as containing secondary follicles with initial antral formation indicating early tertiary or vesicular formation. Most of these follicles undergo atresia but some will continue to grow; consequently a distinct cortico-medullary junction is defined. At the advent of puberty, some of the vesicular follicles will enlarge and mature. The epithelium is cuboidal at this time with connected epithelial cords extending through the tunica albuginea.

Various descriptions of the superficial or secondary epithelial cords (Pfluger cords) attribute varying activity and function to these cords. Recognition of such cords is relatively common; however, function of these cords is in dispute.

Harrison and Matthews (1951) described crypts in the germinal epithelium as a mammalian ovarian characteristic. These are tubular (lumenized) invaginations of the epithelium which penetrate into or through the tunica albuginea as distinguished from Pfluger "egg cords" which are solid downgrowths from the germinal epithelium. In the dog, crypts and tubes originate in the germinal epithelium, terminating in islands, tubes, and small follicles of epithelial cells, not related to the oocytes which are deep within the

the tunica. Harrison and Matthews (1951) described comparable conditions in pinnipeds, fissiped Carnivores, Marsupialia, Insectivora, Procavia, and primates. Cord activity was reported to be cyclic during the reproductive period in all these species.

Andersen (1970) described lumenized (common in the cortex) and solid (sometimes penetrating into the medulla) epithelial cords in the dog with the activity of these cords varying with the stage in the estrous cycle, and the cords often containing oocytes. Some proliferation and degeneration of the germinal epithelium was described for all stages of the cycle (Evans and Swezy, 1931).

The contributions of the germinal epithelium and associated cords to the ovarian structure varies from apparently no function, i.e., present but having no ova (Schottlander, 1893; Regaud and Policard, 1901) to the production of ova and follicular cells (Bonnet, 1891; Felix, 1906; Evans and Swezy, 1931; Hargitt, 1930b; Harrison and Matthews, 1951; Marion and Gier, 1971).

Three sources of ova from the germinal epithelium have been described in the dog. One type, most abundant during estrus, consists of an epithelial thickening with some cells being cut off from the germinal epithelium, and subsequent enlargement of these cells. Another is invaginations of the germinal epithelium being pinched off with accompanying mitotic changes which result in the enlargement of the oocyte. Thirdly, invaginations of solid germinal epithelial cords (Pfluger tubes) becoming disassociated from the epithelium and subsequent germ cell enlargement (Evans and Swezy, 1931). Individual enlarged cells or groups of cells (epithelial nodules) with mitotic figures have also been considered a source of new oocytes (Barton, 1945).

Epithelial cords from the germinal epithelium are considered to be a source of follicular cells (Harrison, 1962; Marion and Gier, 1971). A dual role in the origin of the follicles has been given to the germinal epithelium and epithelial cords. Germinal epithelial proliferations into tubes or cords give rise to some cells which enlarge and produce oocytes and some that do not. The cord then breaks up into follicles (Hargitt, 1951).

With increased age, the canine ovary develops clefts or crevices which are assumed to be continued proliferations of the cortex accompanying atrophy of the medulla. In old age, cortical proliferation is common but the degree of normalcy of follicles varies greatly. The number of tertiaries is greatly reduced (Andersen, 1970).

Anovular follicles have been reported as detached epithelial cords in which the oocyte has degenerated, or as a primary follicle in which, through atresia of the follicle, the oocyte has undergone degeneration (Gerard, 1920; Barton, 1945; Hargitt, 1950b).

In proestrus, differentiation of the germinal epithelium is rare, the number of oocytes being directly proportional to the amount of proliferation (Evans and Swezy, 1931). Cords are often surrounded by mature follicles (Andersen, 1970). The number of small and medium follicles in the cortex, many of which are atretic, has increased. Ovulatory follicles greatly exceed the other in size (5 to 6 mm), showing granulosal folding (Evans and Cole, 1931). Granulosal folding when first observed was considered to be a premature formation of the CL (Bischoff, 1845). Corona radiata is the detached from the granulosa (Barton, 1945). The CL from the previous cycle is maintained throughout proestrus although in a regressed condition (Evans and Cole, 1931).

In early estrus, the epithelial cords are numerous, surrounded by mature follicles, with some detachment from the germinal epithelium. During late estrus, the cords become tubular (Andersen, 1970). Some degenerative changes are seen in the cords and "primaries"; locally the cuboidal germinal epithelium is stratified in preparation for new germinal epithelial activity (Barton, 1945). The degeneration initiated in proestrus has effectively reduced the follicular numbers, leaving yet a considerable number of medium size atretic follicles (Evans and Cole, 1931).

During early metestrus, little difference in ovarian activity is noted between pseudopregnancy and pregnancy (Evans and Swezy, 1931). The epithelium varies from squamous (Barton, 1945) to low cuboidal (Andersen, 1970) in the area of the forming CL, probably due primarily to stretch of the epithelium. Epithelial cord activity has been reported from low (Barton, 1945), to the development of tubular diverticulae which lose their connections with the epithelium (Andersen, 1970), to abundant cords and anovular follicles (Evans and Swezy, 1931). The number of oocytes and follicles continues to increase throughout metestrus (Evans and Swezy, 1931). At 10 days, the CL reaches maximal size with little change until 30 days into metestrus at which time the CL begins to degenerate. During late pregnancy, the production of ova and follicles is uniform, large follicles are degenerating and the proliferation of the epithelium is extensive (Evans and Swezy, 1931).

During the anestral period, the germinal epithelial activity increases, giving rise to new oocytes (Barton, 1945). This is the period of maximal degeneration when few follicles complete their growth. This period is similar to late pregnancy. The CL is regressing although retained until the next proestrus. Many ova and small to medium follicles are present (Evans and Swezy, 1931).

In summary, the follicular waves are considered to be cyclic. The development of the follicles and the CL occurs within a short time, when maximal follicles are produced. Massive atresia occurs when one or more follicles reach maturity. Ovulation occurs, a CL is formed and a new cycle is initiated. If no ovulation occurs, mature follicles degenerate and the cycle begins again. Ovulation is accompanied by a wave of degeneration of follicles preceding or coincident with ovulation (Evans and Cole, 1931).

Atresia or degeneration can occur in any stage of follicular growth. Follicular cells may be gone with an oocyte retaining its entity, but the follicle has undergone degenerative changes such that ovulation will not occur (Evans and Swezy, 1931).

The origin of the interstitial cells and glands has received little mention but generally the origin is attributed to the thecal elements of degenerating follicles (Greunwald, 1942). Evans and Swezy (1931) in their classic paper on the dog did not find the formation of interstitial glands from the thecal elements of degenerating follicles. During the juvenile period in the rat, interstitial cells were reported to be granulosal outgrowths and ingrowing of cords from the germinal epithelium, but in the adult, interstitial gland origin was described as the theca interna cells of atretic follicles (Rennels, 1951; Dawson and McCabe, 1951). Tsukaguchi and Okamoto (1930) reported the origin of the interstitial glands as activated stromal cells or as being detached from the medullary cords. They reported that interstitial glands occurred definitely in the two to six months old dog and only occasionally in the adult.

Leydig-like cells have also been reported in the human ovary, variously named hilus cells, Berger cells and sympathicotrophic hilus glands according

to Berger (1922) and Harrison (1962). Such interstitial cells are reported to be limited to the area of the ovarian hilus and adjacent mesovarium. Sternberg (1949) demonstrated a close correlation between Leydig cells of the testis and the Berger cells by correspondence of nuclear and cytoplasmic detail, and similarity of lipochrome pigment and crystalloids of Reinke. He made the suggestion that these cells secrete androgens.

MATERIALS AND METHODS

The majority of the ovaries used in this study were obtained by ovariectomies of dogs maintained in a colony at Kansas State University from 1952 through 1965. The number of each age category studied included 11 fetuses, 22 pups, 3 puberal (Table 1), and 42 mature adults (Table 2). Fetal ovaries from 34 days gestation to birth were examined (Table 1). Pup ovaries examined ranged from birth to pre-estrus. Animals in puberty were those approaching the first estrous cycle, and nearing sexual maturity. Mature adults were examined in various stages of the estrous cycle: proestrus, estrus, metestrus (pseudopregnant and pregnant), and anestrus. The age of these animals varied as did the number of previous cycles and number of previous pregnancies.

Age of the animals was determined from laboratory records. The stage of the estrous cycle was established by means of vaginal smears. Ovaries were removed surgically, weighed and measured, then two or three cuts were made into the ovary to insure infiltration of the fixative. Ovaries were then immersed in either Bouin's fluid for 24 hours or Flemming's fixative for 4 hours. In some ovaries, perfusion of fixative was made through the ovarian artery. After adequate fixation, the ovaries were washed in water 8 to 10 hours, then excess picric acid was removed by ammoniated 70%

Table 1. Fetal and immature dog ovaries examined, with age and condition.

Dog number	Age days	STAGE Fetal	Immature	Proestrus	Estrus
363L	36	X			
363RB	38	X			
169LA	40	X			
322LA	49	X			
155LC	53	X			
273 E	54	X			
331RA	55	X			
225 C	57	X			
266-2L	1 da		X		
267-1L	8		X		
266-1L	24		X		
268-1L	30		X		
267-2L	30		X		
267-3L	35		X		
268-3L	63		X		
392-2R	90		X		
392-2L	90		X		
219-1L	98		X		
392-3L	120		X		
320-3R	150		X		
392-4L	150		X		
271L	165		X		
392-4R	165		X		
392-5L	180		X		
250-6R	180		X		
272L	195		X		
271R	210		X		
392-6L	210		X		
392-6R	225		X		
382-2L	240			X	
375-2R	390			X	
407-1	310			late	
407-2	310			late	
249R	270				1st 1 da
73L	245				1st 3 da
149L	600+				2nd 4 da
153L	480				2nd 4 da
97L	600+				3rd 4 da

Table 2. Mature dog ovaries examined, with age and condition.

Dog number	Age, months	STAGE			
		Metestrus-I	Metestrus II		Diestrus days, P.O.
			Pregnant	Pseudopreg.	
149R	21	2nd		0	
365-R	15	2nd		0	
365-I	15	2nd		2	
373L	15	1st		3	
234R	14	2nd		3	
97R	23	3rd		7	
137L	9	1st		2	
137R	9	1st		4	
373R	15	1st		6	
405AL	13		8		
405AR	13		8		
390R	26			12	
390L	26			12	
388 L	31		12		
388R	31		12		
260R	37		17		
398L	17		19		
398R	17		21		
384L	15		22		
241L	21		25		
384R	15		27		
377L	11		31		
226R	12		43		
262R	37				66 NP
223L	16				70 NP
266L	23				90
214R	16				121
408-1	16				160
408-2	16				160

isopropyl alcohol. Dehydration was accomplished in baths of 70, 85, 90, 100% isopropyl alcohol and 8:1:1 100% isopropyl alcohol, xylene, oil of wintergreen clearing solution for at least 2 hours in each bath. Infiltration was accomplished by passing the tissues through two 54° C paraffin baths for 3 hours each, then after 15 minutes in a vacuum infiltrator, they were embedded in 57° C paraffin.

Tissues were serially sectioned at varying thicknesses from 7 to 25 mu. A variety of stains was employed: Periodic Acid - Schiff's reagent counterstained with Harris Hematoxylin; Harris Hematoxylin followed by Masson's Trichrome, Mallory's Triple or acid fuchsin - orange G; and Iron Hematoxylin. Best overall results were obtained with Mallory's Triple and Harris Hematoxylin. Periodic Acid - Schiff's reagent with Harris Hematoxylin proved helpful in differentiation of membranes.

One section from each of three separate slides of each ovary was selected to be counted as a representative sample. An average number of follicles in the ovary and the total number of follicles in each class was calculated by the formula proposed by Marion et al (1968):

$$\frac{\text{ovary length in mm. X no. of follicles counted}}{\text{average diameter X no. of sections counted}}$$

The average nuclear diameter was utilized in calculation of primordial and primary follicle numbers. The average oocyte diameter was used in calculation of secondary follicles, and the average follicular diameter, limited by the membrana propria, was used in calculation of tertiary follicle numbers.

Follicles were classified (Table 3) according to the scheme proposed by Marion et al. (1968):

A) Primordial follicles: 1. early; 2. late

B) Primary follicles: 1. early; 2. middle; 3. late

C) Secondary follicles: 1. early; 2. middle; 3. late

D) Tertiary or vesicular follicles were categorized as to normal or atretic and by size class (<0.5, 0.5-1, 1-2, 2-3, 3-5 mm). Stages of atresia were categorized (Table 4) on criteria slightly modified from those proposed for the bovine by Marion et al. (1968).

In order to establish a more functional evaluation of the ovarian cycle, in addition to follicular counts, the epithelial cord activity of each ovary was classified as: (1) no cord activity; (2) cord activity but no oocytes; (3) high cord activity with production of new oocytes and primordials; (4) decreasing, but still active cords with many new primordials; and (5) less cord activity, with progression of some primordials to primaries (Table 5).

Mitotic and meiotic activity was observed as well as formation of interstitial glands and interstitial cells or Leydiglike cells.

OBSERVATIONS AND DISCUSSION

Fetal ovaries

Migrating primordial germ cells (PGC's) are clearly visible (Fig. 1) at 21 days gestation (36 somite) canine embryo (Marion and Gier, 1971). At this time, the PGC's are located lateral to the dorsal aorta and ventral to the mesonephric tubules. By 22 days, the PGC's have become incorporated into the coelomic epithelium, now a germinal epithelium, which will serve as a source for new follicular cells and oocytes. The PGC's are no longer

Table 3. Classification of follicles of the dog ovary, modified from the scheme developed for bovine ovaries (Marion et al., 1968).

Class	Subclass	Diameter (microns)	Oocyte (microns)	General Characteristics
A Primordial				incomplete layer of follicle cells;
	1 early	15-25	12-20	only 2 to 5 follicle cells in section;
	2 late	25-50	20-40	4 to 20 follicle cells in a section.
B Primary				a single cell layer complete;
	1 early	50-70	40-60	follicle cells flattened to cuboidal;
	2 middle	65-85	55-70	follicle cells cuboidal to low columnar;
	3 late	85-110	70-80	follicle cells simple columnar
C Secondary				multiple layers of follicle cells;
	1 early	110-125	80-90	follicle 2 cells thick;
	2 middle	120-160	85-95	follicle 2 to 3 cells thick;
	3 later	150-250	90-100	follicle 3 or more cells thick; theca distinguishable.
D Tertiary (Vesicular)				follicle with lumen separating cumulus and granulosum;
	1 transition	240-500	100-110	one or a few disconnected intercellular spaces;
	2 early	500-1000	105-115	continuous lumen, 2 to 4 times diameter of cumulus
	3 middle	1000-2000	115-125	cumulus frequently stalked; lumen 4 times diameter of cumulus;
	4 maturing	2000-3000	120-130	rapid lumen expansion, granulosum thinning.
	5 mature	3000-5000	125-140	stratum granulosum undergoing pre-ovulation folding.

Table 3A. Typical Normal Oocyte Counts in Dog Ovaries

Stage	Total Oocytes	OOCYTES IN FOLLICLES			
		Primordial	Primary	Secondary	Tertiary
Newborn	1,600,000				
20 days	800,000				
1 month	130,000	10,000			
2 months	80,000	20,000	15,000		
3 months	100,000	50,000	25,000	150	
6 months	45,000	20,000	4,000	500	30
9 months	30,000	20,000	4,000	800	80
Estrus	35,000	25,000	6,000	1000	100
Mid pregnancy	25,000	20,000	3,000	200	50
Late pregnancy	120,000	100,000	6,000	800	60
Mid diestrus	140,000	140,000	7,000	1200	30
Proestrus	130,000	120,000	5,000	1000	100

Table 4. Classification of stages of follicular atresia, modified from the scheme proposed for bovine ovaries by Marion et al. (1968).

Stage	Characteristics of the follicular structures			
	theca	membrana propria	stratum granulosum	cumulus
1	normal or swollen	intact or broken	loose; irregular; some cells in lumen; Call-Exner bodies.	normal, or few atretic bodies on margin; Call-Exner bodies.
2	thickened by contraction; glandular cells absent	usually disrupted	many atretic bodies; remaining granulosa irregular	loose, irregular; atretic bodies around margin.
3	glandular cells absent except in incipient cystic follicle; beginning contraction	fragmentary except in incipient cystic follicles	reduced to a single cell layer, possibly discontinuous.	remnant cells only; oocyte vacuolated or granulated.
4	glandular cells absent lumen greatly reduced by thecal contraction	absent	discontinuous cell layers possibly being compressed into "interstitial gland."	gone; oocyte bare if present.
5	lumenal border hyalinized, to complete thecal hyalinization.	absent	completely gone	gone: possibly zona pellucida persisting.

Table 5. Characteristics of stages of epithelial cord activity.

Stage	Germinal epithelium	Length of cord	Oocytes in cords	Primordial follicles
1	cuboidal to flat	0 - 15	none	none
2	cuboidal	15 - 50	early meiosis	none
3	low columnar	40 - 100	numerous, in deep cords	a few stage 1
4	columnar to cuboidal	75 - 125	numerous	numerous, stages 1 and 2
5	cuboidal	15 - 0	none	numerous, stage 1 to early primary

EXPLANATION OF FIGURES

Figure 1. Section of a 21 day (36 somite) dog embryo in the region of somite 18. Primordial germ cells (PGC's) have concentrated between the mesonephric tubules (MT). Some of the PGC's have migrated to the coelomic epithelium (CE). Bouin fix, Hx orange-G stain, 200X.

Figure 2. Section through the gonadal ridge of a 22 day (40 somite) dog embryo. The germinal epithelium (GE) has formed by the incorporation of the PGC's into the coelomic epithelium (CE). Primary epithelial cords (PEC) have begun invagination into the gonadal connective tissue.

Figure 3. Enlarged portion of the cortex of a 34 day dog embryo. Secondary epithelial cords (SEC) from the germinal epithelium (GE) are growing into the cortical connective tissue. Medullary cords (MC) have broken connection with the germinal epithelium constitute much of the medulla (M) and are disintegrating. Bouin fix, Hx orange-G stain. 160X.

Figure 4. Well differentiated 34 day fetal canine ovary. The cortex (C) is composed of secondary epithelial cords and a germinal epithelium. The medulla (M) consists of degenerating primary epithelial cords. The rete (R) remains connected from the ovary to several mesonephric tubules (MT). The metanephros (MN) is forming dorso-lateral to the gonad. Bouin fix, Hx orange-G stain. 20X.

Figure 5. At birth, the secondary epithelial cords are packed with oocytes (O), many of which show degenerative changes: some are undergoing apparently normal meiotic changes (Me). Progressive enlargement of oocytes are found deeper in the cords; interstitial glands (IG) are developing at the ends of the cords deep within the cortex. Bouin fix, Hx Mallory's tripple stain. 46X.

Figure 6. Cortex of one day dog ovary. The secondary epithelial cords (SEC) are continuous with the germinal epithelium (GE). Many oocytes (O) in the cords show degenerative changes such as clumping of nuclear material, vacuolation of the cytoplasm, and disintegration of the cell membrane. The basement membrane (BM) of the germinal epithelium is continuous around the cords. Bouin fix, PAS Hx stain. 184X.

Figure 7. Mitotic metaphases (m) and anaphases (a) in this eight day pup ovary are found in some oocyte nuclei. These mitotic diviosions are unexplained but apparently indicate cells doomed to destruction. Other nuclei are degenerating (DN). Non-germ cells (FC) (potential follicular cells) are prominent in this cord. Bouin fix, FeHx stain. 200X.

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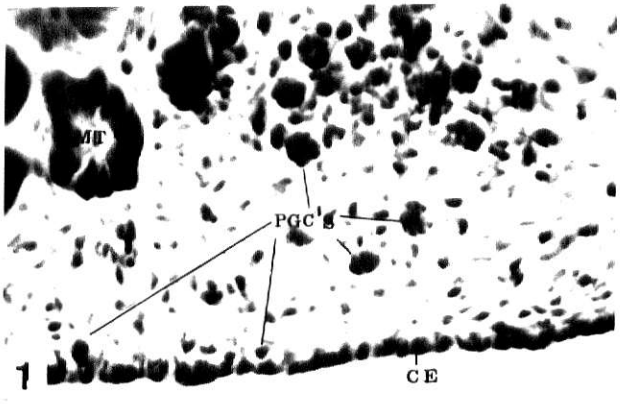

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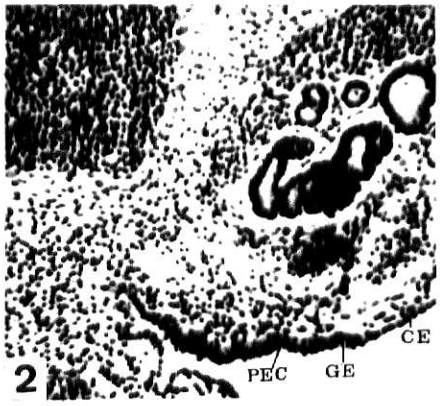

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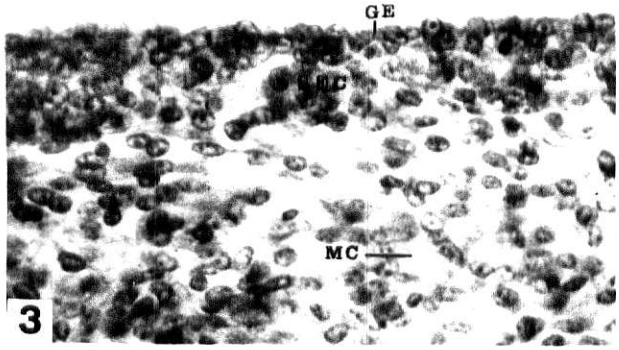

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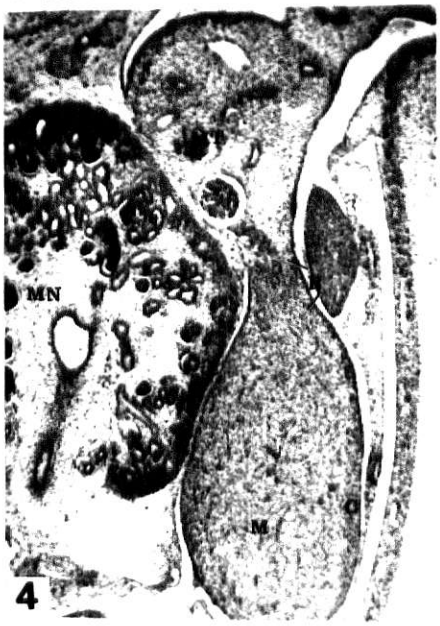

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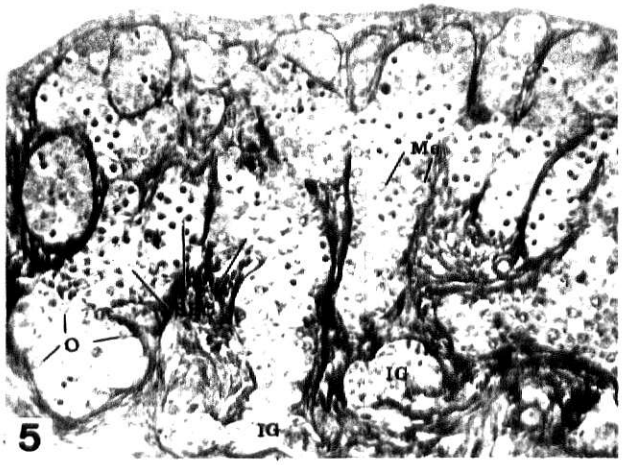

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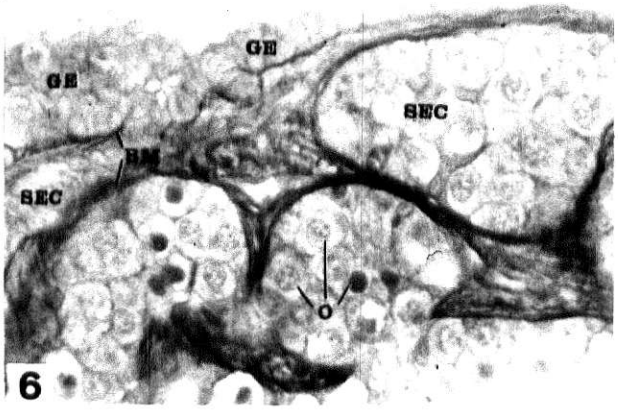

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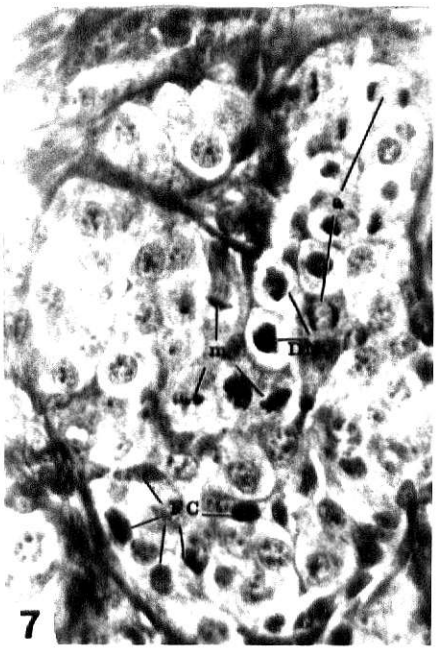

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detectable, except for a thickening of the epithelium where the PGC's were previously observed. At 23 days (40 somite), gonadal ridges can first be observed, consisting of a distinct germinal epithelium and thickened mesenchyme between the germinal epithelium and the mesonephric tubules. About 12 hours after formation of the germinal epithelium, epithelial invaginations (Fig. 2), the primary epithelial cords, extend into the gonadal mesenchyme. Primary sex cords (medullary cords) detach from the germinal epithelium at 29 days gestation in the dog. These cords will form the medullary portion of the ovary as previously reported by Brambell (1932).

At 33 days gestation, sexual differentiation is definite in the canine gonad. In the ovary, a second set of epithelial cords develop (Fig. 3), pentrating the cortical mesenchyme, forming the ovarian cortex as previously reported by Brambell (1932) and Hargitt (1936d). Medullary and cortical portions of the ovary are well defined by 34 days (Fig. 4). At 38 days gestation, the ovary shows a distinct demarcation between medulla and cortex, a separation which was detectable at 34 days but which is now even more prominent. The medulla is essentially fibrous with a few remnants of the primary epithelial cords. The cortex is much thicker, with secondary epithelial cords (Pfluger's cords or cortical cords) continuing to expand into the cortical area of the ovary. No oocytes are visible at this time. The basement membrane of the germinal epithelium is continuous around the epithelial cords. The rete is persistent throughout the lifetime of the animal (Fig. 4). The germinal epithelium is a single layer of cuboidal to columnar epithelium (Fig. 3).

Oocyte differentiation within the secondary epithelial cords has begun at 40 days. Indeed, these oocytes are differentiated into clusters

with a membrana propria (germinal epithelial basement membrane derivative) surrounding each cluster. Oocyte differentiation in the cords has taken place, expanding the epithelial cords throughout the ovarian cortex. Secondary cord activity at this time will produce "baby" cords present at birth (Figs. 5 and 6) which are approximately two-thirds germ cells as opposed to later secondary cords which will contain only one-third germ cells. All oocytes within these cords are destined to undergo atresia and degeneration (Figs. 5 and 7), although persisting cells of some cords form interstitial glands (Figs. 5 and 8).

Secondary epithelial cords continue to expand and produce more oocytes, which show evidence of meiotic changes (Fig. 7). Oocytes do not differentiate beyond this stage before birth, in contrast to the development of follicles to tertiary or vesicular stages as reported in larger animals such as the cow (Marion and Gier, 1971) and the giraffe (Harrison and Matthews, 1951). At birth the secondary epithelial cords are greatly expanded, packed with oocytes undergoing meiotic or degenerative changes (Fig. 5). The deep ends of each cord begin shortly to break up into primordial follicle groups (Fig. 8, 9. and 10). The deep cell groups referred to by Raps (1948) are merely tangential sections near the edge of the ovary, cutting the ends of the secondary epithelial cords which are limited at all times to the cortical portion of the ovary, never extending into the medulla. The tunica albuginea is perforated by expanding cords to the point of complete disruption (Fig. 9 and 11). Remnants of cords without oocytes form interstitial glands at the ends of the cords (Fig. 12).

Ovarian growth is accomplished by expansion and building on of the cortex through continued proliferation of the secondary epithelial cords,

EXPLANATION OF FIGURES

Figure 8. Within an expanded cord in this eight day dog ovary is a gradation from obviously degenerating oocytes (DO) with clumped nuclear material and vacuolated cytoplasm (VC), to apparently normal oocytes (NO) undergoing meiotic changes, to the formation of interstitial glands (IG). Germinal epithelium was beyond the left of the epithelial cord pictured. Bouin fix, FeHx stain. 220X.

Figure 9. Section of ovary of a 24 day old dog. The number of secondary epithelial cords (SEC) and oocytes (O) within the cords are reduced. Cords are beginning to break up, some fusing at the deeper ends of the cords. Fusion occurs where basement membranes touch. Down the cord there is a gradation from oocyte (O) to primary follicle (PR.) Bouin fix, Hx-Mallory's triple stain. 50X.

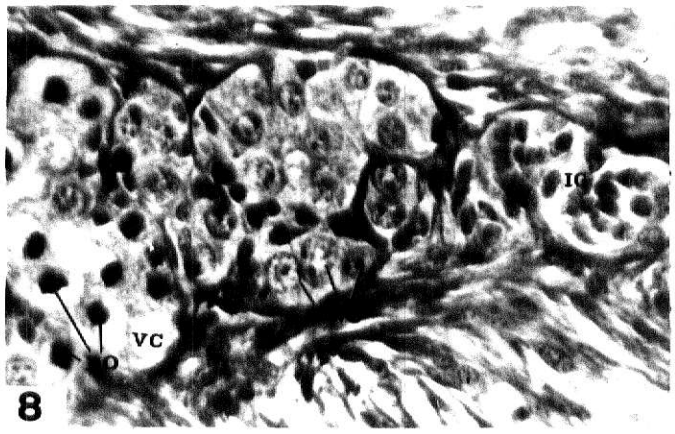
Figure 10. Enlargement of epithelial cord in a 24 day dog ovary. Differentiation occurs within the cords, progressing from oocyte (O), to primordial follicle (PF), to primary follicle (PR). Such a progression is indicative of maturation within the cord. Bouin fix, Hx-Mallory's triple stain. 176X.

Figure 11. Section from a 24 day dog ovary. The basement membrane (BM) is maintained around the secondary epithelial cords (SEC), and will be maintained around the follicle as the membrana propria. Cords are continuous with the germinal epithelium (GE). Bouin fix, PAS-Hx stain. 50X.

Figure 12. Two types of interstitial cells are present in the ovary. In this 30 day dog ovary, the membrane-bound interstitial glands (IG) with enclosed non-germ cell remnants of the secondary epithelial cords (EpC) can be distinguished from the non-membrane bound interstitial cells (IC) derived from the stroma. Bouin fix, PAS-Hx stain. 176X.

Figure 13. Cortex (C) and medulla (M) are distinct in this five week pup ovary. The medulla contains rete (R), blood and lymph vessels, and remnants of the primary epithelial cords which persist for a short time as interstitial glands (IG). Bouin fix, Hx-Mallory's triple stain. 16X.

Figure 14. In this enlarged portion of the ovarian medulla from 13, the rete (R) is clearly distinguishable from interstitial glands (IG). Bouin fix, Hx-Mallory's triple stain. X50.



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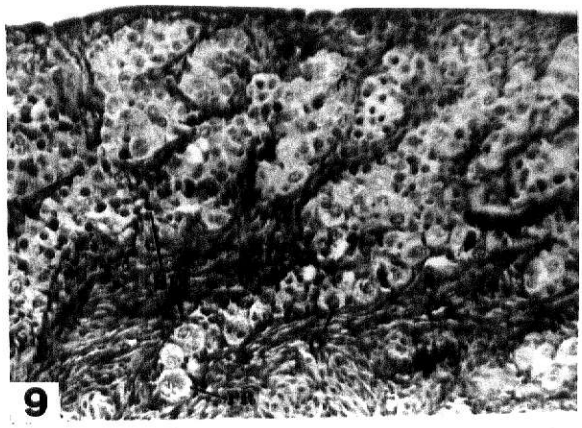

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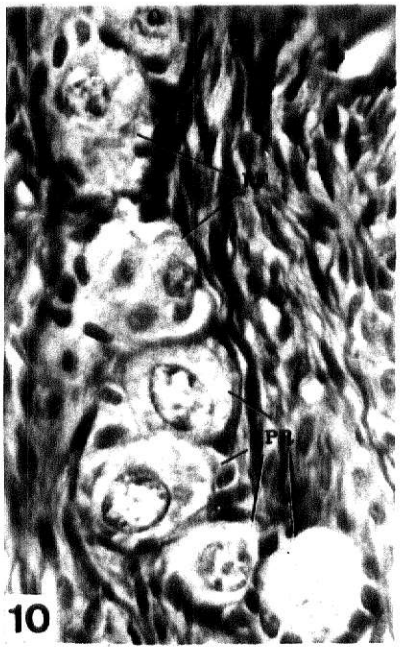

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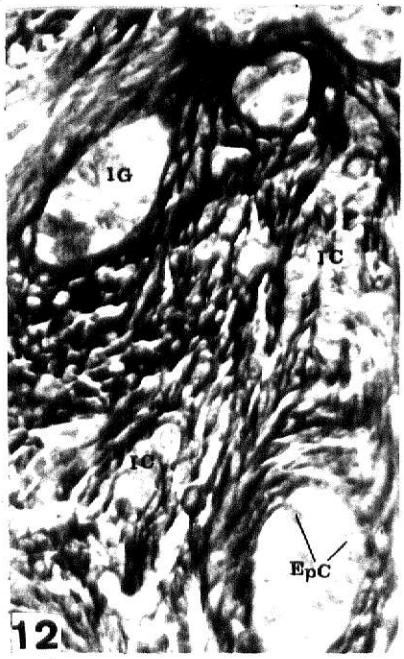

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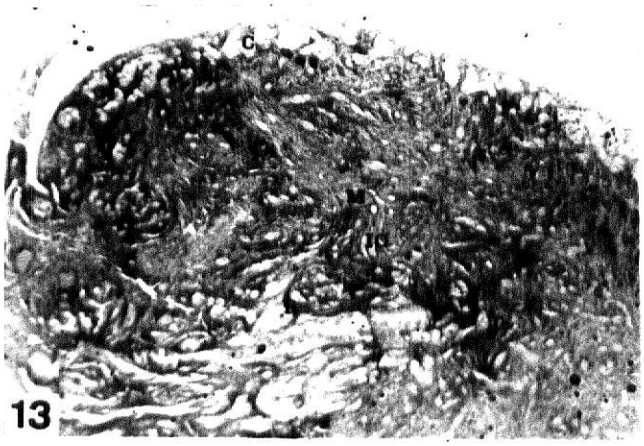

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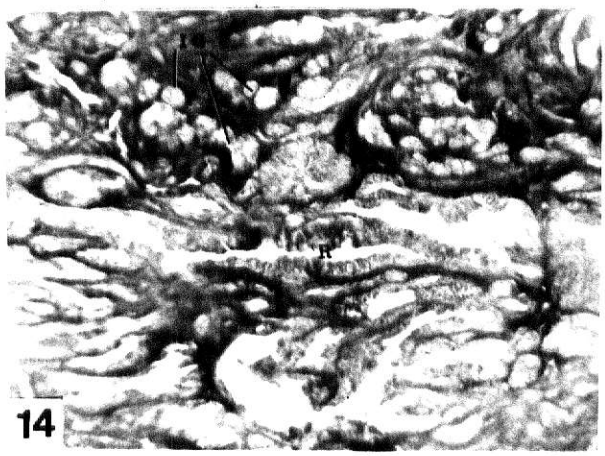

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accompanied by shrinkage of the medulla by degeneration of the medullary cords (primary epithelial cords).

Immature Ovaries

The medulla and cortex are well defined at birth and remain distinct at least until 5 weeks after birth (Figs. 13, 14). The medulla contains connective tissue, blood and lymph vessels, rete and remnants of primary epithelial cords which persist for some time as interstitial glands. Eventually these interstitial glands degenerate, causing shrinkage of the medulla. Interstitial glands are bounded by a basement membrane which was derived from the basement membrane of the germinal epithelium (Fig. 6 and 11).

Another type of glandular cell which is present at this time and throughout the ovarian lifetime has been described variously as Berger cells, Leydig-like cells, sympathicotrophic hilus glands (Berger, 1922; Sternberg, 1949; Harrison, 1962) and will be referred throughout this report as interstitial cells (Fig. 12). These cells are found within the ovarian stroma, between the interstitial glands from both primary and secondary epithelial cells, so are present in both the medulla and the cortex. They occur as single cells or small cell clusters that are not membrane bound (Fig. 12), obviously differentiated from the gonadal mesenchyme, and not from secondary epithelial cords as described by Dawson and McCabe (1951) for the rat. These interstitial cells are distinctly different from the interstitial glands in origin, histological structure and staining properties. Insterstitial cells resemble the Leydig cells of the testis and have been reported to be similar in many ways to the testicular interstitial (Legdig) cells (Berger, 1922; Sternberg, 1949). Berger (1922) reported

that these cells were confined to the area of the ovarian hilus, contrary to what was seen in this study.

The cortex, at one day after birth, is characterized by the presence of large expanded epithelial cords, the "baby" cords of the young ovary (Fig. 5), as distinguished from the functional secondary epithelial cords (Fig. 15). These cords are direct developments of the secondary epithelial cords, which first appeared at 34 days gestation (Fig. 3), and which have now become expanded with some 1.5 million oocytes per ovary. The ovigerous cords extend deep into the ovarian cortex (Fig. 5 and 9) but do not penetrate the medulla as contended by Andersen (1970). The cortical cords (secondary epithelial cords) maintain their connections to the germinal epithelium (Fig. 6, 9 and 11), the basement membrane of which is the limiting membrane of the epithelial cords. Deep within the cortex there is an apparent secondary fusion of basement membranes wherever one cord contacts another (Fig. 9).

Within the ovigerous cords of the 1-day ovary, oocytes are undergoing nuclear changes (Fig. 6, 7, 8, 9, and 11). Many of these changes appear to be meiotic changes as reported previously by Andersen (1970) and Jonckherre (1930) in the dog, and Winiwarter and Sainmont (1908) in the cat. The normalcy of these apparent meiotic changes is in question. Raps (1948) reported only mitotic changes occurring within these oocytes. Several investigators (Lange, 1896; Kirkham, 1916; Kingery, 1917; Butcher, 1927; Brambell, 1930; and Hargitt, 1930b) suggested that these oocytes undergo maturation changes and subsequently degenerate, most or all original oocytes degenerating. Our observations indicate that many of the oocytes do not undergo changes before degeneration and few ever reach the later

stages of meiotic changes. Nuclear changes appear aberrant, and degeneration is massive (Fig. 7 and 8). Disintegration or total disappearance of the cytoplasm, absence of the cell membranes, and aberrant nuclear changes were observed in at least 75% of the oocytes (Fig. 5, 7, 8, and 9). Some nuclei were represented only by clumps of nuclear debris which have classically been described as pachytene stage of meiotic changes. Rather, these represent condensation of nuclear material as a stage toward total destruction. Many oocytes containing such condensed nuclear material showed severe vacuolization or absence of cytoplasm, certainly an indication of irreversible degeneration, and not indicative of normal meiotic changes. Some oocytes, usually at the ends of the ovigerous cords nearest the germinal epithelium, do not show degenerative changes at this time (Fig. 6, 7, 8 and 10).

Unexplained and apparently senseless mitotic figures sometimes occur in nuclei of some oocytes (Fig. 7) as noted by Raps (1948). The progress of mitosis follows the normal process of mitosis through prophase, metaphase, and anaphase within cells which are distincly oocytes, apparently proliferating oocytes that are doomed to degenerate, as none of the original oocytes persist more than five weeks after birth.

Interstitial glands form at the deep ends of the ovigerous cords, by the persistence of non-germ cells of the epithelium, within the ovigerous cords after all oocytes in that particular segment have degenerated. The interstitial gland is pinched off beyond the more peripheral oocyte-containing portion of each cord, thus producing separate, independent, membrane-bound interstitial glands deep in the cortex, but not penetrating into the medulla (Fig.5, 8, and 12).

Cells within the secondary epithelial cords of 1-day ovaries showed a gradation from small newly formed oocytes near the connection of the

cord to the epithelium, to larger oocytes undergoing nuclear changes or degeneration near the middle and deeper cords, to disappearance of oocytes and the formation of interstitial glands at the deep ends of the cords (Fig. 8).

Essentially there is no change in the cord and oocyte arrangement from the day of birth (Fig. 5) to the eighth day at which time the oocyte count is still near 1.5 million oocytes per ovary. The number of cords is slightly reduced, but otherwise progression within the cords are similar to those observed in the 1-day ovary. Within the cortex a gradation exists from the germinal epithelium, with undifferentiated cells in the cords next to the epithelium, to an expanded region containing oocytes with clumped nuclei and extensive vacuolation indicative of degeneration, to apparently normal oocytes in later stages of meiotic changes. Interstitial gland formation appears to continue unabated at the deep ends of the cords through the ninth day, increasing the number of such glands in the deep cortex (Fig. 8).

By 24 days after birth (Fig. 9), the oocytes in the cords have been reduced drastically, to about 700,000. Between nine and 24 days, degenerative changes of oocytes are more rapid than replacement by the generation of new oocytes from the germinal epithelium. The massive expanded "baby" cords are greatly reduced in length, extending for a shorter depth into the ovarian cortex. The cords are broken up and many of them are separated from the epithelium. A few oocytes remain in the cords but far more non-germ cells than oocytes are present. Debris from the degenerating oocytes constitutes a considerable part of the deeper cords.

Degeneration of the oocytes within the original secondary epithelial cords continues unabated until about 30 days after birth. A new generation of secondary epithelial cords begins to differentiate from the germinal

epithelium, giving rise to a new type of cord comprised of few oocytes and more non-oocytes than were found in the original ovigerous cords. Both types of cords are present in ovaries of 35 day old dogs (Fig. 15).

The original secondary cords with their masses of oocytes have nearly disappeared by 35 days, leaving only non-oocyte remnants as interstitial glands deep in the cortex (Fig. 13 and 14). The germinal epithelium continues to be highly active. The new set of ovigerous cords has become dominant, with only two to five oocytes present in a cross section of any cord (Fig. 15). Within the cords, there is a progression of oocyte differentiation from near the germinal epithelium inward: undifferentiated cells in the narrow portion near the germinal epithelium; new oocytes in the deeper, tubular portions (anovular follicles of Evans and Cole (1931), Barton (1945) and others); distinct oocytes with nuclei in meiotic changes in the major part of the cord; to oocytes in diplotene or dictyate interspersed with non-germ (follicle) cells. At the deep end of each cord one or two oocytes and a few follicle cells surrounded by the basement membrane separate from the cord as a primordial follicle (Fig. 9). Primary follicles differentiate through subsequent multiplication of the follicular cells around a primary oocyte. The oocyte count at 35 days is approximately 130,000 within the cords, plus 10,000 primordial follicles (Table 6).

By the end of the second month after birth, most of the original oocytes and primordial follicles have disappeared. Epithelial cord activity is high (Table 5, stage 3) with continued production of oocytes and primordial follicles. The number of primordial follicles progressing into primary follicles is also increasing, with as many as 18,000 developed in the deep cortex at the ends of the cords (Fig. 16). Interstitial gland formation continues at the deep ends of some cords. Oocytes produced during this

EXPLANATION OF FIGURES

Figure 15. Five weeks after birth, two distinct sets of epithelial cords are present in the dog ovary. A few of the "baby" cords (BC) persist with degenerative oocytes (DO). New secondary epithelial cords (SEC) have developed from the germinal epithelium (GE). Bouin fix, Hx-Mallory's triple stain. 50X.

Figure 16. The "baby" cords have disappeared in this nine week pup ovary: proliferation of a new set of cords accompanies degeneration of the "baby" cords. Some oocytes within the cords have progressed to primary follicles (PR) at the deep ends of the cords. Interstitial glands (IG) remain from degeneration of previous cords. Bouin fix, Mallory's triple stain. 200X.

Figure 17. Section of an ovary from a 4-month old dog. During puppyhood, an ovarian period of quiescence is characterized by relatively low cord activity, low germinal epithelium (GE) and the presence of a crop of new primordial follicles (PF). Some of these primordial follicles later in this period show varying stages of progression. Primary follicles range from early (E) to middle (Mi) to late (L) stages. Bouin fix, Hx-Mallory's stain. 50X.

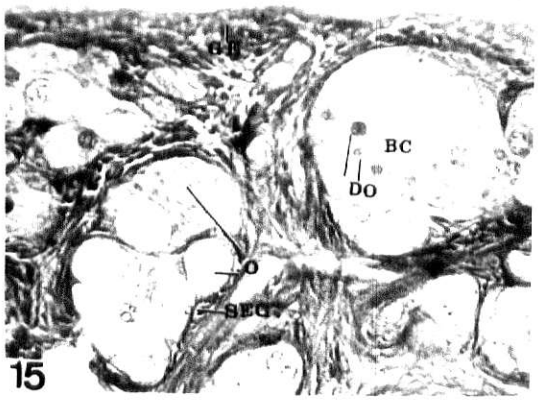
Figure 18. Section of an ovary from an 8-month old dog. In adolescence, activity is high, epithelial cords (EC) are proliferating, and all stages of follicles are present: primary (PR), secondary (SF) and tertiary follicles. From the left, three stages of tertiary follicles are illustrated: a normal follicle with an oocyte (NF); atresia: Stage 4 (S 4); and atresia: Stage 3 (S 3). Bouin fix, Hx-Mallory's triple stain. 19X.

Figure 19. Another section of the 8-month ovary shown in Fig 18. The pup ovary during the adolescent period is characterized by high cord activity (EC). A new set of primordial follicles has been differentiated from this new cord activity. Bouin fix, Hx-Mallory's triple stain. 50X.

Figure 20. Portion of a section of an ovary. Stage 5 atresia is a condition in which the theca interna cells are hyalinzed. In this particular follicle, the zona pellucida (Z) and degenerating oocyte (O) remain intact. Bouin fix, Hx-Mallory's triple stain. 50X.

Figure 21. By 66 days after ovulation, the corpus luteum (CL) has begun to show signs of regression. Interstital glands (IG) are present in various stages of development. A gradation of cord activity from the epithelium is characteristic of all ovaries. Bouin fix, Hx-Mallory's triple stain. 50X.

Figure 22. This ovary contained no corpus luteum, although the other ovary contained five CL's at 75 days after ovulation. As there was no functional CL to repress follicular growth, a greater number of secondary follicles (SF) and tertiary follicles were present than in ovaries of the same period which contained CL's. Cord activity is high and a new set of primordial follicles (PF) has been proliferated. Bouin fix, Hx-orange G stain. X40.



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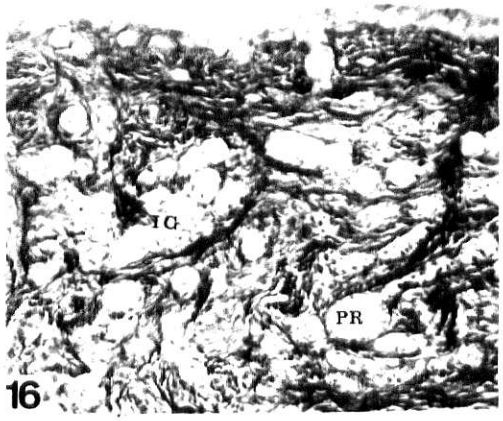

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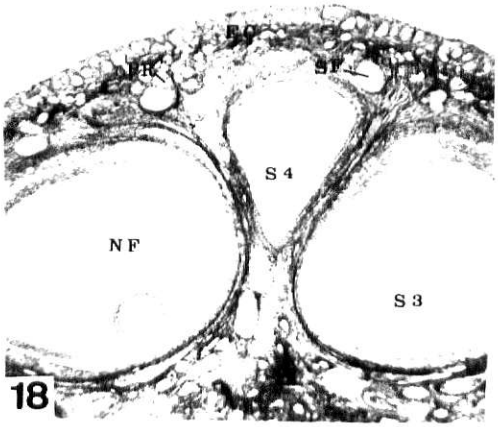

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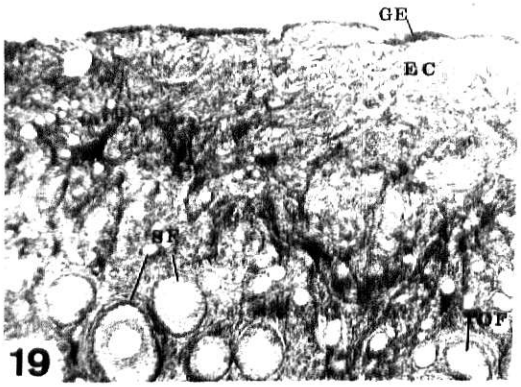

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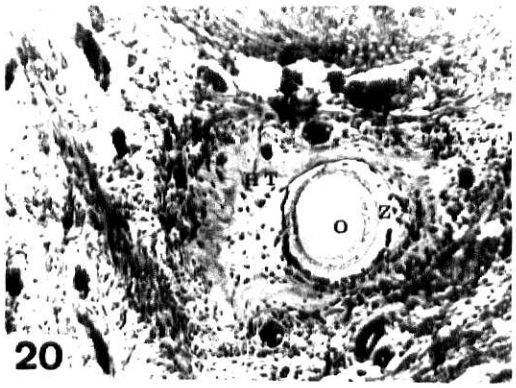

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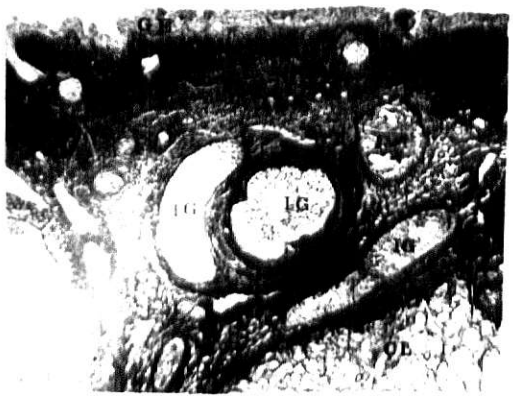

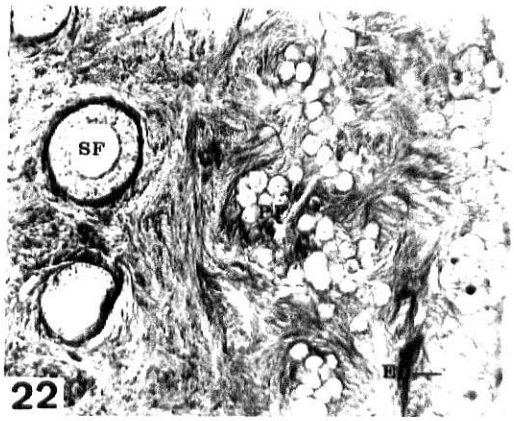

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Table 6. Number of follicles in dog ovaries, subdivided as normal or atretic for each category. Numbers given were determined by counting and tabulating all follicles in at least three sections, then calculating the various categories $\left(\frac{\text{ovary length x follicles counted}}{\text{average diameter x section counted}}\right)$.

Age (Weeks)	Primordials		Primaries		Secondaries		Tertiaries					
							1 mm		1-3 mm		3-5 mm	
	Normal	Atret.	Normal	Atret.	Normal	Atret.	normal	atret.	normal	atret.	normal	atret.
5	10,000	2000										
9	48,000	2800	17,000	1600								
14	45,000	3000	26,000	2500	140	0						
20	38,000	1600	5,000	2300	300	200						
30	16,000	2500	5,000	2000	400	500	0	40				
35	30,000	4000	3,000	1200	35	50	12	11	0	40	0	20
40	10,000	2000	4,000	1000	800	700	8	80	3	12	0	0
45 (Estrus)	28,000	1400	7,000	500	800	600	12	40	3	25	5	0
48 (Preg.)	22,000	900	3,000	100	130	50	50	300	0	21	0	3
52 (Preg.)	100,000	1000	6,000	400	750	200	0	200	0	0	0	0
60 (Diest.)	73,000	3500	7,000	1500	1000	400	9	150	0	40	0	0
75 (Diest.)	140,000	1000	7,000	1200	1400	300	10	30	0	0	0	0
120 (Diest.)	200,000	800	20,000	500	2000	100	10	100	0	0	0	0
130 (Proest.)	150,000	2200	8,200	800	1200	400	8	120	6	30	1	3

period of ovarian activity progress through follicular stages of primordial, primary, and some into early secondary (Table 6) at about three and one-half months (Fig. 17). Some of the oocytes produced during this early cord activity may endure up to five months but none progress beyond the late secondary or early tertiary follicular stage (Fig. 15A). At this time, production of new oocytes has reached a level approximating the rate of atresia of follicles, so that the total oocyte numbers remain nearly constant for a time, at about 100,000 in all categories (Table 6).

Ovarian activity decreases noticeably at about 2.5 to 3 months and maintains a low level of epithelial activity to about 4.5 to 5 months (Fig. 15A). During this period of quiescence, cord activity is greatly reduced (Table 5 stage 5) thus eliminating the increase of oocytes. A new crop of primordial follicles has differentiated from previous secondary epithelial cord activity. Early, middle and late stage primary follicles have differentiated by 3 months (Fig. 17). Secondary follicles (Table 3) first appear about 14 weeks after birth. Proportional degeneration of all class follicles continues. Primordial follicles, differentiated from cord activity at 2 to 3 months, provide the primary, secondary and early tertiary follicles (Table 3) present during the early adolescent period. None of the oocytes differentiated during early cord activity persist to the first estrous cycle, the last of that series of follicles degenerate at 5 to 6 months.

Epithelial cord activity is renewed at 5 to 6 months of age (Fig. 15a) after which cord activity appears to be continuous, with some cyclic variation: one area of the cortex may show high cord activity while another area shows little or none. Oocytes are first differentiated within the new secondary epithelial cords at 5.5 to 6.5 months. New primordial

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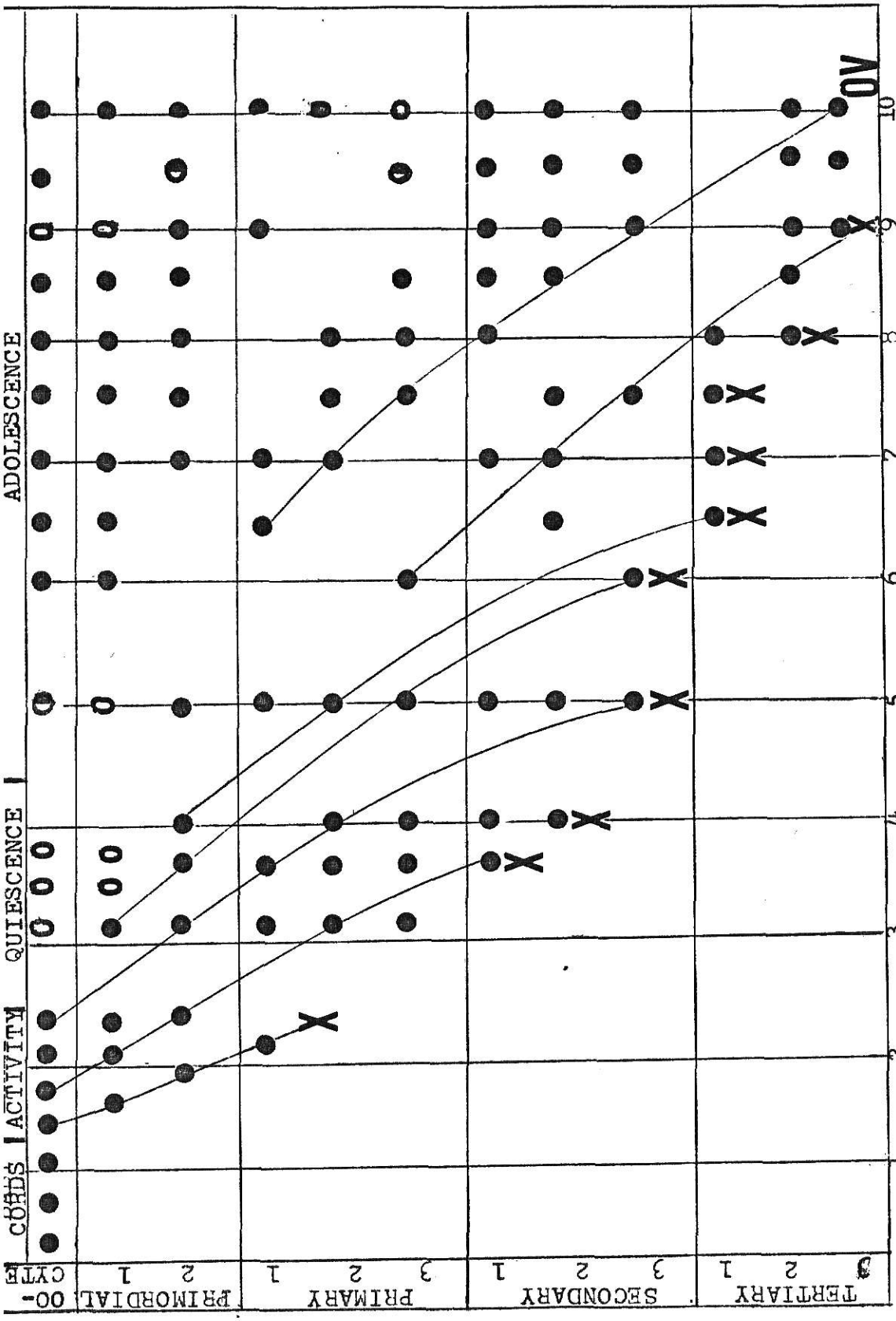

Figure 15A: Progression of follicular types from birth to first ovulation. Symbols: X = Total atresia of class; O = absence of class; ● = low number in class; ● = large number in class. This figure is a representation of numbers derived through follicular counts (Table 6). Oocytes differentiated in early cord activity, 5 - 10 weeks after birth, all degenerate before first ovulation. Ovulatory follicles are derived from new proliferations of epithelial cords at about 6 months.

follicles form (Table 5, stage 4) by the basement membrane of the epithelial cord constricting around an oocyte and accompanying follicular cells, thus pinching off the cell cluster from the cord. Follicular growth continues by the multiplication of the follicular cells and enlargement of the oocyte.

Tertiary follicles (Table 3) were first found at 5.5 to 6.5 months, as reported by Andersen (1970) by growth of follicles from the earlier cord activity of 5 to 10 weeks (Fig. 15A). Because these first tertiary follicles are found during the period of renewal of epithelial cord activity, they must necessarily be derived from earlier oocytes. All of the tertiary follicles found in adolescent bitches, up to 8 months of age, were distinctly abnormal so could not be the follicles that would ovulate later.

Several distinct generations of oocytes can be illustrated in an ovary at any one time (Fig. 18 and 19). In the ovaries of a bitch 7 to 8 months old, regularly there can be seen (1) active epithelial cords, with new oocytes in the deep portions of the cords; (2) primordial follicles from the immediately previous cord activity; (3) late primordial and primary follicles from the first cycle after renewed activity; and (4) secondary and early tertiary follicles remaining from the oocyte formation at 5 to 6 months. There is some overlap of follicular classes because cord activity is not uniform around the surface of the ovary, thus confusing the issue within any one ovary.

Oocytes which develop from cord activity during the adolescent period give rise to some follicles which will progress to late tertiary stages, a few of which will reach ovulatory size, and ovulate between 10 and 12 months (Fig. 15A).

Atresia is a constant factor throughout ovarian activity (Fig. 15A, Table 6) affecting 1 to 90% of the follicles of all stages at any one time. Sometimes atresia of tertiary follicles proceeds to the point that the theca interna is hyalinized but the oocyte may persist, although not in a viable condition (Fig. 20). Considering the continuing atresia present at and a few weeks after birth, the results of this study cannot substantiate the contention that oocytes present at birth constitute the total oocyte supply for life. That those oocytes remain dormant until maturity does not seem feasible as the life span of the oocyte is obviously not that long.

The different classes within a follicular type are reduced to zero at times (Fig. 15A), substantiating the concept that oocytes differentiated in the original secondary epithelial cords are destroyed. For example, in the pup period of quiescense, no class 1 (Table 3) primordial follicles are present. During estrus, only, are normal 3-5 mm follicles present. The abscence of older primordial and primary follicles during proestrus and estrus proves the necessity for a renewal of cord activity and differentiation of new oocytes.

Several generations of oocytes can be observed in one ovary. Cortical growth occurs through a buildup process by near-continuous epithelial cord activity. As a set of oocytes grows and moves deeper into the ovarian cortex, another set of oocytes is differentiated nearer the surface. Atresia of some follicles produces new interstitial glands, resulting in reduction of the deep cortex. Simultaneously, the medullary interstitial glands are shrinking and are not replaced: therefore, the size of the medulla decreases as the size of the cortex increases. At maturity the ovarian cortex is much thicker than the ovarian medulla, a situation opposite to that in the young ovary.

Progression of follicular stages as seen in the series examined and described indicates that when any follicle stops growing, it undergoes atresia. Such continuous growth was also interpreted from a study of bovine ovaries (Marion and Gier, 1971).

Interstitial glands form from the deep ends of the epithelial cords long before any follicles are formed with an oriented theca interna. Interstitial glands are always membrane-bound from the basement membrane of the epithelial cords or the membrana propria of the follicle (Fig. 5, 8, and 21). Interstitial glands were observed to originate from two sources: (1) the persistence of follicular cells after degeneration of oocytes within the epithelial cords (Fig. 5); or (2) the persistence of the stratum granulosa cells in atretic tertiary follicles (Fig. 21) after the oocyte and cumulus oophorus degenerate. The interstitial glands formed from the original medullary cords persist for a few months, then degenerate (Fig. 13 and 14). Interstitial glands in the cortex degenerate and are replaced by new interstitial glands.

Tsukaguchi and Okamoto (1930) reported interstitial glands as being present in the dog ovary from two to six months of age, but only occasionally in the adult. They also reported continuance of the medullary cords throughout ovarian history. The medullary cords were described as giving rise to the interstitial glands or contributing to follicle formation in some stages of development. Neither medullary origin of interstitial glands, nor the retention of medullary cords can be accepted. The only interstitial glands ever found in the medulla are those that form by degeneration of the primary epithelial cords and they normally disappear within the first year of life. Interstitial glands are present in the

cortex throughout the life of the ovary, and are not restricted to two to six month period after birth.

In addition to interstitial glands, interstitial cells (Berger Cells) occur throughout the ovary, between interstitial glands (Fig. 12) as single cells or in loose clusters and resemble the Leydig cells of the testis. Some atretic follicles, rather than forming interstitial glands, show hyalinization of the theca interna cells; granulosal cells are absent (Fig. 20).

Mature Ovaries

After the first pregnancy, or pseudopregnancy, the ovaries are mature in all respects and show the same characteristics that are found in ovaries after subsequent estrual periods. Anestrus (Table 7) is a stage of relative inactivity of the ovary lasting approximately 100 days, starting from parturition or the end of pseudopregnancy to the onset of proestrus. Anestrus is the beginning of a new estrous cycle. Six ovaries were examined for this period ranging from 66 to 150 days post ovulation (Table 2).

The CL at parturition shows degenerative changes which progressively reduce the structure throughout anestrus. At 66 days post ovulation (PO), or 6 days post partum (PP) (Fig. 21) the cells of the CL are loosened and the diameter of the CL has decreased from its maximum of 6 to 7 mm at 55 days to about 5 mm. By 85 days, blossom cells of the CL are highly vacuolated and apparently non-functional; and the diameter of the CL is further reduced to about 4 mm.

One ovary, 75 days post ovulation, contained no CL (Fig. 22). The opposite ovary contained three regressing CL's but the animal had not been pregnant. The major difference between the two ovaries was in the advancement of the follicular growth. The one ovary escaped the CL

repression, so contained a greater number of late secondary and tertiary follicles. In the ovary which contained three CL's, there were many less late secondary and tertiary follicles. Both ovaries showed Stage 4 epithelial cord activity with many new primordial follicles under the germinal epithelium (Table 5).

During metestrus II, epithelial cord activity persists only in the folds between the CL's (Fig. 23). Early in anestrus, epithelial cord activity is renewed in the area of the regressing CL (Fig. 24). The epithelium becomes more diversified because of regression of the CL (Fig. 25), with columnar epithelium to Stage 1 cord activity immediately over the CL and Stage 4 or 5 cords in the areas between CL'S. At 121 days post ovulation, there are many Stage 4 cords (Table 5) and a new set of primordial follicles (Fig. 26) which will probably be the source of the next ovulatory follicles. The number of secondary follicles remains stable at about 1400 with slightly less atresia (about 35%) (Table 6) than in the previous two months.

About 97% of the tertiary follicles are atretic at 120 day PO (60 weeks old), with only an occasional normal follicle, all under 1 mm diameter (Table 6).

Proestrus follows the long anestrus and lasts on the average eight days (Table 7). It is characterized by tertiary follicles enlarging to such size that they produce enough estrogen to initiate vulvar swelling, bloody vaginal fluid, and preparation of vulva, vagina and uterus for copulation and sperm transport. A small number of 2 to 3 mm tertiary follicles begin to show preovulatory changes, such as granulosal folding and more rapid increase in size (Fig. 27). As Evans and Swezy (1931) reported, these preovulatory follicles are much larger than other tertiary follicles present in the ovary. Only the largest follicles have any

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Figure 23. Cord activity (EC) is increasing in this diestral dog ovary (85 days post ovulation): activity is particularly high in the epithelial folds (EF) between the corpora lutea where activity is repressed least by the CL. Bouin fix, PAS-Hx stain. 40X.

Figure 24. In this dog ovary (85 days post ovulation), the cord activity is beginning again in the area of the CL, where the germinal epithelium was repressed during the time the CL was functional. The germinal epithelium (GE) remains cuboidal but small invaginations (I) of the epithelium are evident at this time. Bouin fix, PAS-Hx stain. 40X.

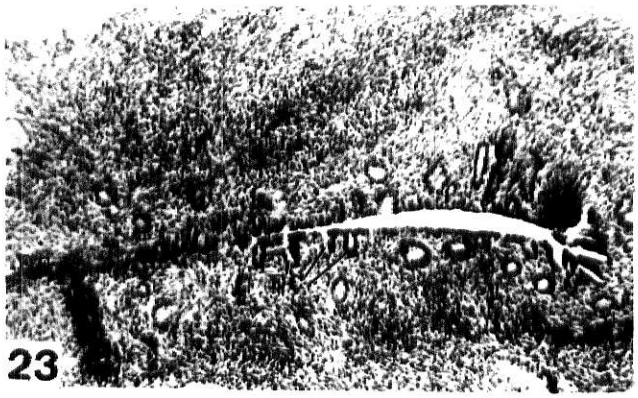
Figure 25. In this late diestral ovary (115 days after ovulation), cord activity has increased. A regressing corpus luteum (CL) is present, and degenerating tertiary follicles (TF) are abundant. Bouin fix, Hx-Mallory's triple stain. X13.

Figure 26. In this 121 day post ovulation ovary, epithelial cords (EC) are in Stage 4 activity with a new crop of primordial follicles (PF) recently differentiated. Variation of cord activity around the surface of the ovary is well illustrated here. Secondary follicles (SF) remain from the previous cord activity of early diestrus. Bouin fix, Hx-Mallory's triple stain. 40X.

Figure 27. A pre-ovulatory sized follicle with granulosal folding characterizes the proestrus ovary. Most of the tertiary follicles present are atretic; a new set of primordial follicles is present, and cord activity is decreasing. Bouin fix. HX-orange G stain. 16X.

Figure 28. Section of ovary from a bitch in estrus. Oocytes (O) are differentiating in active cords (EC) of the ovary in estrus. Late primordial follicles (PF) are present deeper in the ovary. Bouin fix, HX-Mallory's triple stain. 200X.

Figure 29. Estrus is characterized by high cord activity and the production of a new set of primordial follicles (PF). Cord activity varies around the surface of the ovary from low to moderate (Mo) to high (H). Bouin fix. HX-Mallory's triple stain. 50X.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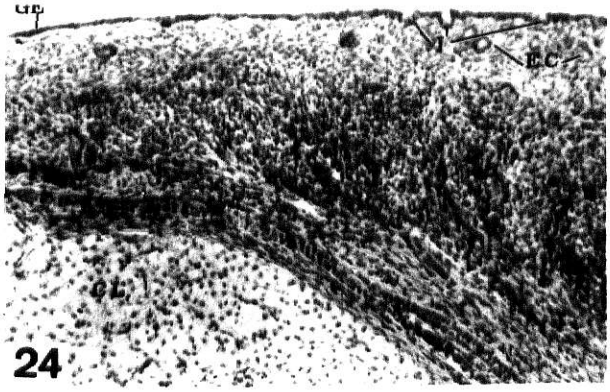

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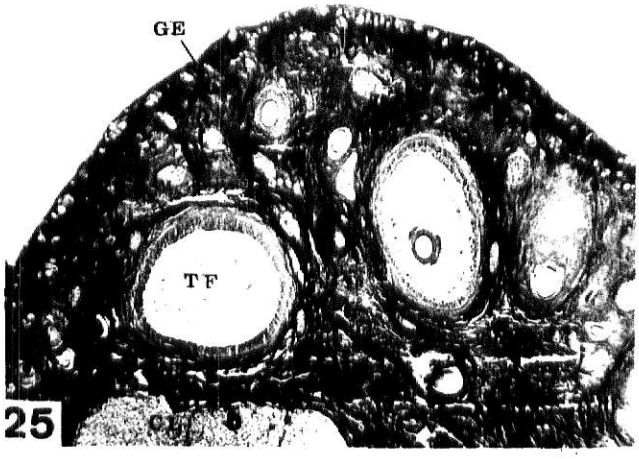

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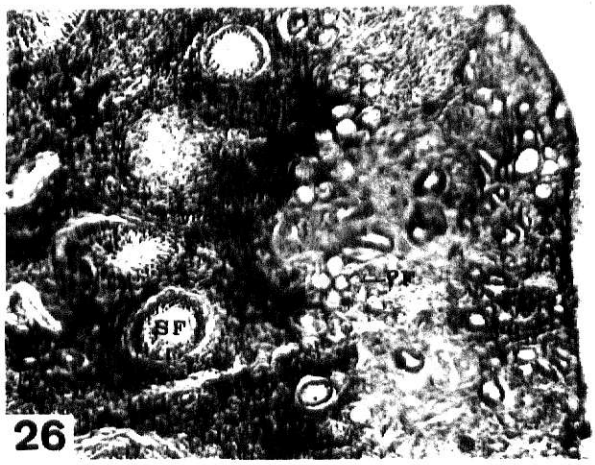

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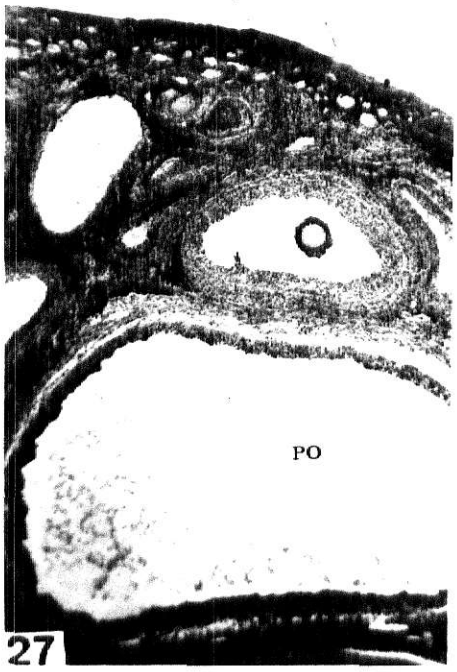

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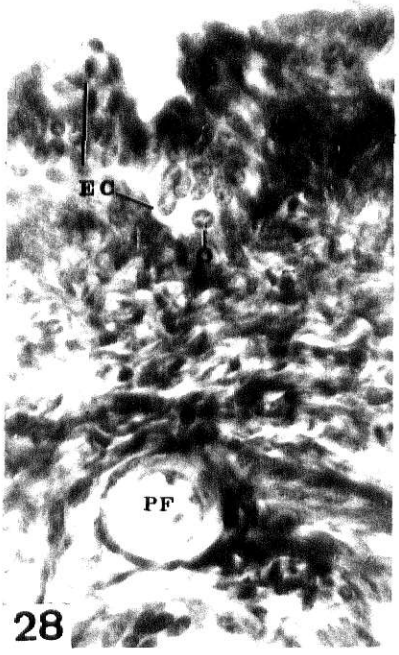

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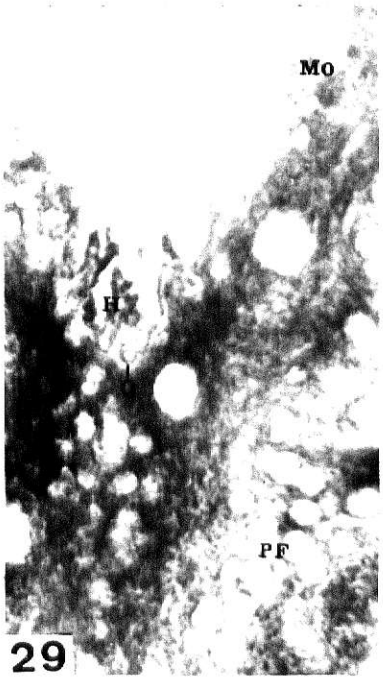

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Table 7. Characteristics of stages of the estrous cycle of the dog, as defined by Gier (1960).

Stage	Onset	Duration days	(avg)	Corpus luteum	Follicles	Vaginal epithelium	Vaginal fluid
Diestrus	parturition	100-120	(104)	inactive (CR)	many, < 1.0 mm.	2 cell layers	sparse
Proestrus	vaginal cornification, bloody discharge	2-20	(8)	inactive	1.2 - 1.8 mm.	5-10 cell layers, some cornified	bloody, copius
Estrus	receptivity	2-20	(9)	inactive	1.8-5.0 mm. granulosum folding, new primordial	20+ cell layers; entire lining cornified	bloody to chocolate; ep. cells and RBC, only
Metestrus I	ovulation	6-8	(7)	forming	all remaining tertiary follicles atretic	cornified cells sloughing	becoming white, sticky many leucocytes
Metestrus II	completion of corpus luteum.	50-54	(52)	functional	late atresia, only; new series primordial to early tertiary	2 cell layers; occasional stratification	mucus, sparse; many leucocytes

possibility of ovulation, and they enlarge from about 1.8 mm at the onset of estrus to 4.5-5 mm at ovulation.

During proestrus and estrus, epithelial cords show near maximal activity, with the formation of new oocytes and primordial follicles. The germinal epithelium ranges from low cuboidal to multilayered cuboidal and columnar, varying around the surface of the ovary. New oocytes (Fig. 28) can be seen in various stages of meiotic changes, differentiating within the cords which are in stage 3 of cord activity (Table 5). About one-third of the tertiary follicles present are normal, and two-thirds are in various stages of atresia: 15% stage 5, 62% stage 4, 12% stage 3, and 25% stage 1. About 50% of the secondary follicles are atretic at this time (Table 6).

As reported by Evans and Cole (1931), the CL's from the previous cycle are still present in the ovary although in a regressed state and appear as red bodies, or corpora rubra (CR).

Estrus is a period ranging from 2 to 20 days (Table 7), averaging nine days in length. During estrus, the bitch becomes attracted to and will accept a male. Estrogen production by the large follicles is maximal at this time. Estrus is terminated 48 to 72 hours after ovulation. Six estrual ovaries were examined from five animals (Table 2).

Cord activity varies in individual ovaries throughout the estrous cycle as well as within the cortex of any one ovary (Fig. 29). Cord activity during estrus ranges from shallow to deep cords with oocytes differentiating within the cords (Fig. 28 and 29). Many circular cross sections of the highly convoluted epithelial tubes (Pfluger tubes) commonly known as "anovular follicles" (Barton, 1945), are present in preparation of ovaries taken at the time of high cord activity.

Germinal epithelial activity varies around the ovary. Due to distention of the germinal epithelium by expanding preovulatory follicles, epithelial cord activity in the area of large tertiary follicles is low (Fig. 20) as reported by Evans and Cole (1931), Evans and Swezy (1931), and Barton (1945). The germinal epithelium over the large follicles is low cuboidal, not squamous as reported by Barton (1945), similar to the condition of the epithelium and cortex as a result of distention of the CL during metestrus. The growth of the follicle stretches and thins the germinal epithelium to the point of limiting activity and transforming the germinal epithelium to a low cuboidal condition.

The number of early primordial follicles increases through proestrus and estrus, accompanied by massive atresia of secondary and tertiary follicles. A new crop of follicles provides the source of future secondary and tertiary follicles.

Approximately 50% of all secondary follicles were atretic, the number of secondary follicles being slightly less than in other periods of the cycle (Table 6). Ninety percent of the tertiary follicles were atretic. Some hyalinized follicles (Fig. 20) were present but this stage (stage 5) of atresia was much less prominent than other stages as most tertiary follicles undergo atresia before reaching the 1 mm size. Of the normal tertiary follicles observed, 3% were ovulatory size (3 - 5 mm) during mid-estrus; 14% were 2 - 3 mm; and 83% were less than 1 mm.

Just before ovulation, the cumulus oophorus becomes re-oriented as the corona radiata and granulosal folding becomes accentuated (Fig. 30).

Metestrus I (M-I) is a transitional period (Table 2) in which high estrogen levels are replaced by high progesterone levels due to the

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Figure 30. Section through two ovulatory follicles (OF) in an estral ovary. The stratum granulosum shows extensive folding (GF). Epithelial cord activity (EC) varies around the ovary. Between the large follicles activity is higher than in the area adjacent to a large follicle. Bouin fix, Hx-orange G stain. 16X.

Figure 31. In an ovary three days after ovulation, the corpus luteum (CL) is maturing but remains loosely organized. Massive atresia is present in all remaining tertiary follicles (TF). Primordial follicle (PF) and primary follicles (PR) show a moderate rate of atresia. Bouin fix, Hx-Mallory's triple stain. 20X.

Figure 32. Dog ovary 13 days after ovulation, with corpora lutea (CL). Epithelial cord activity (EC) is limited to the area between the CL's. The epithelium (GE) near the CL is low cuboidal and cord activity is absent. Bouin fix, Hx-orange G stain. 16X.

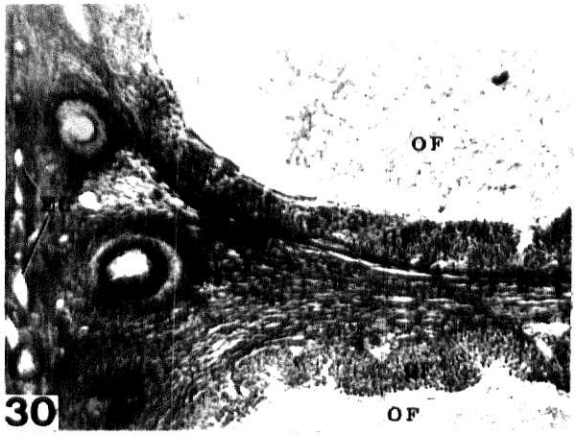
Figure 33. By 22 days after ovulation, cord activity is absent in the area of the CL; the germinal epithelium (GE) is low cuboidal due to the repressive factor of the CL. Cord activity is limited to the area of epithelial folding (EF). Bouin fix, Hx-Mallory's triple stain. 16X.

Figure 34. The germinal epithelium (GE) is greatly repressed by the presence of large functional corpora lutea. When the CL is maximally functional, the epithelium is low cuboidal. Bouin fix, Hx-orange G stain. 50X.

Figure 35. Thirty-one days after ovulation, the corpora lutea (CL) are highly functional. Interstitial glands (IG) from atretic tertiary follicles are present. Bouin fix. Hx-orange G stain. 16X.

Figure 36. A new set of primordial follicles (PF) has been proliferated in this ovary from a bitch 43 days pregnant. Proliferation of the new cords and differentiation of primordial follicles occurred between 31 and 43 days gestation. Primary follicles (PR) are present. An early (ES) and late (LS) secondary follicle are present. Bouin fix. Hx-Mallory's triple stain. 16X.

Figure 37. The corpus luteum from an animal 43 days pregnant appears to be fully functional Bouin fix. Hx-Mallory's triple stain. 200X.



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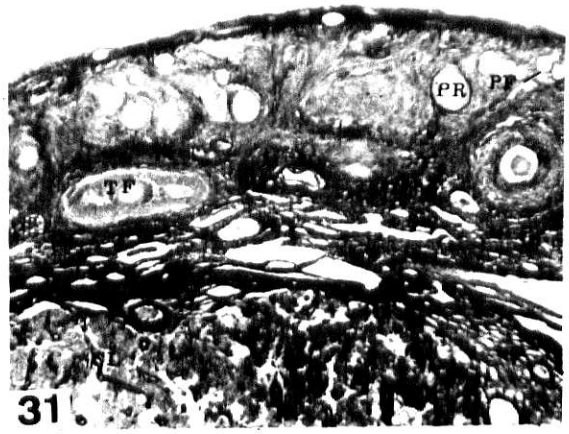

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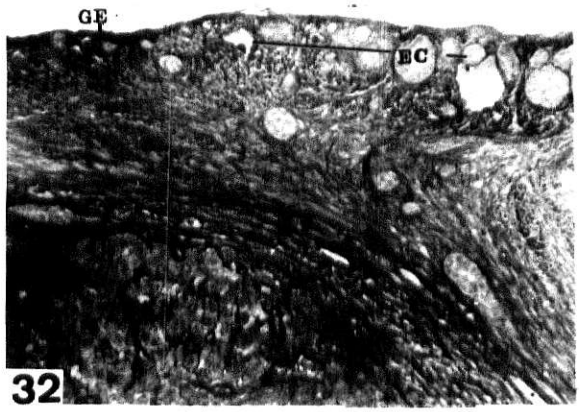

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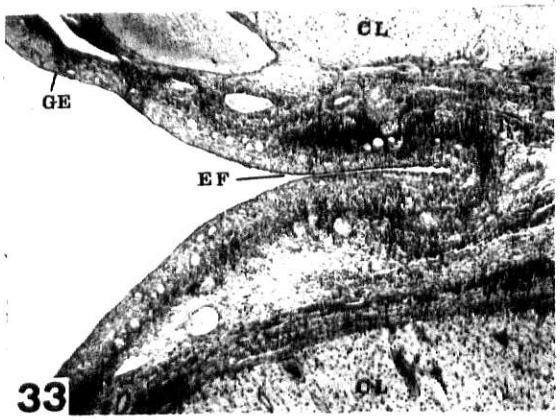

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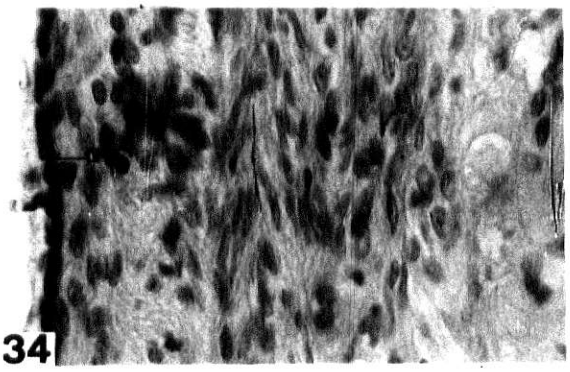
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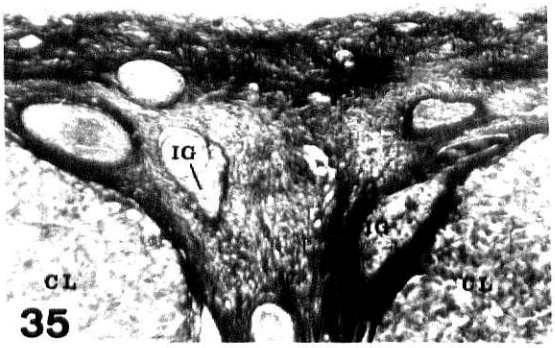

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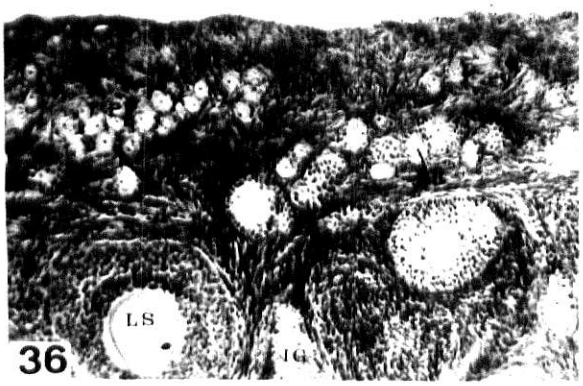

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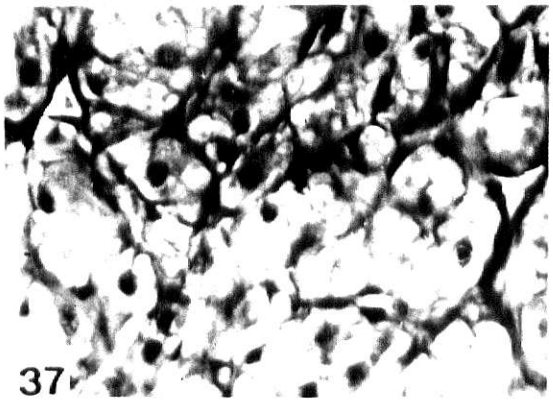
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development of a functional, progesterone-secreting CL and to atresia of the non-ovulatory estrogen-secreting tertiary follicles. M-I begins with ovulation, overlapping late estrus by about 3 days and lasts about eight days after ovulation. Twelve ovaries from eleven dogs were examined for this period, removed by surgery from the day of ovulation to eight days after ovulation (Table 2).

No appreciable ovarian differences were observed between pregnant and pseudopregnant animals, provided a functional CL was present in the ovary (Fig. 31 and 32). If the CL was absent, cortical activity was greater than seen in ovaries with active CL's. With the repressive factor of the CL absent, cord activity was high throughout most of the cortex. A large number of small normal tertiary follicles (<0.5 mm) were present, with two-thirds of them atretic; 37% of the atretic follicles were in stage 1, the remaining 63% were in stage 4 (Table 6). Stages 2 and 3 were absent, indicating that stage 4 atretic follicles originated from an earlier cycle of cord activity than that of the early atretic follicles and small normal tertiary follicles (Fig. 15A).

Each follicle that ovulates in late estrus forms a CL during M-I which becomes fully functional 7 or 8 days after ovulation. As the CL expands, the activity of the epithelium and the epithelial cords is suppressed, probably by two factors: (1) stretching of the epithelium over the CL, eventually resulting in a thin, stretched tunica albuginea and inactive germinal epithelium (Fig. 33 and 34) and the formation of folds of thicker tunic between CL's with active epithelium and epithelial cords of stages 1 and 2 (Table 5); and (2) direct repressive effect of progesterone produced by the CL upon the cord activity. Absence of cords over the CL, presence of cords between CL's and over the surface of ovaries or parts of ovaries

without CL's, suggests that progesterone diffuses directly from the CL through the thinned tunic to the germinal epithelium, thus repressing cord growth. The larger the CL and the nearer the germinal epithelium is to the CL, the lower the cord activity.

Cord activity varies from stage 2 to 5 (Table 5) with new cords, beginning proliferation of a new set of oocytes, to cords with decreasing activity after new oocytes and primordial follicles have differentiated. As the M-I period ends, the CL nears maximum size and epithelial cord activity is severely repressed.

Early in M-I, the germinal epithelium is more variable than in late M-I or in M-II, varying from low cuboidal, to columnar, to multilayered or stratified cuboidal. At the close of M-I, most of the germinal epithelium is low cuboidal, with stratification only between the CL's. Most tertiary follicles are atretic (93%) in late M-I, mostly in stages 3 and 4 (Table 4) indicating that atresia began during estrus. Only 3% of the tertiary follicles, all under 1 mm, were interpreted as being normal, and they probably contain oocytes that differentiated during late anestrus or proestrus. As observed in other stages, the number of secondary follicles remains relatively constant with 50% of them atretic (Table 6).

During M-I, the CL blossom cells, formed from granulosa cells, and companion cells from theca interna cells, are loosely arranged with much space between cells. Vascularization of the CL has begun (Fig. 31).

Metestrus II (M-II) is the luteal phase of the estrous cycle, with functional corpora lutea in one or both ovaries (Table 7). The M-II phase begins 7 or 8 days after ovulation, lasts 50-53 days and is terminated with parturition or the end of pseudopregnancy. Twelve ovaries, from 11 animals, were examined from this period (Table 2).

Where cord activity is not repressed by the presence of a CL, cords are in stage 5, or 1, or absent (Fig. 32 and 33, Table 5). Late primordial and early secondary follicles may be abundant, developed from primordial follicles that differentiated during estrus or M-I. Between 38 (Fig. 35) and 50 (Fig. 36) days P.O. (late pregnancy or pseudopregnancy), another wave of cord activity occurs, as the cords at 50 days are in cycle stage 4 or 5 (Table 5), with greatly increased numbers of new primordial follicles. Throughout M-II, the germinal epithelium is primarily low cuboidal (Fig. 34) except in the folds between C1's, where the new cords and primordial follicles are located.

Secondary follicle counts continue to be relatively constant with atresia continuing at near 50%. Continued massive degeneration of all follicle classes, coupled with the maintenance of a nearly constant number of secondary follicles indicates that repeated production of oocytes is necessary to maintain follicle numbers.

Only 3% of the tertiary follicles, again limited to the small class sizes, were determined to be normal; 97% of the tertiary follicles are atretic, mostly in stage 4 (Table 4). Follicles continue to develop but are constantly being destroyed, so none exceed the 1 mm stage.

The CL reaches its maximum size and function during M-II, with cells compact, vascularization complete, and possibly a persistent central lumen (Fig. 33 and 35). It persists in apparently functional condition until parturition, contrary to a report by Evans and Cole (1931) that the CL begins regression approximately 36 days after ovulation. A 50 day CL (Fig. 37) showed no regressive changes.

As in the immature ovary, the ovarian medulla and cortex are well defined in the mature ovary. The medulla contains rete, blood and lymph vessels, and connective tissue. The cortex contains all the follicles, a germinal epithelium and associated epithelial cords, a tunica albuginea and other connective tissue and interstitial cells and glands. Cords, follicles, interstitial glands and CL's are confined to the cortex (Fig. 42).

Polyovular follicles occur commonly in dog ovaries (Fig. 19 and 40), with two to seven oocytes per follicle. No polyovular follicles were observed to reach ovulatory size, all having undergone atresia or losing the supernumary oocytes before reaching this size. Polyovular follicles are formed by the basement membrane of the epithelial cord enclosing more than one oocyte as it separates from the cord. The follicle grows, possibly to vesicular stage, with follicular cells surrounding several oocytes within a single membrana propria.

As previously reported (Evans and Cole, 1931), the CL's from the previous cycle are retained although in a greatly regressed state. The CL continues to regress and is retained for several cycles as a corpus rubrum (CR). Three different cycles of CR's and a new CL are illustrated in Fig. 43. The CR's continue to degenerate until they disappear about 3 or 4 cycles after the CL was formed. A second cycle CR is illustrated in Fig. 44.

EXPLANATION OF FIGURES

Figure 38. Two types of atresia are illustrated here: (1) Complete degeneration of the follicle with no retention of granulosa cells and (2) retention of some granulosal cells after degeneration of the oocyte, the follicle collapses and forms an interstitial gland (IG). The arrow points to a follicle in which the granulosal cells are loosening and being shed into the follicular cavity. Bouin fix. Hx-Mallory's triple stain. 13X.

Figure 39. Enlargement of Figure 38 showing the formation of interstitial glands from atretic follicles (2). An intermediate stage of atresia shows loss of granulosa on one side and retention on the other in a large atretic tertiary follicle. Bouin fix. Hx-Mallory's triple stain. 40X.

Figure 40. This polyovular tertiary follicle containing four oocytes (O) represents an intermediate stage in the formation of an interstitial gland. An interstitial gland (IG) has developed at the end of the follicle (F) while the other end of the follicle is undergoing atresia. The membrana propria is intact. Bouin fix, Hx-Mallory's triple stain. 50X.

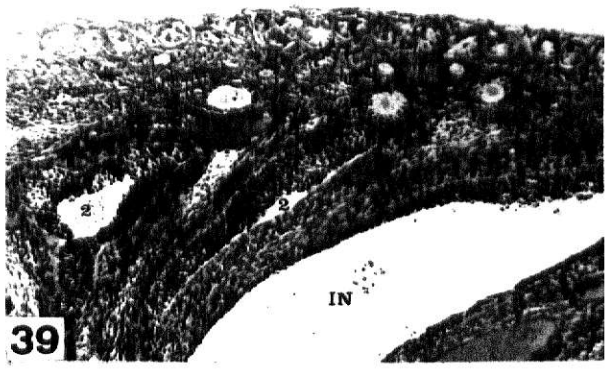
Figure 41. A progressive maturation of follicles is illustrated from primary (PR) to middle (MSF) and late secondary (LSF) to early tertiary follicles (ETF) with a forming antrum (A). Among these follicles are both interstitial glands (IG) and interstitial cells (IC). Bouin fix. Hx-Mallory's triple stain. 50X.

Figure 42. Cortex and medulla are well defined in this adult dog ovary. The small medullary portion contains blood vessels (BV), lymph vessels, connective tissue and rete (R). The larger cortex contains the germinal epithelium (GE), secondary epithelial cords and follicles. Follicles and corpora lutea (CL) are confined to the cortex. Bouin fix, Hx-Mallory's triple stain. 2X.

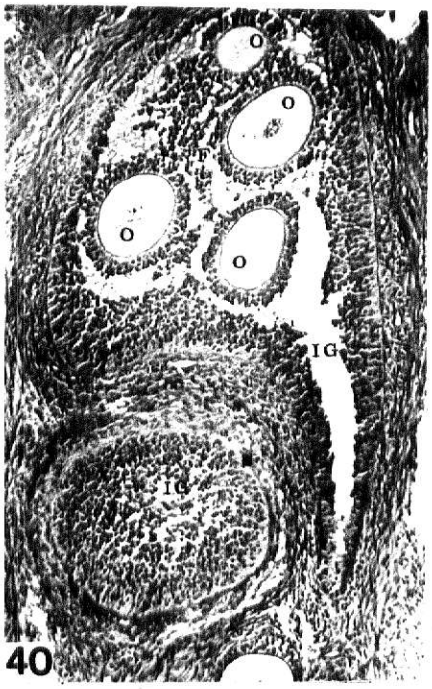
Figure 43. The CL after regression is retained in the ovary for several cycles as a red body or corpus rubrum (CR). Three cycles of CR's and a corpus luteum (CL) are present in this ovary: (CR 1, CR 3, and CR 4). Hx-orange G stain. 16X.

Figure 44. The corpus rubrum (CR 4) present in this ovary is from two estrous cycles prior to the present. Epithelial cords (EC) do not seem to be affected by the presence of the CR. Bouin fix. Hx-orange G stain. 16X.

38



39



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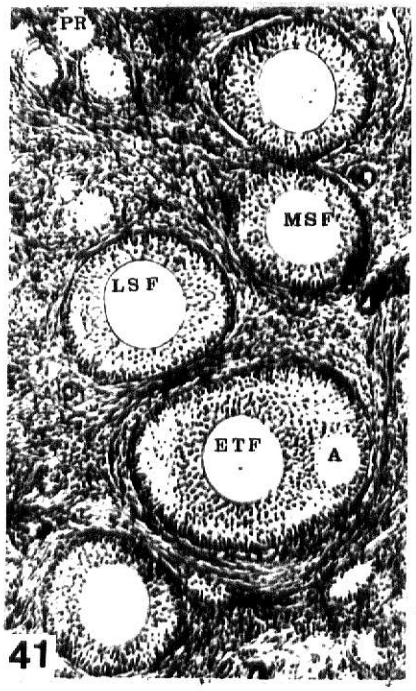

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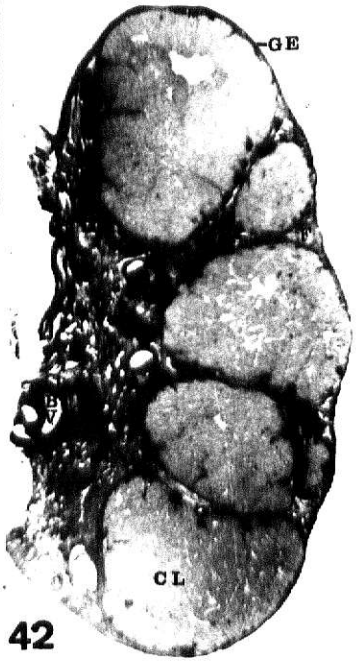

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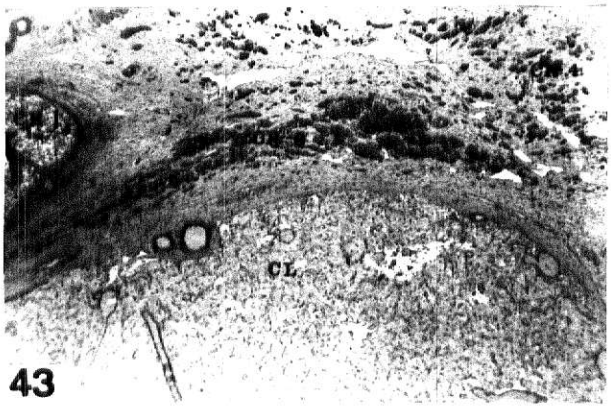

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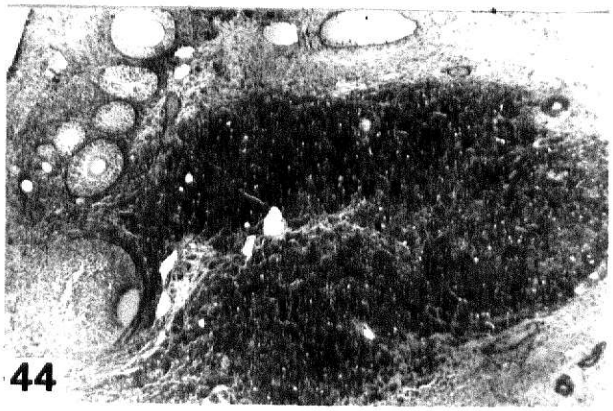

44

Follicular numbers

Counts of pre-follicular oocytes and follicles of all classes were completed on 68 ovaries taken from birth to proestrus; nine during proestrus and estrus; 22 during metestrus; and nine during diestrus (Table 1).

At birth, about 1.6 million oocytes are packed within the expanded secondary epithelial cords of each ovary, with no differentiation beyond the oocyte stage. A slight reduction in numbers occurs by eight days when each ovary contains about 1.5 million oocytes. Massive degeneration results in a 50% reduction in oocyte number by 24 days. The rate of atresia apparently is increased at about one month as a drastic reduction in the number of oocytes occurs between 24 and 63 days: 24 days, 719,000; 30 days, 359,000; 35 days, 134,000; 63 days, 80,000 (Table 6). After 9 weeks, the cords are no longer expanded as they were at birth, and oocyte numbers continue to decrease although new oocyte production continues. Ovaries from 9 week old dogs included remnants of the original packed cords, plus new oocytes and primordial follicles differentiated from recent cord activity.

Primordial follicles were first found in 4-week ovaries. At 5-weeks, about 12,000 primordial follicles per ovary are present. The number of primordial follicles after degeneration of the original packed cord population is relatively constant throughout the juvenile period, at about 24,000 from 9 weeks to 8 months (Table 6).

Primary follicles are first differentiated at about 4 weeks after birth. After 6 weeks, the number varies within a range of about 1600 to 8000 averaging 4300 per ovary. Secondary follicles first appear about 14 weeks after birth, after which the number remains within a range of 100 to 1000, averaging about 400 per ovary (Table 6). Such stability

in numbers coupled with observed atresia supports the concept that new proliferations germinal epithelial are constantly replacing older follicles and the lifetime of the oocyte is relatively short.

Tertiary follicles were first observed at 165 days after birth averaging less than 60 per ovary (Table 6). Numbers of tertiary follicles increase as the ovary nears the pre-estrus condition to an average of about 150 per ovary. The average tertiary follicular count for ovaries between 5.5 months and puberty was: normals, 14; atretics, Stage 1, 36; Stage 2, 10; Stage 3, 16; and Stage 4, 27. The number of follicles was directly proportional to the percentage of atresia. For example, one younger ovary had a total of 22 tertiary follicles with 50% atretic. An older ovary contained 210 tertiary follicles with 97% atretic (Table 6).

In the near-mature ovary, the number of follicles, particularly primordial follicles, vary with cord activity and CL activity. The number of primordial follicles is most variable, large numbers being present during and immediately after intense cord activity. The number of secondaries is the most stable (Table 6). The average numbers of particular follicular types within an ovary is: primordial, 130,000; primary, 5000; secondary, 1100; and tertiary, normal, 72; atretic 250 (Table 6). Reduction in follicular numbers from primordial to tertiary supports the concept of continual proliferation of new oocytes.

The largest numbers of both normal and atretic tertiary follicles are in the two smallest class sizes. Normal follicles reach the 3-5 mm class only during estrus. In one ovary of a bitch in proestrus, 95% of all tertiary follicles were atretic, ranging from early to late atresia (Table 6).

Atresia is correlated with follicular size and follicular stage. As the follicle matures, the probability of atresia increases, i.e., atresia in the primordial follicles is less marked than atresia in the tertiary follicles. As the primordial follicle matures, the chance of atresia and degeneration of the follicle increases; the follicle is less likely to reach maturity as it progresses toward maturity. The average percentage of atresia varies with follicular type, being least in the primordial follicle and greatest in tertiary follicles: primordial, 0.5%; primary, 8%; secondary, 50%; and tertiary, 97%.

By following through the progression of stages (table 6 and Fig 15A) it can be deduced that none of the oocytes from the embryonic cords ever progress beyond the primordial stage, that the oocytes formed during the early activity period (1.5 to 3 months) may develop into the early tertiary stage at 6 or 7 months and that the quiescent period (2.5-4.5 months) effectively separates the early follicles from the later developing (5 to 7 months) oocytes which may progress to ovulation at about 10 months.

The rapid disappearance of atretic follicles during metestrus provides proof that atresia is not a lingering process, and that no follicle is detectable in histological preparations for more than a few (10 to 20) days after onset of atresia, except those large follicles that reach the hyalinized condition of stage 5 atresia. The occurrence of atresia in 25% of the primary follicles, 50 to 80% of secondaries and 80 to 97% of tertiary follicles (100% during M-2) gives adequate evidence that the 10,000 primordial follicles present at 5 weeks, or even the 48,000 at 9 weeks could never provide the 80,000 at first estrus or the 232,000 found during late diestrus, plus providing for replacement in the 5,000 to 22,000 primaries and 1300 secondaries that are continuously progressing to higher stages,

or degenerating. All the evidence found in this study points to cyclic production of new oocytes and primordial follicles that progressively develop into more advanced stages, with extensive atresia all the way, so that the full life span of any one oocyte is no more than two or three months from primordial follicle to ovulation or atresia.

Indications from the counts available point to the probability that, at most, 25% of the primordial follicles ever progress for enough to become primaries, 10% of the primaries may reach secondaries, less than 10% of the secondaries ever become tertiaries, and 1% of the tertiaries may ovulate. To be more specific, 100,000 primordials may produce 25,000 primaries, 2500 secondaries, 250 tertiaries, and 2.5 ovulations, considering that the calculations are made for that crop of primordial follicles that lead to ovulation, in contrast to a series that normally reaches completion during mestestrus or diestrus and all follicles end in atresia. If we consider that during late puberty and proestrus, there are no more than 40,000 normal follicles of all categories, 70,000+ during metestrus, and 150,000 in late diestrus before onset of second estrus, with a fluctuation of primordial follicles from 10,000 to 232,000, it becomes obvious that new follicles must be produced somewhere. We have followed the production of new oocytes within the epithelial cords, which are pinched off with a few non-germ cells to make primordial follicles, each enclosed within its own membrana propria that is derived from the basement membrane of the epithelial cord. Each primordial cord so formed then progresses in development toward maturity or destruction. We have found no evidence to support the contention of Zuckerman (1957) that the original oocytes may lie dormant, nor that of Barton (1945) that the newly formed oocytes do not progress to the extent of possible ovulation.

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THE FOLLICULAR CYCLE IN THE DOG

by

KIT ELIZABETH POLING

B.A., Alaska Methodist University, Anchorage, Alaska, 1973.

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ABSTRACT

Dog ovaries used in this study consisted of 11 fetal, 25 immature and 41 cycling. Ovaries were serially sectioned and stained with hematoxylin and PAS or Mallory's triple. Counts and measurements were made of oocytes and primordial, primary, secondary, and tertiary follicles from three sections of each ovary to provide an estimate of the total follicles in each class. Progressive changes were noted in germinal epithelium, epithelial cords, follicles, and interstitial glands.

Differentiation of the ovary occurs by 33 days gestation. Oocytes were detected at 40 days; 1.5 to 2 million oocytes were present in 450 $\pm$ 50 epithelial cords in each ovary, with progressive reduction to 130,000 at 5 weeks; 80,000 at 9 weeks and all expanded cords and original oocytes gone at 15 weeks. Between 3 and 12 weeks after birth a new generation of oocytes differentiates within the original basal unexpanded segment of the epithelial cords progressing to primordial follicles by 32 days and primary follicles by 37 days. New oocytes undergo meiotic changes within the epithelial cords and differentiate rapidly. Clusters of cells separate from the cord as primordial follicles if oocytes are included, or as interstitial glands if no oocytes are in the separation segment.

No new oocytes or follicles are formed from 12 to 20 weeks, although follicular growth continues. At 5 months, cord activity increases; new primordial follicles differentiate; follicles develop to the vesicular condition by 7 months and to pre-ovulation state at 10-12 months.

Ovarian cord activity is continuous but cyclic; cords develop as invaginations of the germinal epithelium, oocytes develop within the cords, are pinched off at the ends of the cords, the cords disappear or become inactive. Cord length varies from 10 mu at Stage 1 to 250 mu at Stage 3, and back to 10 mu or less in Stage 5.

During middle and late anestrus, cord activity is high. Cord length ranges from 100 to 250 mu with new follicles differentiating. Cords remain active through Metestrus I and decrease in activity early in Metestrus II after the CL becomes functional. Cord activity during Metestrus II is restricted to folds between CL's with the height of cords 10 to 30 mu.

Primordial follicle numbers vary from 10,000 at 5 weeks, to 70,000 at 21 weeks, and 30,000 at 33 weeks. Secondary follicles are relatively stable at 750 $\pm$ 250 after 16 weeks. Tertiary follicles first appear about 28 weeks, increase to 200 $\pm$ 75 during proestrus, decrease to near zero during M-2, then increase after mid anestrus.

Follicular atresia during proestrus averages 0.5%, 8%, 50%, and 97% respectively for primordial, primary, secondary and tertiary follicles, directly proportional to size and maturity of follicles.

Interstitial glands are epithelial in origin, either from epithelial cords or from granulosal remnants of atretic follicles. Leydig-like interstititial cells (Berger cells) of stromal origin are interspersed throughout ovarian stroma from first differentiation to old age.

Polyovular follicles, commonly observed in the dog ovary, are formed by enclosure of groups of oocytes within one membrana propria during separation from the cord. All oocytes within a polyovular follicle usually degenerate by early tertiary stage.

Oocytes present in the dog ovary at birth all degenerate by 9 weeks, with only a few progressing as far as the primordial follicle stage. Functional oocytes are differentiated from new epithelial cord activity in waves at 2 to 3 months, 6 to 8 months; late metestrus or early diestrus; and proestrus. Total counts of follicles show little variation from puberty to old age, but counts by follicular class show continuous progression from primordial follicle to ovulation or atresia, thus necessitating the production of new oocytes, from germinal epithelium, throughout life.