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Ovarian Development in the Golden Hamster

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OVARIAN DEVELOPMENT IN THE GOLDEN HAMSTER

by

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LIFE

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INTRODUCTION

There is scarcely an aspect of early gonadal development on which investigators are unanimous. The relation of the germinal epithelium to the stroma, the continuity between primordial germ cells and oöcytes, and the changes which take place in the ovarian cortex throughout the embryonic and postnatal periods to the time of sexual maturity are still controversial.

The purpose of this investigation was to study the embryological and histochemical aspects of development of the ovary in the golden hamster (Cricetus auratus) with special emphasis on the formation of the secondary ovarian cortex. The factors considered were the determination of the age at which the secondary cortex begins to form, the structure of cells involved in the formation of the cortex, and the relationship between the so-called primary and secondary cortex. The aim was to contribute further information to the salient points of cortex formation in the ovary and to observe the development of the mammalian oöcyte in a Eutherian mammal.

Through the use of selected stains, this investigation attempted to follow the multiplication of embryonic ovarian cells in order to determine what definitive structures they might form. Using Masson's trichrome stain it was possible to trace the daily increase in connective tissue growth, to note the proliferations of the germinal epithelium, and to follow the changes

within the primary oöcyte. Employing the periodic acid-Schiff technique, attempts were made to differentiate cells by the intensity of the staining. Histochemical reactions were tried to show the presence of alkaline phosphatase in the hamster's primordial germ cells, as it is seen in other mammalian forms. Using the closely related azure B, methylene blue, and toludine blue stains, the binding capacity of the various cells was compared.

REVIEW OF LITERATURE

When attempting to study the formation of the secondary cortex in Eutherian mammals, it is necessary to consider the origin of the oöcytes as well as the formation of the epithelial (sex) cords, since both are intimately involved in its formation. While there has been a concensus of opinion with regard to the origin of the mammalian eggs from the sex cords, the origin of the cells that give rise to these cords has been a controversial subject for many years.

Since the earliest investigators (Pflüger, 1863), the majority of researchers tended to assume that the mammalian oöcytes are the direct descendants of the sex cords. These cords have been found to originate from the germinal epithelium in a variety of animals, e.g. in the lamprey (Butcher, 1927); in the mouse (Lange, 1896), (Allen, 1905), (Brambell, 1927); in the rat (Swezy, 1933); in the guinea pig (Schmidth and Hofman, 1941); in the cat (Winiwarter and Sainmont, 1909); and in the human (Gruenwald, 1942), (Forbes, 1942).

One school of thought believes that the primordial germ cells arise

during embryonic development through the proliferation of the so-called germinal epithelium that covers the gonad, as described in the lamprey (Butcher, 1927); in the fish (Balfour, 1878), (Foulis, 1875), (Wolf, 1929); in the bird (D'Hollander, 1904), (Semon, 1887), (Waldeyer, 1870); in the mouse (Allen, 1905, 1923), (Allen and Cresdick, 1937), (Allen, Smith and Gardner, 1937), (Brambell, 1927), (Everett, 1942), (Kingery, 1917), (Lange, 1896), (Stein and Allen, 1942), (Stein and Foreman, 1949); in the rat (Arai, 1920), (Deane, 1952), (Evans and Swezy, 1931), (Firket, 1920), (Hargitt, 1925, 1930), (Harz, 1883), (Swezy, 1933), (Vincent and Dornfeld, 1948); in the guinea pig (Bookhaut,1945), (Papanicolaou, 1924), (Rubaschkin, 1909), (Schmidth and Hoffman, 1941); in the armadillo (Hamlett, 1935); in the cat (Kingsbury, 1913); in the dog (Barton, 1945), (Pflüger, 1863); and in the human (Felix, 1912), (Forbes, 1942), (Gaillard, 1950), (Gruenwald, 1942), (Winiwarter, 1901), (Winiwarter and Sainmont, 1909).

However, the opponents of the germinal epithelial origin of oöcytes state that the germ cells become segregated before the formation of organ systems in the embryo, as appears to be the case in the amphibian (Humphrey, 1928), (Hegner, 1914), (Kuschakewitsch, 1910), (Witschi, 1929); in the fish (Johnston, 1951); in the bird (Benoit, 1930), (Berenberg-Gassler, 1913), (Dantschakoff, 1908, 1932), (Goldsmith, 1928, 1935), (Nussbaum, 1880), (Pearl and Schoppe, 1921), (Reagen, 1916), (Swift, 1914, 1915), (Willier, 1937); in the mouse (Mintz, 1957), (Rudkin and Griech, 1962); in the rat (Cowperthwaite, 1925), (Mandl and Zuckerman, 1951), (Moore and Wang, 1947); in the cat (Kingsbury, 1938), in the armadillo (Enders, 1960); (Vanneman,

1917); and in the human (Fuse, 1912), (Gillman, 1948).

Waldeyer (1870) was the first to present the problem of the embryonic origin of the definitive germ cells in his work on the domestic fowl. He surmised that definitive germ cells and follicle cells arose from the modified peritoneal covering of the embryonic gonad. However, he did not claim to have seen this transformation. A contemporary of his, (Foulis, 1875) shortly afterward demonstrated the difficulties to be encountered when attempting to interpret the dynamic development of the ovary from sections by stating that although the ovum seemed to be derived from the germinal epithelium in the fish embryo the follicle cells were formed from ordinary connective tissue stroma.

Nussbaum (1880) was the first to formulate the theory that "sexual cells do not come from any cells that have given up their embryonal character or have gone into building any part of the body, nor do sexual cells ever go into body function."

Swift (1914, 1915) reported that the "entodermal wandering cells" (Dantschakoff, 1908) which arise in the crescent area of 16 to 24 hour chick embryos are primordial germ cells. He traced these via the blood stream and their own amoeboid movements to the root of the developing mesentery in the region of the gonad. He stated that only the follicle cells are derived from the coelomic epithelium.

Rubaschkin (1909) and Berenberg-Gossler (1913) were able to find primordial germ cells in the entoderm and splenchnic mesoderm lateral to the coelomic angle in the 22-23 somite chick embryo. However, it was only

surmised that the primary germ cells entered the gonad, since neither Swift, Rubaschkin, nor Berenberg-Gossler have actually seen them enter it.

Reagan (1916) and Goldsmith (1935) both tried to determine the effect of excision of the crescent shaped area of the extraembryonic blastoderm in the chick. The embryos were entirely devoid of germ cells after the operation even though there persisted sometimes a germinal epithelium. Danschakoff (1932) cauterized the crescent area and concluded that migratory entodermal cells were necessary for normal gonad formation and that the potential of the coelomic cells to form germ cells is highly doubtful.

Balfour (1878), on the other hand, occasionally saw specially large cells in the outermost layer of germinal epithelium of the embryonic fish which he believed perhaps deserved the appelation primitive ovum. He also noted the striking resemblence in size and character of the cells of the germinal epithlium to the solid cords of epithelial-like cells which grow from the periphery toward the center of the ovary. In addition Marz (1883) observed in many embryos cells growing around the enlarged epithelial cell to form follicles, and these follicles being pushed deep into the ovary. Lange (1896) in the mouse embryo found that the follicular structure can also be produced by the epithelial cords, but Gillman (1948) states that at no stage in human gonad development are typical sex cells present in the active coelomic epithelium. In the armadillo Hamlett (1935) and Enders (1960) report that there is no neoformation of odcytes from the germinal epithelium in the developing or mature ovary, because of the absence of mitosis and more specifically the absence of meiosis in the germinal epithelium.

Wolf (1929) in the fish, Semon (1887), and Allen (1905) in the mouse, rabbit and pig saw transitional forms which linked the peritoneal cells with the primitive sex cells found in the sex cords of embryonic ovaries. However, Allen thought that the granulosa cells arise only from the medullary cords, which are the first proliferation of the germinal epithelium.

A similar origin was assumed in the lamprey (Butcher, 1927), in the mouse (Brambell, 1927), in the rat (Swezy and Evans, 1930), in the guinea pig (Bookhaut, 1945), in the cat (Winiwarter and Sainmont, 1909) and in the human (Deane, 1952), (Felix, 1912), (Firket, 1920), (Hargitt, 1925, 1930), and (Winiwarter, 1901) mainly because the authors saw large cells in the embryonic germinal epithelium as well as in the inner aspects of the ovary. These large cells were assumed to be primordial germ cells. The impression was that the daughter cells of these large cells degenerated and that the germinal epithelium later yielded the definitive ova.

Allen (1923) showed evidence that there are germinal epithelial proliferations, in the adult mouse, which are of a cyclic nature correlated with reproduction. Allen and Cresdick (1937) and Allen, Smith and Gardner (1937) by using colchicine on adult mice demonstrated mitosis in the germinal epithelium and the height of its apparent cyclic activity appeared at estrus. Stein and Allen (1942) succeeded in stimulating the proliferation of the germinal epithelium by local injection of estrone into the ovarian capsule of adult mice. Stein and Foreman (1949) found that thyroxine inhibits germinal epithelium proliferation, but the extract of the entire thyroid stimulates proliferation. This is in disagreement with Papanicolaou (1924) who was able

to accelerate ovarian proliferation in the adult guinea pig by irradiation of the thyroid gland.

Vincent and Dornfeld (1948), using the possible relationship between the presence of high concentrations of nucleic acids, especially ribonucleic acid, and the rapid proliferation of certain regions near the hilus of the germinal epithelium in prepubertal rats, regard any cell in the germinal epithelium as a potential egg cell.

Gaillard (1950) cultivated fragments of the embryonic ovarian cortex noting that the germinal epithelium encapsulated the fragments with one layer of cuboidal or columnar cells. The primordial follicles appeared to be alive after all parenchymal elements, including the egg cells, had degenerated and disappeared. After a few days the epithelium began to proliferate into the interior of the explants, and numerous young ova developed within the cords. Thus a histogenetic relationship was indicated between the germinal epithelium, the follicle cells of the primordial follicle and the egg cells.

Despite the apparently widely demonstrated validity of these findings, important papers have appeared which contradict these tenets. By counting ova in different age groups, Arai (1920) observed a decrease in the oöcyte population in the ageing rat. Mandl and Zuckerman (1951) and Moore and Wang (1947) did not observe any decrease in the oöcyte population following the destruction of the germinal epithelium in the mouse.

Witschi (1929) in the embryos of frogs also found that a destinctive characteristic of the primordial germ cells is their large size. He is

convinced that translocation of the germ cells was effected by active, innate movements of the cells themselves. From the appearance of the surrounding tissue he concluded that "the gonia make extensive use of their apparent capacity for lytic destruction of membranes, of intracellular substances, and even of entire cells that block their way. The direction of movements is possibly influenced by the release of some substance from the peritoneum of the gonadal area."

Hamlett (1935) presents an interesting abnormal picture which has a definite bearing on this discussion. In the adult armadillo ovary he found tissue projecting abnormally from the surface of the ovary. It was composed of Pflüger's cords proliferating outward instead of inward from the germinal epithelium. In cellular composition the proturberance was seen to be identical with the proliferating cortex of the ovary, having epithelial cells, oöcytes and oögonia. The tunica was neither disrupted nor invaded in the slightest degree by the growing mass. It could be concluded from this observation that cyclic oögenesis, such as has been claimed for the rat, is not possible in mammals having a well developed tunica albuginea.

Forbes (1942) also in the human embryo traces the primary epithelial proliferations into the medulla. He stated that the later epithelial proliferations, the future cortex, develope rapidly, dwarf the primary sex cords and crowd their remnants to a central position.

Mintz (1957) reports that phosphatase-positive cells are present in the embryonic mouse ovary, but since no phosphatase-positive cells are proliferated from the germinal epithelium after birth, the tissue does not

appear to be a secondary source of germ cells, and the primary cells may be regarded as the definitive ova.

Rudkin and Griech (1962) intraperitoneally injected labelled thymidine into female mice midway through the gestation period. It was demonstrated by autoradiographic methods to be incorporated into the nuclei of oöcytes of female embryos; it was also found in the oöcyte nuclei of the daughters of similarly treated females at maturity. These observations support the theory that the primordial germ cells are the only source of oöcytes.

A sharp division of opinion still exists on the fundamental issue of the role of coelomic epithelium in gonad development. Either, new ova are formed throughout life, from the embryonic period through the time of sexual maturity from the germinal epithelium; or, ova are formed in the embryos in large numbers and stored in the ovary as a reserve to be drawn on throughout life. This paper serves to contribute to the clarification of this controversy.

MATERIALS AND METHODS

The Golden hamster (Cricetus auratus) was chosen for this study because the hamster has a shorter gestation period than any other commonly available laboratory mammal, and as a consequence the hamster developes faster than any other Eutherian animal on record (Greves, 1945), having a gestation period of 15 days. For example the 13 day old hamster embryo is equivalent in development to the 10 day old rat, the 24 day old pig, or the 60 day old human embryo (Magalhaes and Briggs, 1962).

Males and females were kept in an air conditioned room in standard separate cages. Due to the need of a high protein diet, the hamsters were fed Dog Chow pellets, occasionally green lettuce, and tap water ad libitum.

In an effort to determine the sexual cycle of our females, an attempt was made to record the 4 day estrus cycle of the animals by obtaining vaginal smears with a fine cotton swab. This method proved to be unsatisfactory since the females became pseudopregnant for 8 to 13 days as the result of vaginal irritation. Subsequently the pipette method, in which a drop of saline is placed in the vagina and removed with a fine glass pipette, was tried and ultimately found to eliminate the pseudopregnancies, but it still did not contribute sufficiently to effectively tracing the cyclic vaginal changes. In the hamster about one centimeter from the vulva, the vagina forms two vento-lateral pouches which as a rule are almost completely filled with layers of cornified epithelium. Since this is in a constant state of growth and sloughing, cyclic changes are difficult to observe (Deanesly, 1958).

Satisfactory matings were obtained by putting one male into a cage with two females, which had previously been tagged with an earpunch for identification purposes. Each morning between 7 AM and 10 AM vaginal smears were taken with the pipette method, and stained with hematoxylin-eosin stain. The vaginal smears were examined for the presence of sperm. Knowing that females accept the male only when in estrus, if sperm were present it was assumed that fertilization had taken place the night before, putting the mating approximately between 2 AM and 4 AM (Harvey, Yanagimachi and Chang,

1961).

The age of the embryos was computed from the date of mating, and the specimens ranged from 8 days post coitum to 15 days post partum. The technique involved: subjecting the pregnant hamsters to terminal ether anesthesia, removing the embryos from the uterus and freeing them from their membranes. Entire 8 and 9 day old embryos were placed into fixative. The apparently indifferent gonadal region of the 10, 11, and 12 day old embryos was dissected out with the aid of a dissecting microscope. After opening the abdomen of the 13 day old embryos, sex could be determined grossly, so thereafter only the ovaries were removed and preserved.

If the animals were to be obtained for study after birth, virgin females 3 to 4 months old were mated. Young females were used because it was found that the older the mother, the more cannibalistic she was toward her young, as was reported also for mice by Deckle, Atkinson and Pete (1957). The mother and her litter were left undisturbed in breeding cages until the young were of appropriate age. Up to and including 7 days post partum animals, both the males and females were taken from the mother since the external genitalia were not developed sufficiently in order to differentiate the two sexes. At 8 days the female showed mammary glands along the abdomen, and this served as a useful tool in utilizing only the female animals thereafter. Several cervical vertebrae were crushed with forceps to kill the young. During the post partum period the dissecting microscope was still needed to remove the very small ovaries, which were found with the oviduct at the ends of the uterine horns.

A total of 40 adult, mated, female hamsters, which yielded 147 female fetuses, were utilized in this study.

The dissected gonadal region or ovary was immediately placed in Bouin's solution—if it was to be studied with histological stains; or, in cold acetone, if histochemical techniques were to be employed. The tissue was then dehydrated, imbedded in celloidin-paraffin, and serially sectioned at 4 μ in order to obtain a three dimensional picture.

Sections were stained with hematoxylin (Harris') and eosin to obtain a general histological picture of the tissue. Masson's trichrome (Davenport, 1960) stain was used mainly to follow the infiltration of connective tissue into the ovaries, and also served well in showing the transformations of the ovary from one stage to the next.

In order to determine the presence of mucoproteins and polysaccharides the periodic acid-Schiff reaction was employed (Pearse, 1961).

Gomori's calcium cobalt method for alkaline phosphatase and Fredrieson's modified Gomori method for alkaline phosphatase were both tried to show the sites of enzyme activity (Pearse, 1961).

The similar stains of azure B, methylene blue, and toludine blue were used with the hope of revealing certain cells which have a stronger affinity for one or more of these stains due perhaps to their containing a greater amount of ribonucleic acid or deoxyribonucleic acid than their neighboring cells (Pearse, 1961).

EXPERIMENTAL RESULTS

Our investigation was primarily designed to observe the morphology of the developing hamster ovary, by studying in great detail the proliferations of the germinal epithelium in the hope of determining exactly what cells it forms, or does not form.

10 day embryo, (9 specimens studied). The primordia of the sex glands appeared as accumulations of epithelial cells forming a very small, (approximately 60 μ in diameter) indistinct genital ridge, which was beginning to be separated by a shallow groove from the lateral mesonephric fold (Fig. 1). The cells bounding the coelom in this early stage formed a continuous layer on the coelomic side only. Basally the cells exhibited long processes like those of the underlying mesenchyme cells, and there was no basement membrane separating them from the deeper mesenchyme. The cells all appeared to be of the same type. Embryonic red blood cells varied in size and shape, but their nuclear and cytoplasmic characteristics were so distinct that they could not be confused with any other cell. They were smaller and their nuclei were pycnotic.

10 1/2 day embryo, (8 specimens studied). The gonad primordium was more advanced at this stage. The genital ridge was now distinct; its division from the mesonephric fold was marked by a deep groove (Fig. 2). A definite coelomic epithelium as manifested by the appearence of a bounding basal membrane was established. The gonad was more compact than the surrounding tissue; the cells of the cuboidal epithelium were staining

deeper than the cells within.

Sex could not be determined through the microscopic inspection of the gonad, although on closer examination the presence of two distinct dark staining bodies in the nucleus of the majority of cells, one the nucleolus and one the Barr body (Barr, 1949), or female sex chromatin, revealed that the animal was to develope into a female (Fig. 2). Connective tissue was visible in the surrounding area, but none was evident in the gonad although the presence of capillaries and blood cells was observed. The nuclear membrane and some of the nuclear substence were strongly PAS-positive, while the cytoplasm showed only a moderate PAS reaction. The nuclear membrane, nuclear organelles and the cytoplasm stained intense blue with methylene blue stain at a pH of 7.

11 day embryo, (13 specimens studied). In dissecting the gonads from the embryo it was still practically impossible to distinguish between the males and females, but with the help of histological sections the males could be separated from the females by the presence of cord-like structures and the elongation of cells below the germinal epithelium assuming the shape of tunica albuginea cells in the testis.

Despite the fact that relatively few mitotic figures were visible, the ovary increased in size (approximately 340 μ in length and 130 μ in width) during the 24 hours and appeared separated from the genital ridge. It consisted basically of an extension of the indifferent gonad (Fig. 3).

The surface epithelium was composed of flat to cuboidal shaped cells, from one to four cells thick and frequently presented an irregular

outline. Throughout the entire ovary no cell membranes were clearly defined. Most of the cells comprising the genital ridge appeared small (5-7 μ) with the exception of some larger ones (8-10 μ) which contained more PAS-positive material and possessed a greater affinity for toludine blue, azure B, and methylene blue stain when buffered in solutions of pH 4 to pH 7. The larger cells (possibly primordial germ cells) had large, vesicular nuclei. No similar cell type was observed in the germinal epithelium. In the inner part of the genital ridge (the blastema) they were observed in clumps of 2 to 4, possibly indicating a recent mitotic division. Connective tissue was present throughout as fine single fibers.

12 and 13 day embryo, (19 specimens studied). In this phase of development the embryo's sex could be determined by inspecting the genital ridge. Although there are similar histological characteristics between the two sexes the distinctive separation of the testis tubules served as a ready diagnostic aid.

The ovary (approximately 400 μ in length and 170 μ in width) consisted of a central medulla composed of indistinctly delineated islands and cords of large vesicular (Primordial germ) cells which were individually surrounded by a loose connective tissue capsule. Without, the ovary was covered by a flat to cuboidal germinal epithelium which in places appeared to be continuous with cell cords that separated the islands of primary (primordial) germ cells from one another (Fig. 4). The nuclei of the germinal epithelial cells were relatively large (though smaller than those of the primary germ cells) and moderately dense; their cytoplasm was so

scanty that the epithelial cords seemed to consist of a mass of nuclei in a syncytium.

Although there was little mesenchyme or connective tissue between them, some small blood vessels were readily observed. Likewise no tunica albuginea separated the epithelial cords from the coelomic epithelium, although in some discontinuous areas an unmistakeable, wavy, PAS- positive basement membrane was observed. Some of the primary germ cells retained the staining properties of 11 day development, the cytoplasm contained PAS-positive material and has shown an affinity for azure B, toludine blue, and methylene blue stain.

The primary odgonia were usually situated well below the cortex, but occasionally some were observed between the epithelial cords. Primary odgonia were characterized by large vesicular nuclei (only a few mitotic figures were in evidence) with comparatively scanty, deeply staining cytoplasm around it. Such cells were not encountered in the coelomic epithelium.

14 and 15 day embryo, (20 embryos studied). The closely packed cells of the epithelial or sex cords were now more clearly defined, their boundaries standing out sharply (Figs. 5 and 6). They were pressed against the germinal epithelium, which was found to vary from a thin layer of cuboidal or squamous cells to several cells in thickness, with only a partial tunica albuginea separating them from the epithelium.

The most striking characteristic of this age was the occurence of nests of large lightly stained germ cells between the cords. Between some of the cords only a few scattered germ cells were seen, whereas between

others many clusters of primary germ cells were accompanied by a few indifferent cells. The primary oögonia were equally abundant in all regions of the ovary, in the medulla some of them were in the leptotene stage of meiotic prophase, whereas those in the cortex appeared to be in interphase. Mitotic figures were less common than in the previous stage, but still occurred.

The epithelial (sex) cords originated at undefined intervals from the proximal layer of the germinal epithelium, and sometimes fused to form relatively thick cords. Close examination showed that these cords were wavy in their course toward the hilus of the gonad. In the outer layers they were clearly separated and retained their continuity with the germinal epithelium covering the ovary. Growth was apparently maintained through the activity of the coelomic germinal epithelium. In the cortex the cords were closer together and were less easily recognized, owing to the reduction of the mesenchyme and the absence of the very large primary sex cells. The cells in the periphery of the ovary were smaller because they were multiplying. In the center of the ovary where the sex cords met, they appeared to form tubules.

The mesenchymal connective tissue cells appeared small, round and lightly stained. They were forming a sheath around the sex cords. In some sections it seemed that the sex cords were separated from the epithelial zone by a layer of connective tissue, but in examining serial sections it became evident that this connective tissue did not form a continuous layer in the ovary. The ovary (approximately 540 μ in length and 400 μ in width)

at this stage was covered by a well formed capsule.

1 and 2 days post partum, (11 specimens studied). The ovaries (approximately 670 μ in length and 400 μ in width) resembled those of the two previous stages, except that meiosis was more advanced. The majority of the primary germ cells in the medulla (Fig. 9) were now in the zygotene stage and the majority of the ones in the cortex (Figs. 7 and 8) were in the leptotene stage. In the medulla nests of primary germ cells were found surrounded by the innermost cords from the germinal epithelium. The ovary was now penetrated by thick bundles of connective tissue radiating from the hilus.

3 and 4 days post partum, (12 specimens studied). The germinal epithelium was not sharply demarcated from the underlying layer, in which were found large primary sex cells and smaller cells derived from and resembling the epithelium (Figs. 10 and 12). In the medulla these elongated epithelial cells encapsulated groups of primary germ cells, and could be called pregranulosa cells (Figs. 11 and 15). Interspersed between the primary germ cells and the epithelial cells, small round mesenchymal cells could be seen. In looking at the whole ovary (740 μ in length and 540 μ in width), the impression was received that the germinal epithelium proliferated cells which migrated through to the center of the ovary. There was no sharp demarcation between the cortex and the medulla, and the germinal epithelium was in a state of continuous proliferation.

Most of the primary germ cells of the medulla were in the pachytene stage of meiosis, and the primary germ cells in the cortex were in the

zygotene stage. A few of the medullary germ cells were degenerating and appeared as deeply staining bodies.

The vascular and connective tissue growth took the path of the cords which separated the nests of primary germ cells.

5, 6, 7 and 8 days post partum, (24 embryos studied). The ovary is now approximately 800 μ in length and 540 μ in width. The germinal epithelium was flat and one cell thick in most regions (Figs. 14 and 17). In places where sex cords were still being proliferated from the germinal epithelium there were about four layers of flattened cells and one outer layer of cuboidal cells (Fig. 14).

The cords and the "egg tubes" (accumulations of oöcytes) between the cords were thick in the cortex and narrower in the medulla. The innermost mass of epithelial cords, occasionally contained a primary germ cell, but as a rule the germ cells of this region had degenerated by this time, and the surrounding cells persisted as the epithelial tubules (Fig. 15).

No follicle, or primary germ cell was surrounded by epithelial cells in the cortex (Fig. 14 and 17), whereas in the medulla granulosa cells appeared to be insufficient to provide a capsule for some oöcytes, and groups of germ cells were encapsulated by sex cords.

Primary oöcytes in arrested meiotic prophase became increasingly more numerous in the medulla of the ovary, whereas those in pachytene and diplotene stages became progressively restricted to the outer cortex. A great number of the primary germ cells in this region were already in the dictyate stage. Primary follicles were beginning to develope in the

medullary region.

Septa now extended throughout the cortex and spread out below the investing epithelium with the epithelial cords, but did not form a continuous tunica albuginea (Fig. 16). If this connective tissue could be considered a tunica, it is a very imperfect one.

9, 10, 11 and 12 days post partum, (24 specimens studied). The germinal epithelium was only a single flat layer, one cell thick (Figs. 18 and 20). The accumulation of epithelial cells into cords was no longer present, nor were the "egg tubes". Throughout the ovary nests of follicles were formed (Figs. 18, 19 and 20). The ovary was now composed of a mass of primitive follicles separated by stroma. The follicles in the 11 and 12 day ovary were larger and the follicle cells were less flat than in the 9 and 10 day ovary. There was still no definite continuous tunica albuginea, but it might conceivably be the case that the hamster ovary never develops a well formed tunica albuginea. The primary germ cells were almost entirely in the dictyate stage.

For all practical purposes the ovary has attained the precursor form of the adult ovary. It now has a "secondary cortex" composed of unilaminar follicles with oögonia in the resting stage before the second meiotic division.

13 days post partum, (7 specimens studied). Follicles two and three cell layers thick surrounded some of the resting germ cells in both the cortex (Fig. 21) and in the medulla (Fig. 22). Other than that there were no changes since the previous stages.

During the development of the ovary, embryos of different ages were tested for alkaline phosphatase activity. We were unable to reveal significant alkaline phosphatase sites in the developing ovary. There was a generalized, non-specific esterase activity over the entire ovary, the primary germ cells not differing in any way.

The germinal epithelium, the sex cords, the follicles, and the ovarian connective tissue were rich in cytoplasmic ribonucleic acid and stained much deeper with eosin stain than the primary germ cells, which apparently contained only moderate amounts of ribonucleic acid in comparison to their size.

Minute amounts of PAS-positive material was found throughout the ovarian strome and particularly in the connective tissue fibers. The cell structure were faintly outlined in PAS-positive material--chromosomes stained deeper than the nuclear and cytoplasmic membranes.

Connective tissue could be easily identified when the ovaries were stained with azure B, methylene blue, or toludine blue stain. The cell membranes, nucleoli and Barr bodies also stained intensely with these stains.

DISCUSSION

For the identification of cell types, even in adult organs, valid and generally accepted criteria are necessary, otherwise, the personal factor interferes with interpretation. Many criteria have been proposed for identifying embryonic germ cells, but their large size appears to be the most

reliable. Despite the objection that all cells round off and become large before mitosis (Hargitt, 1925), obviously oöcytes tend to be especially large and better defined than somatic cells (Allen, 1904; Felix, 1912). The structure of the mitochondria and the Golgi apparatus varies too much according to the physiological activity of the cell to be considered as an identification characteristic.

At no stage in the development of the hamster's ovary were typical sex cells present in the active germinal epithelium, as in the chick where they enter it secondarily (Swift, 1914). At the genital ridge stage cells of the thickened epithelium are cytologically indistinguishable. No cells are present in the genital ridge which can be regarded as primary sex cells, as seems to be the case in the rat (Hargitt, 1925), but even at this early stage a few large cells can be singled out from the compact layer of mesenchyme. These few primary sex cells multiply rapidly and are the characteristic feature of the ovary of older embryos. Nor are there any transitional forms between the epithelial type cells and the primary sex cells present at any time during development as was reported for mice (Allen, 1905). If the primitive sex cells were derived from the coelomic epithelium, it would be reasonable to expect transitional forms, or atleast some cells in the surface epithelium from which the primary sex cells could have been derived; but the primary sex cells as we have seen, appear in the mesenchyme even before the coelomic epithelium has proliferated to any great extent. Our observations did not reveal any similarity to that of the human (Felix, 1912) namely that the primary sex cells were observed to

atrophy and to disappear.

The primary sex cells of the indifferent gonad of the female were traced until they became encircled by a unilaminar layer of cells, which had been formed from the coelomic epithelium.

Although in mammals the evidence for the extragonadal origin is not as positive as in the bird, Swift (1914) reported data which indicates that the primary germ cells do not originate in the coelomic epithelium, but migrate into the gonadal primordium from some extragonadal source.

It is obvious that the coelomic epithelium is not inactive. Its cells invade the underlying structure in the form of germinal cords. Short at first, the cords later are long and anastomose freely, still retaining their connection with the multilayered zone of cells derived from the simple coelomic epithelium. The cells of the cords and of the germinal epithelium are indistinguishable; there can scarcely be any doubt that the cells of these cords came from surface epithelium. In the hamster there appears to be a continuous proliferation of the epithelial cords and one cannot observe a spatial, nor a temporal sequence between successive waves, consequently the description of primary and secondary sex cords, or cortex, could not be made.

The ovarian stroma is derived from mesenchyme. It is foreshadowed as a local condensation around blood vessels, medullary cords, and cortical cords, afterward it spreads peripherally and finally occupies the whole ovary. If the stroma were formed from the coelomic epithelium, such a sequence would probably be reversed.

We were unable to reveal significant alkaline phosphatase activity in the primordial germ cells within the developing ovary. These findings are in agreement with the work of Knigge and Leathen (1956) in the adult hamster. We were led to the conclusion that although the germ cells probably did possess some enzyme activity, the present techniques did not permit the selective staining of such sites in the hamster.

SUMMARY AND CONCLUSIONS

1. The development of the ovary in the hamster begins with the formation of a genital ridge at 10 days after mating.

2. The primary germ cells appear in the gonad before the germinal epithelium has proliferated to any great extent.

3. The germinal epithelium proliferates cells identical with itself, which move toward the center of the ovary, while the primary germ cells are increasing rapidly in number.

4. Germ cells are observed in the meiotic prophase from the day of birth until the 15th day post partum, when the majority of them are found in the dictyate stage of meiosis.

5. The oöcytes in the medulla are generally more advanced in development than the oöcytes in the cortex of the ovary.

6. The proliferations of the germinal epithelium first grow between large areas of oöcytes, then the large regions break up into nests of a few cells each, and finally form the follicle cells around each individual oöcyte.

7. By the 13th day of development the ovary is completely occupied by unilaminar follicles.

8. No evidence was found to interpret the oöcytes as originating from the germinal epithelium—no primary germ cells were observed in the germinal epithelium, no transitional forms were seen, and there was no mass degeneration of oöcytes.

9. The proliferations of the germinal epithelium appeared to be continuous because there was no complete well formed tunica albuginea and also because there were regions of the epithelial cords which were continuous with the germinal epithelium.

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PROTOCOL

Age of Embryo	Stage of Development	Number of Specimens Studied
10 days after fertilization	Genital ridge	9
10 1/2 "	Indifferent gonad	8
11 "	Ovary with no distinction between cortex and medulla	13
12 "	Ovary having a cortex and a medulla	10
13 "	" "	9
14 "	Sex cords and egg nests well developed in both cortex and medulla	10
15 "	" "	10
1 day post partum	Majority of primary germ cells of medulla in zygotene stage; majority of primary germ cells of cortex in leptotene stage.	6
2 "	" "	5
3 "	Majority of primary germ cells of medulla in pachytene stage; majority of primary germ cells of cortex in zygotene stage.	6
4 "	" "	6
5 "	Majority of primary germ cells of medulla in arrested prophase; majority of primary germ cells of cortex in pachytene and zygotene stage.	6
6 "	" "	6
7 "	" "	6
8 "	" "	6

Age of Embryo	Stage of Development	Number of Specimens Studied
9 days post partum	Nests of follicles	7
10 "	"	4
11 "	"	9
12 "	"	4
13 "	Multilaminar follicles	7

TABLE

STAINING OF SPECIMENS

Age of Embryo	No. of Embryos	H.&E.	M.T.	PAS	T.B.	M.B.	AZ	A.P.
10 days after fertilization	17	4	3	3	2	2	2	1
11 "	13	2	2	2	2	3	1	1
12 "	10	2	1	1	2	2	1	1
13 "	9	2	2	1	1	1	1	1
14 "	10	2	2	2	1	1	1	1
15 "	10	3	2	1	1	1	1	1
1 day post partum	6	1	1	1	1	1		1
2 "	5	1	1	1	1	1		
3 "	6	1	1	1	1	1		1
4 "	6	1	1	1	1	1	1	
5 "	6	1	1	1	1	1	1	
6 "	6	1	1	1	1		1	1
7 "	6	1	1	1	1		1	1
8 "	6	1	1	1	1	1	1	
9 "	7	2	2	1	2			
10 "	4	1	1	1	1			

Age of Embryo	No. of Embryos	H.&E.	M.T.	PAS	T.B.	M.B.	AZ	A.P.
11 days post partum	9	2	1	2	1	1	1	1
12 "	4	1	1	1	1			
13 "	7	2	2	1	1	1		

Abbreviations:

H.&E. = Hematoxylin and Eosin
PAS = Periodic acid-Schiffs
M.B. = Methylene blue
A.P. = Alkaline phoshatase
M.T. = Masson's Trichrome
T.B. = Toludine blue
AZ = Azure B

PLATE I

Figure 1. Genital ridge of a 10 day embryo, stained with periodic acid-Schiff reagent (984 X).

Cross section of the genital ridge showing the thickened coelomic epithelium (arrow); the mesenchyme between the genital ridge and the mesonephros is less cellular than in the gonad region.

Figure 2. Genital ridge of a 10 1/2 day embryo, stained with hematoxylin and eosin (984 X).

Mesenchymal tissue concentrated below the coelomic epithelium; the coelomic epithelium is forming short buds into the underlying mesenchyme (arrow). Note that most cells contain a nucleolus and a Barr body.

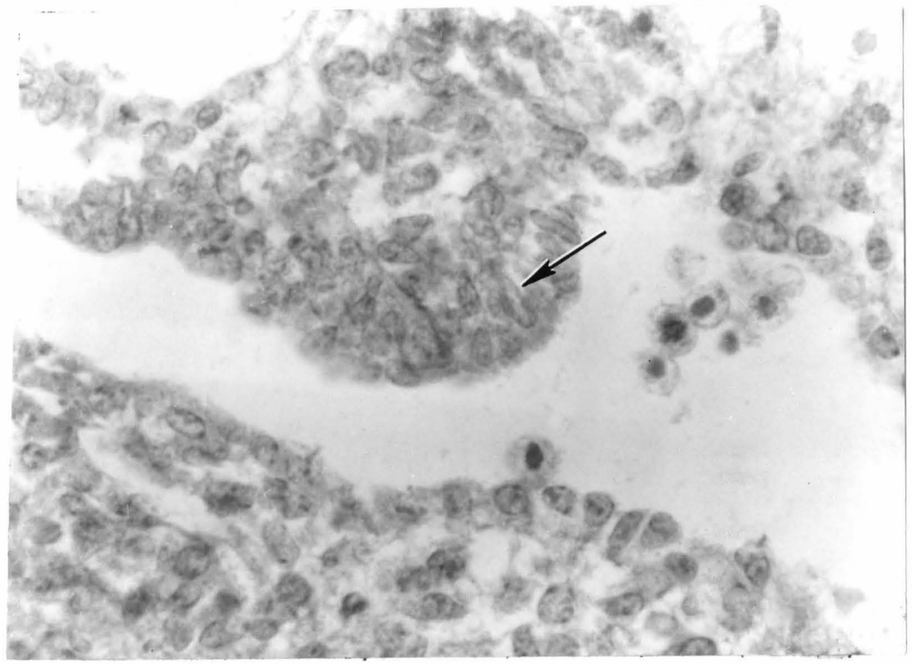

FIGURE 1

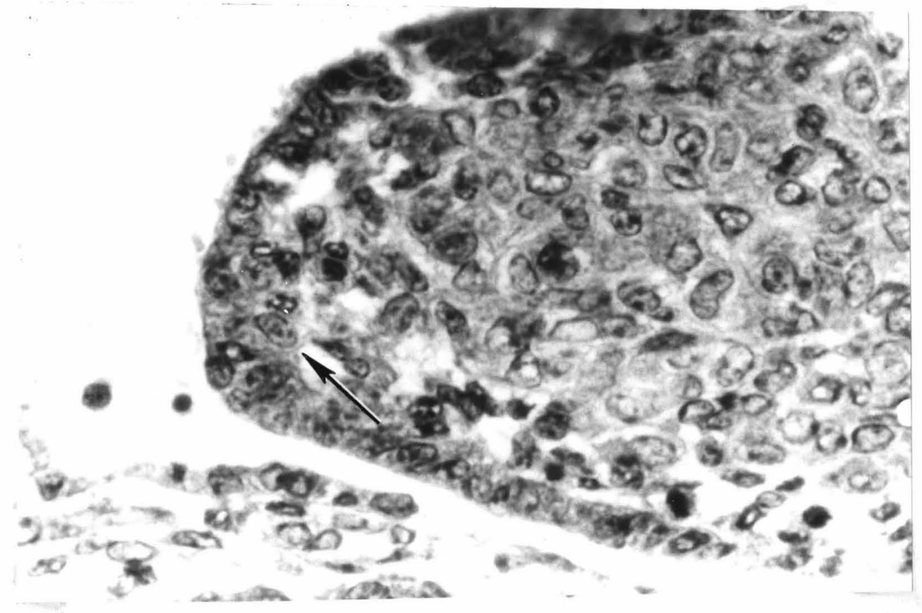

FIGURE 2

PLATE II

Figure 3. Germinal epithelium of the ovary of a 11 day embryo, stained with Masson's trichrome (984 X).

The germinal epithelium forms an irregular boundary, and short cords penetrate into the medulla; there is a great increase in the number of large primary germ cells (arrow) in the medulla.

Figure 4. Germinal epithelium of the ovary of a 12 1/2 day embryo, stained with Masson's trichrome (984 X).

The coelomic epithelium is less irregular than in Figure 3 and is continuous with the inner part of the cortex. The ovary appears denser than in Figure 3 due to the multiplication of primary germ cells (arrow). Note the absence of a tunica albuginea.

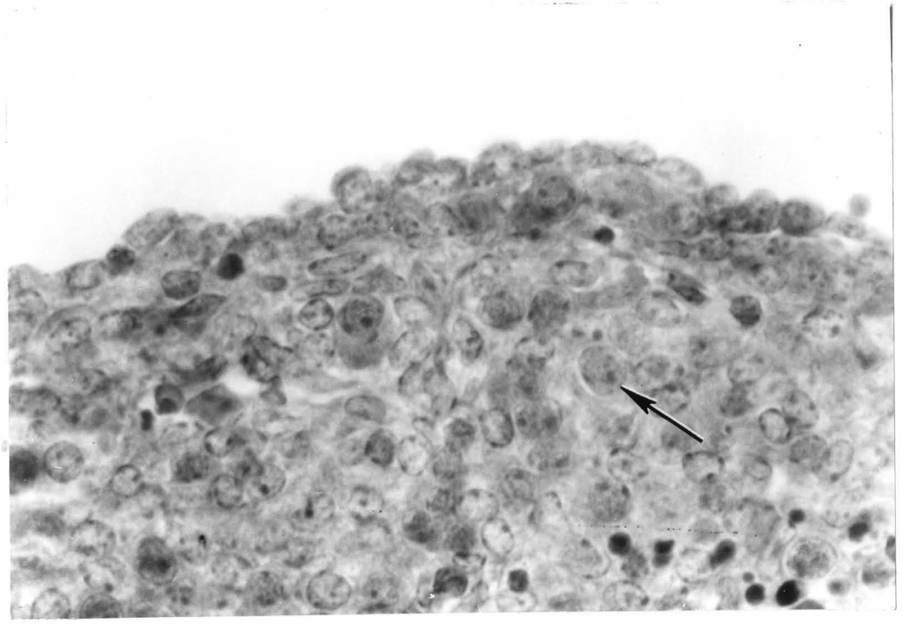

FIGURE 3

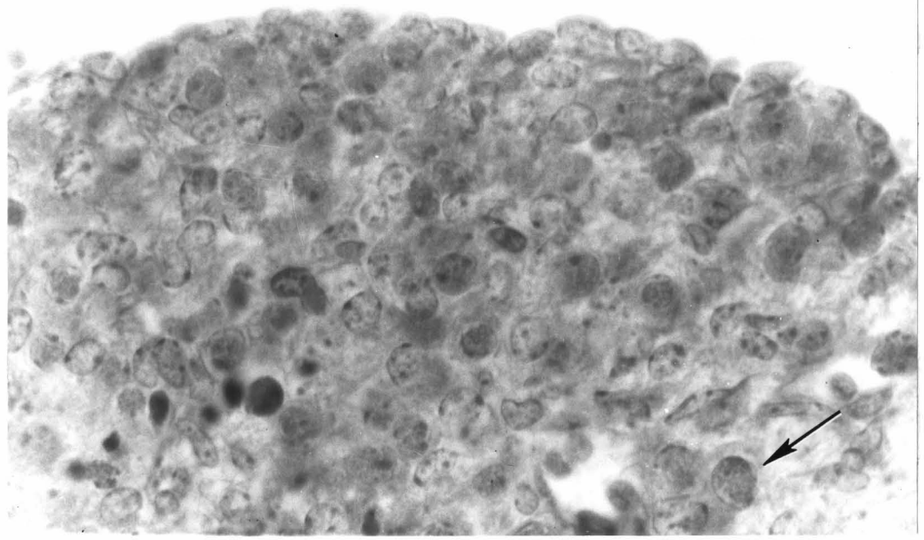

FIGURE 4

PLATE III

Figure 5. Germinal epithelium of the ovary of a 14 day embryo, stained with hematoxylin and eosin (984 X).

The germinal epithelium forms a smooth outline. Cords of epithelial cells seem to penetrate from the germinalepithelium, grouping the underlying primary germ cells, which are in the interphase, into nests (arrow).

Figure 6. Germinal epithelium of the ovary of a 15 day embryo, stained with hematoxylin and eosin (984 X).

Area of the germinal epithelium nearer to hilus than in Figure 5; a greater amount of connective tissue (arrow) is present under the epithelium, and large capillaries are seen penetrating the stroma.

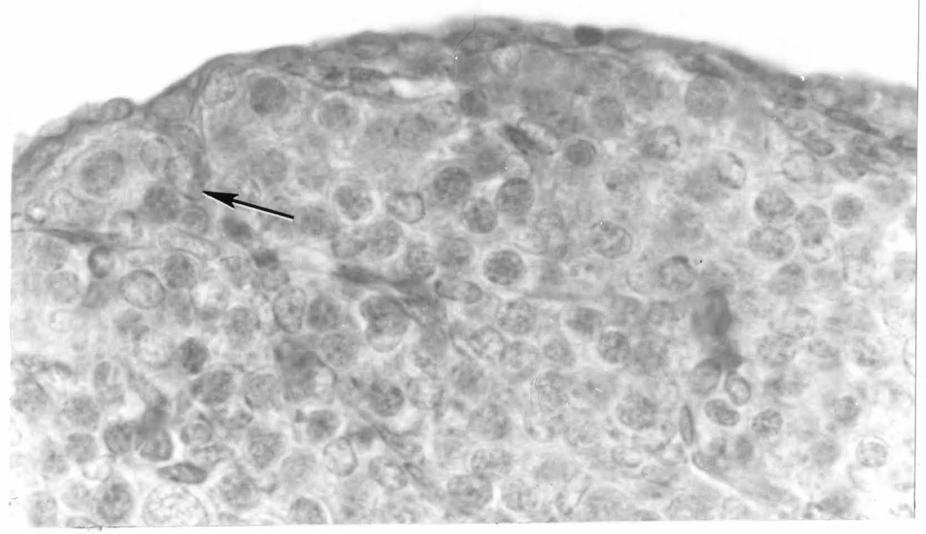

FIGURE 5

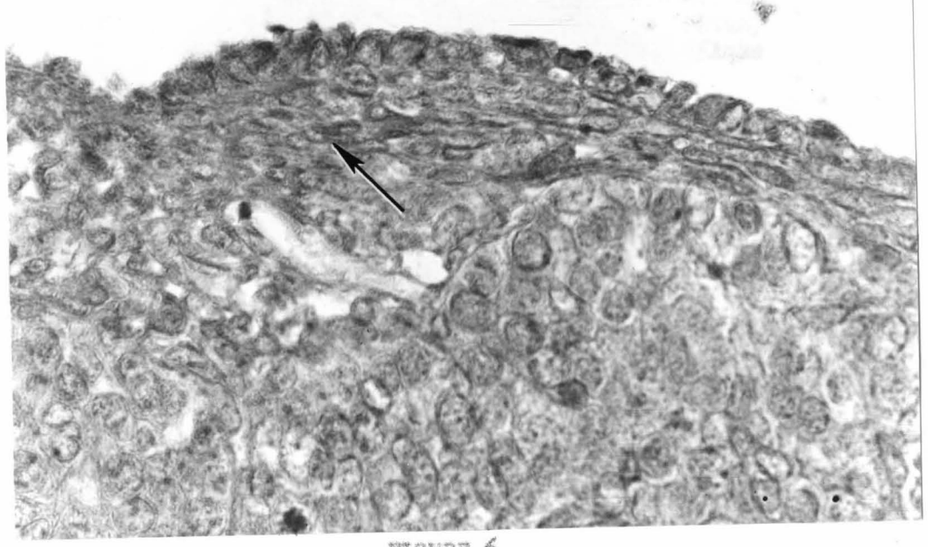

FIGURE 6

PLATE IV

Figure 7. Cortex of the ovary of a 1 day post partum embryo, stained with hematoxylin and eosin (984 X).

This stage shows the same characteristics as in Figure 5 with the exception that most oöcytes are in the beginning of the meiotic prophase (the majority of the oöcytes in the leptotene stage). The ovarian capsule also is visible (arrow).

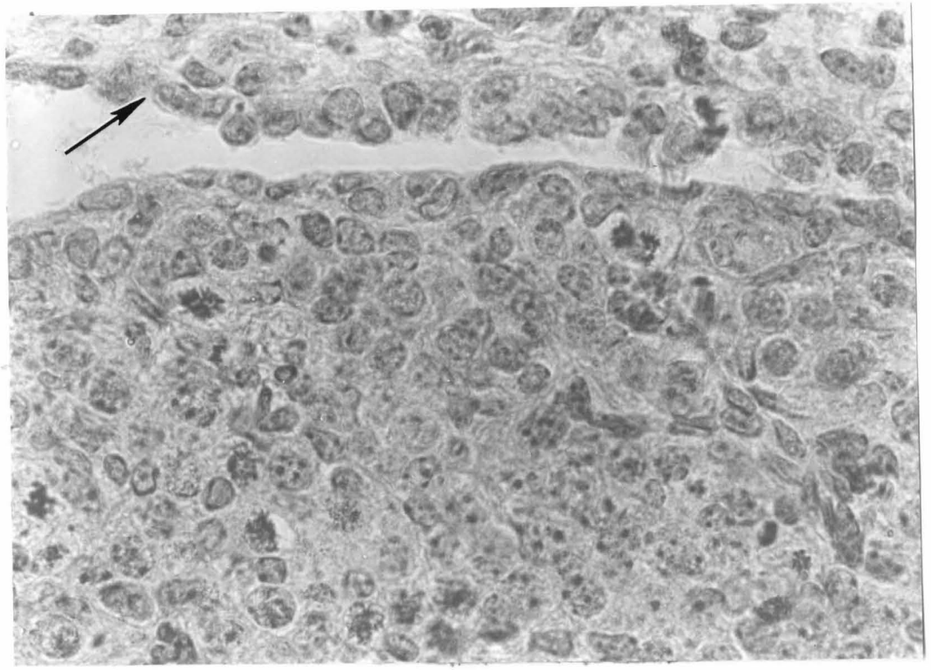

FIGURE 7

PLATE V

Figure 8. Cortex of the ovary of a 2 days post partum embryo, stained with hematoxylin and eosin (984 X).

Oöcytes in the leptotene and zygotene stage of meiosis.

Figure 9. Medulla of the ovary of a 2 days post partum embryo, stained with hematoxylin and eosin (984 X).

The proliferations of the germinal epithelium have reached the center of the ovary where they form tubular strucutres (arrow); they also surround nests of oöcytes, which are in the zygotene and pachytene stages of meiosis.

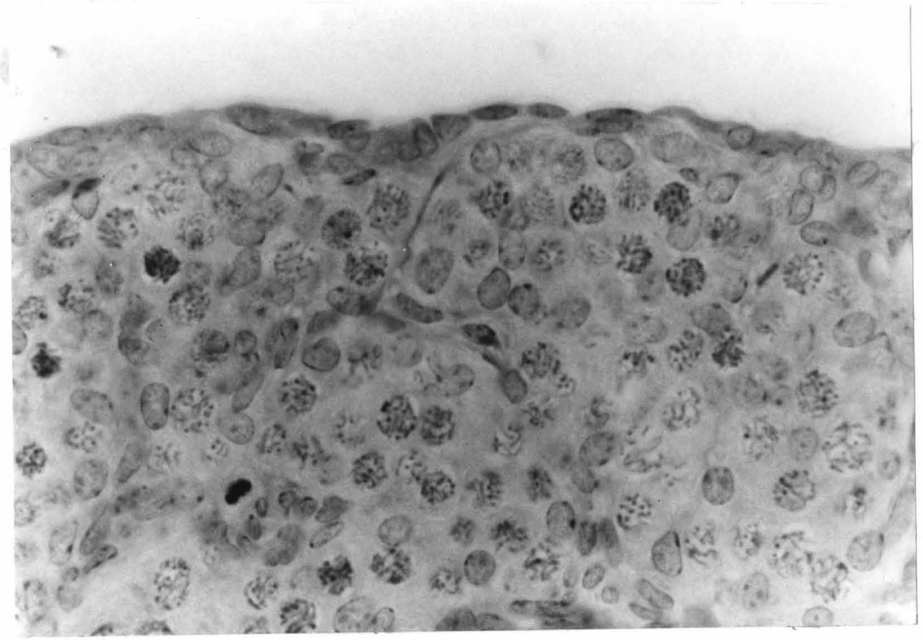

FIGURE 8

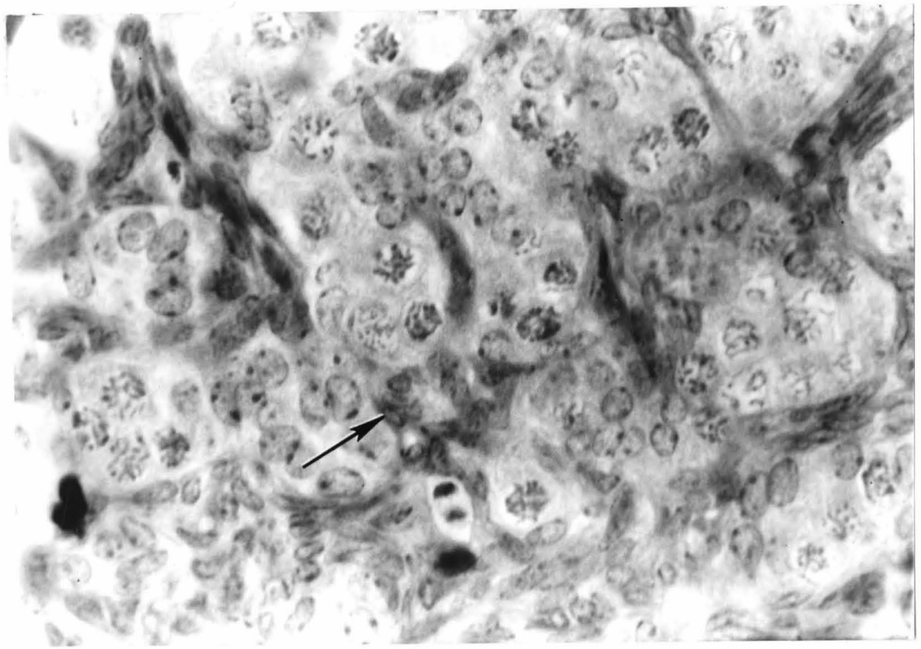

FIGURE 9

PLATE VI

Figure 10. Cortex of the ovary of a 3 days post partum embryo, stained with Masson's trichrome (984 X).

Germinal epithelium cells apparently still proliferating and forming epithelial cords (arrow). The majority of oöcytes are in the zygotene stage of meiosis.

Figure 11. Medulla of the ovary of a 3 days post partum embryo, stained with Masson's trichrome (984 X).

Epithelial cells are beginning to accumulate within the medulla.

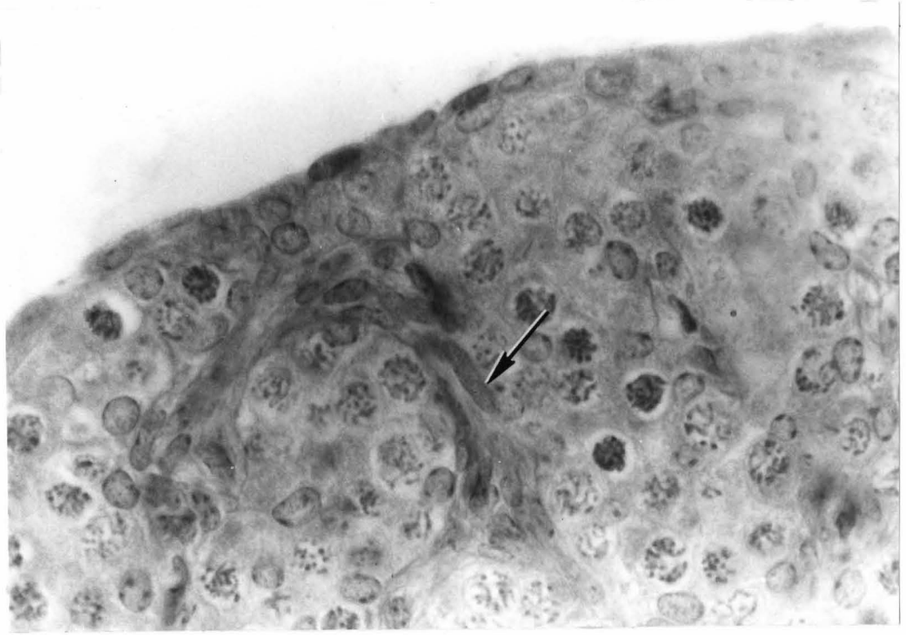

FIGURE 10

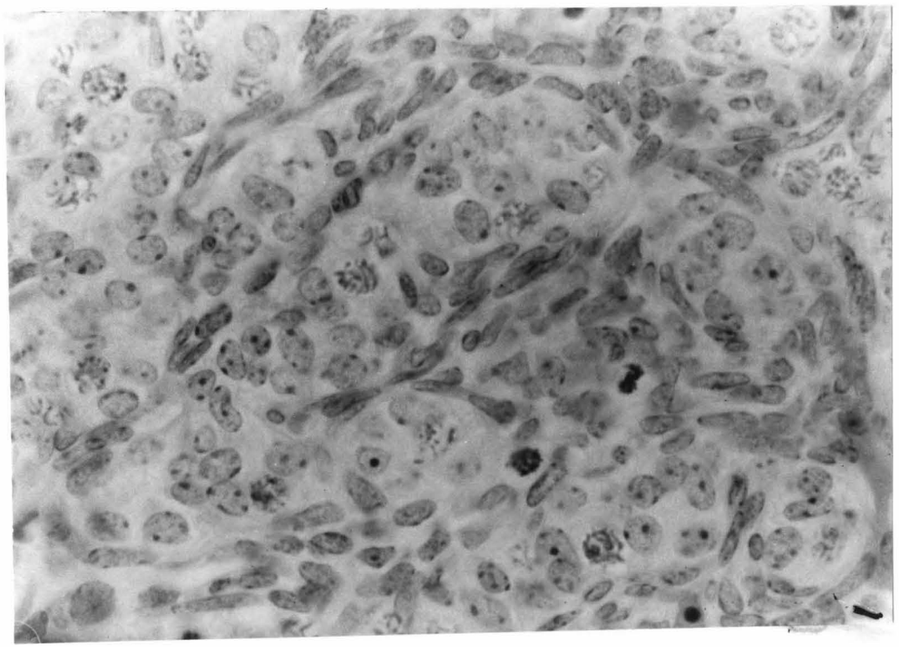

FIGURE 11

PLATE VII

Figure 12. Cortex of the ovary of a 4 days post partum embryo, stained with Masson's trichrome (984 X).

The sharply demarcated germinal epithelium as a rule is one cell layer thick except in areas where proliferation occurs. The nests of zygotene stage oöcytes, resembling the classical Pflüger's egg tubes, are demarcated by the epithelial cells.

Figure 13. Between the cortex and the medulla of a 4 days post partum embryo, stained with Masson's trichrome (984 X).

Growth of the epithelial cells toward the center of the ovary can be seen here in one plane. Between the epithelial cords, oöcytes in the pachytene stage of meiosis and degenerating cells are seen.

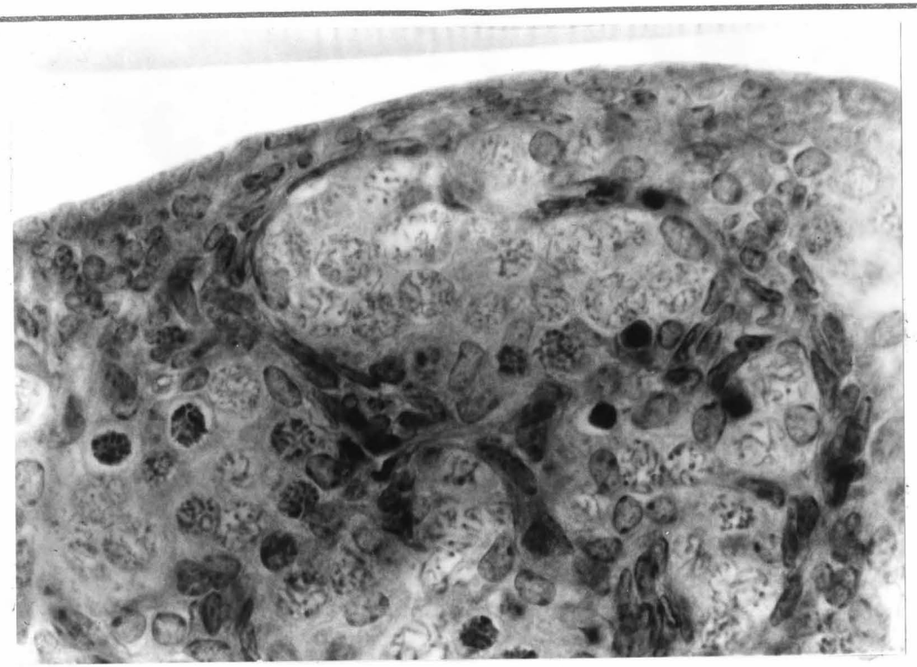

FIGURE 12

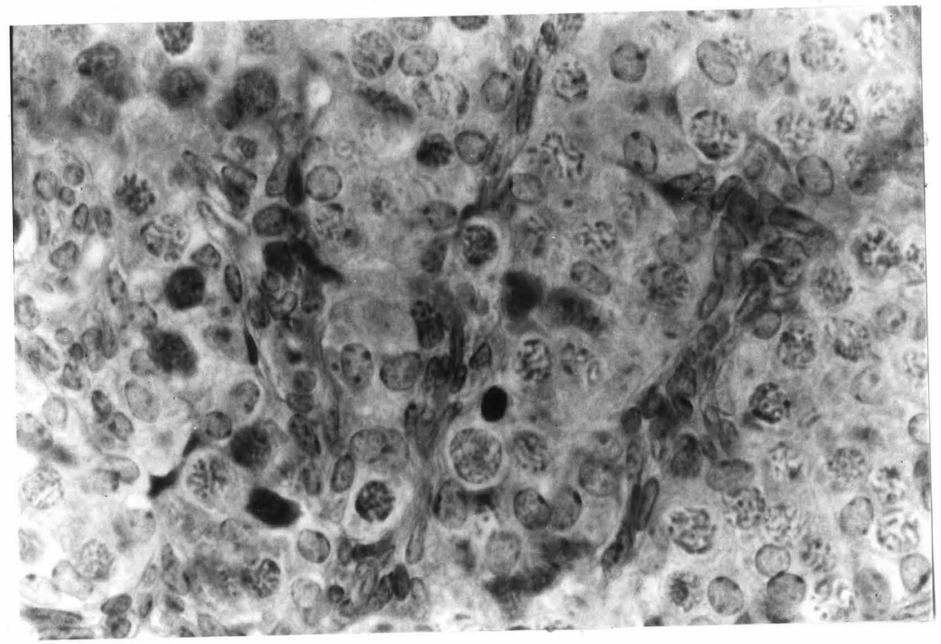

FIGURE 13

PLATE VIII

Figure 14. Cortex of the ovary of a 5 days post partum embryo, stained with hematoxylin and eosin (984 X).

The proliferating epithelium has an outer cuboidal (arrow) and inner flattened cell layers. Many of the oocytes are in the pachytene stage of meiosis.

Figure 15. Medulla of the ovary of a 5 days post partum embryo, stained with hematoxylin and eosin (984 X).

The innermost aspect of the ovary possesses nothing more than epithelial cells at this stage. Surrounding this the oocytes (arrow) are in the dictyate stage of meiosis.

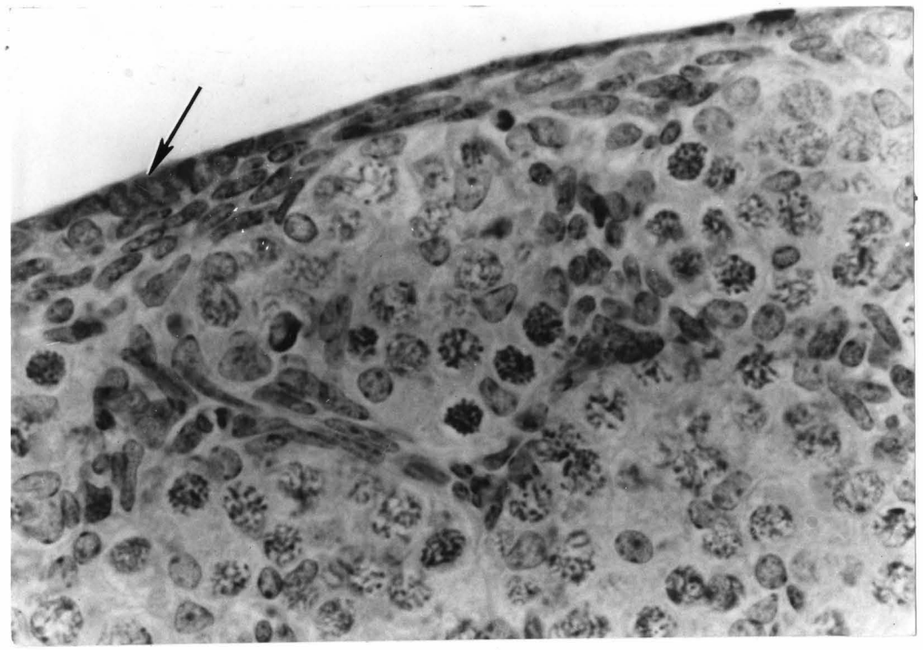

FIGURE 14

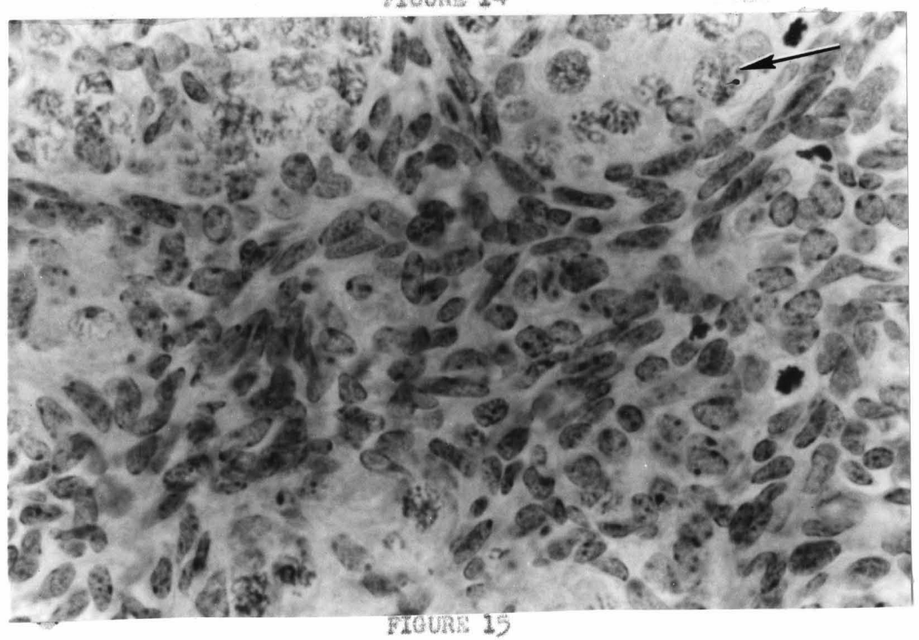

FIGURE 15

PLATE IX

Figure 16. Ovary of a 6 days post partum embryo, stained with PAS reagent (246 X).

Septa extend throughout the ovary and spread out below the investing epithelium, but do not form a continuous tunica albuginea. The path of the connective tissue is between the epithelial cells and not within the "egg nests."

Figure 17. Cortex of the ovary of a 6 days post partum embryo, stained with Masson's trichrome (984 X).

Almost everywhere the germinal epithelium is a flat one cell layer thick. Almost all of the oöcytes are in the diplotene stage of meiosis.

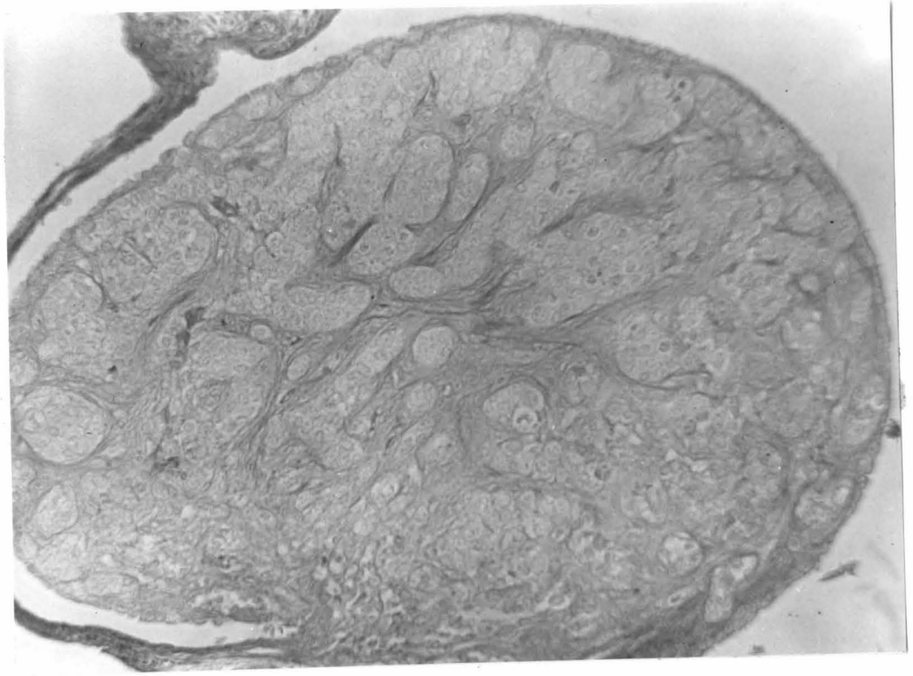

FIGURE 16

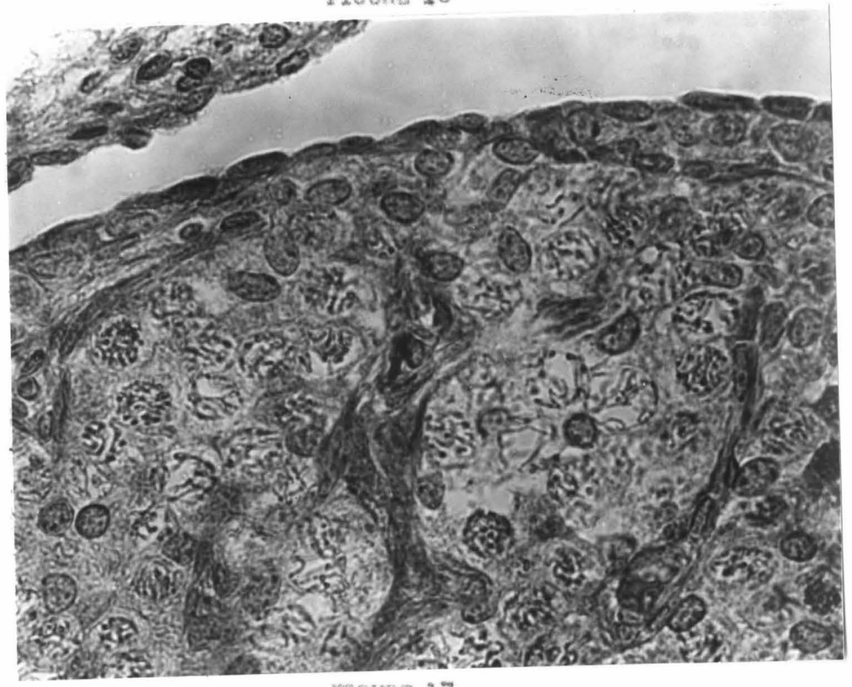

FIGURE 17

PLATE X

Figure 18. Cortex of the ovary of a 10 days post partum embryo, stained with hematoxylin and eosin (984 X).

The cord-like appearance of epithelial and germ cells is no longer visible. Irregular follicles are formed. Oöcytes are in the dictyate stage of meiosis.

Figure 19. Medulla of the ovary of a 10 days post partum embryo, stained with hematoxylin and eosin (984 X).

Same structures as in the cortex (Figure 18), but primordial follicles are better arranged. No degenerating cells visible.

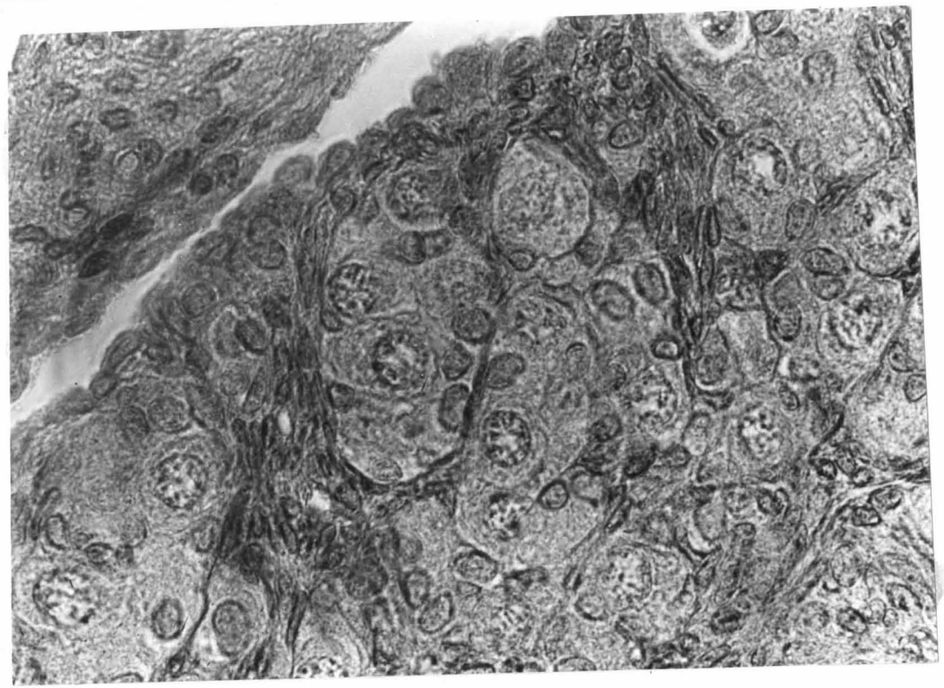

FIGURE 18

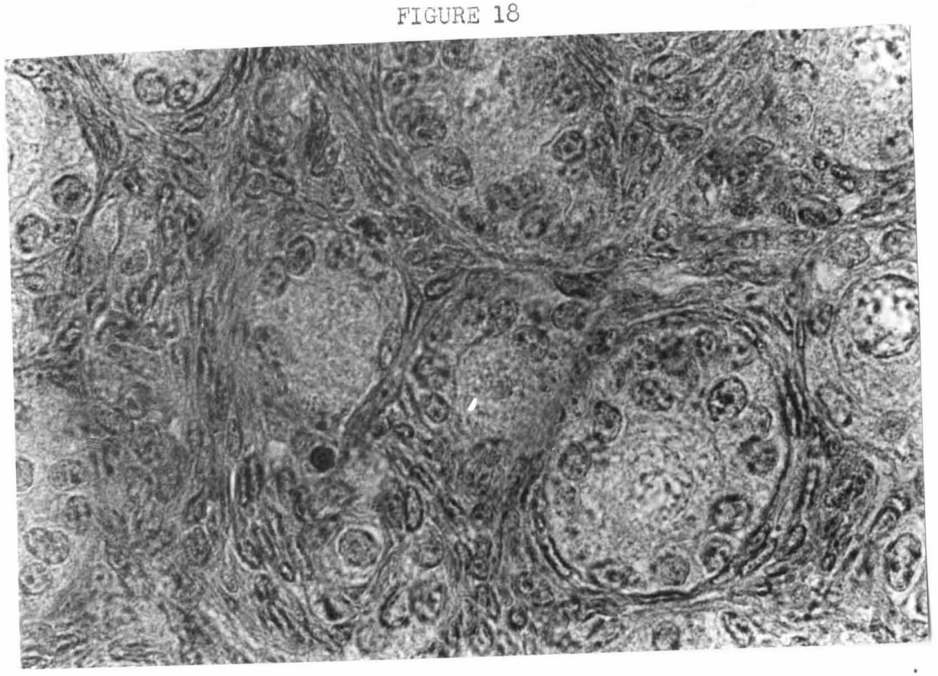

FIGURE 19

PLATE XI

Figure 20. Cortex of the ovary of a 10 days post partum embryo, stained with toludine blue (984 X).

The germinal epithelium is one cell layer thick. Connective tissue fibers are seen between the follicles.

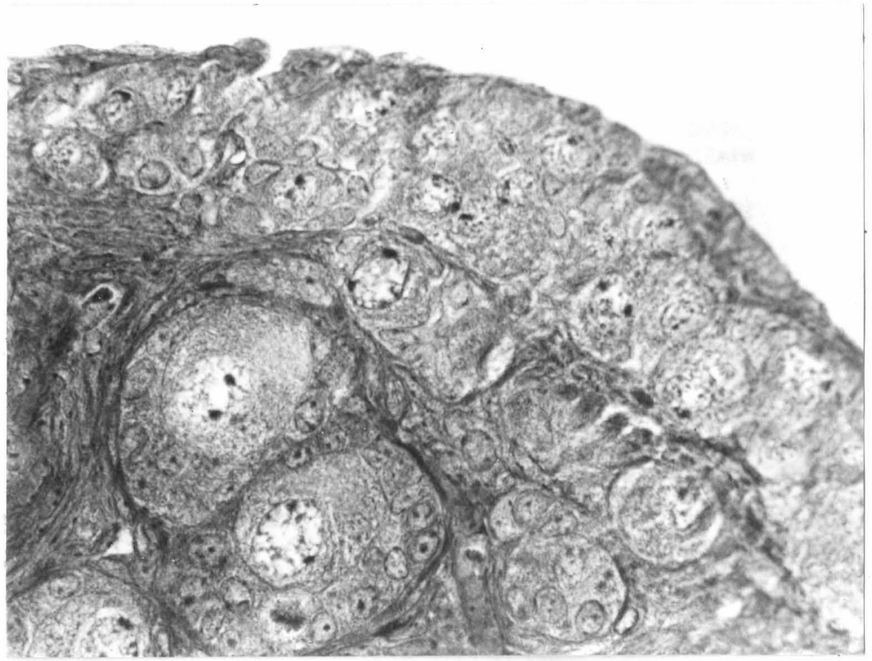

FIGURE 20

PLATE XII

Figure 21. Cortex of the ovary of a 11 days post partum embryo, stained with Masson's trichrome (984 X).

Despite the fact that no tunica albuginea is seen the ovary has now attained the form it will posses at puberty. The oocytes are in the dictyate stage surrounded by follicle cells; the germinal epithelium no longer appears to be proliferating.

Figure 22. Medulla of the ovary of a 11 day post partum embryo, stained with Masson's trichrome (984 X).

Same as Figure 21, but in the medulla.

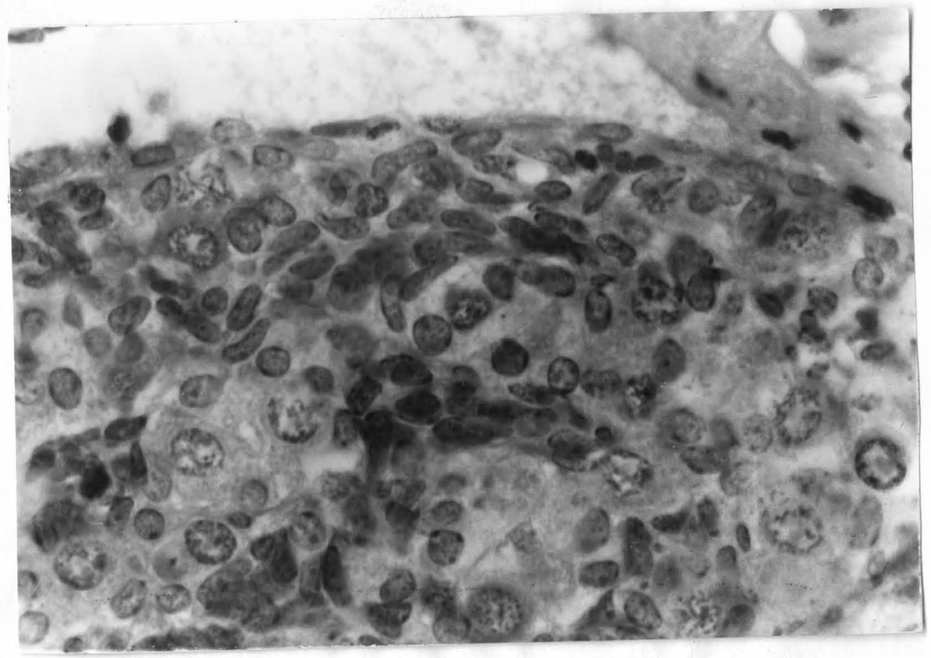

FIGURE 21

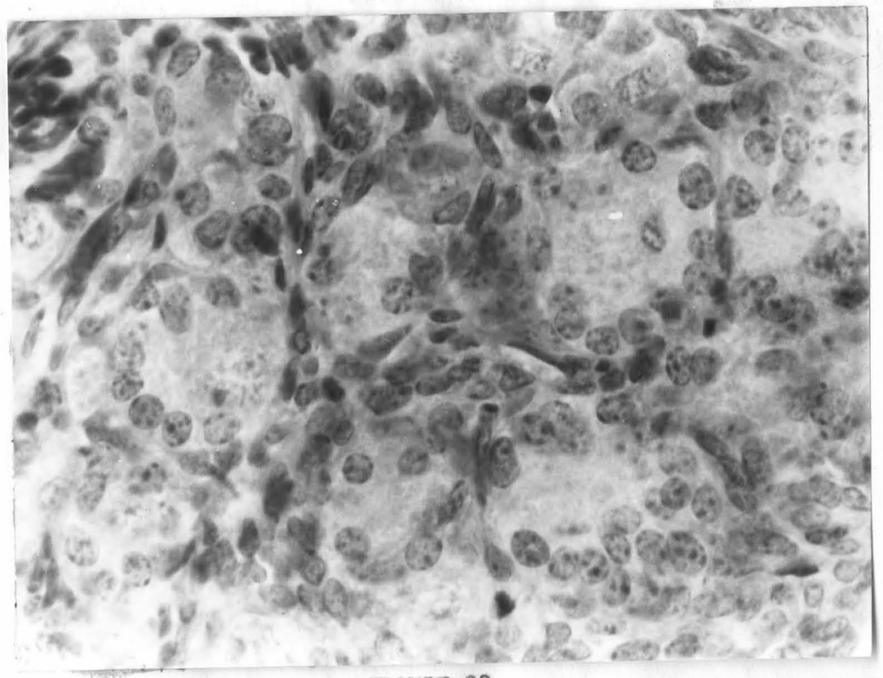

FIGURE 22

APPROVAL SHEET

The thesis submitted by Patricia L. Jaglarski has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

5-25-1964
Date

Robert Hawkr
Signature of Adviser