The teratogenic effects of methoxychlor on the male and female reproductive system of the rat (*Rattus norvegicus*)

by

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DEDICATION

Dedicated to my parents

Ted and Mary Saur

for their continued moral

support during the past

seven years

INTRODUCTION

Methoxychlor, a chlorinated hydrocarbon pesticide, has had the necessary toxicity and carcinogenicity tests for environmental chemicals performed on it, but no teratogenicity tests have been reported. The first effect of methoxychlor on a mammalian system is that of reproductive toxicity, which produces structural changes within the reproductive system, specifically affecting the testis and ovary. This study was conducted to examine the possible teratologic effect of this reproductive toxicity of methoxychlor.

Methoxychlor is one of the pesticides currently used on crops, on and around domestic animals, and in the control of insect vectors of diseases of man. Formerly one pesticide served all of these purposes, DDT. Brooks (1974a) notes that "DDT has been the main agent for control of vectors of an impressive list of diseases, including malaria, Chagas' disease, plague, typhus, yellow fever, dengue/haemorrhagic fever, encephalitis, filariasis, African trypanosomiasis, onchocerciases (sic), and leishmaniasis." Between 1959 and 1970 DDT removed the threat of malaria from more than one billion people. DDT increased crop production by decreasing losses due to insects. Corn borers, cutworms, and the Japanese beetle are some of the more well-known agricultural pests DDT was effective against (Brooks, 1974a,b).

Within a few years after the introduction of DDT, various insects gained resistance to it. By 1946 the housefly in Denmark and Sweden had a 100- to 200-fold increase in DDT resistance. The number of insect species showing DDT resistance increased. There are now at least 57

insect species of public health concern and 29 insect species of agricultural concern that are DDT resistant (Brooks, 1974a,b).

In the late 1960's and early 1970's, other disadvantages of DDT were discovered. DDT was found in the milk of dairy cattle. Coho salmon in Lake Michigan had unacceptable DDT residue levels. A reduction in eggshell thickness due to DDT, and a resultant increase in egg breakage, led to a decline in the number of predatory birds (Brooks, 1974a,b).

In 1969 the USDA recommended that DDT use be restricted to only those applications necessary for the continuation of human health and welfare. The banning resulted in renewed interest in other pesticides for general use, methoxychlor being one of them. Methoxychlor has the advantages of toxicity to insects of its banned analog DDT without the deleterious environmental aspects, and it is less toxic to vertebrates. Its comparative safety, range of effectiveness, and relatively long residual action, when compared to other approved insecticides, make it a useful and safe insecticide (Brooks, 1974a,b; Meister, 1977).

Toxicity studies on methoxychlor have shown the acute oral LD_{50} in the rat to be around 6000 mg/kg (Hodge et al., 1950; Meister, 1977). At lower doses the initial effect of methoxychlor is on the testis. Hodge et al. (1950) showed pathological changes in the testes of adult male rats fed a diet containing 0.1% (122.1 mg/kg/day[1]) methoxychlor

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[1]Approximate values. See Table 1 and Appendices A and B.

for 45 days. In a two year chronic feeding study, diets of up to 0.16% (77.0 mg/kg/day[1]) methoxychlor did not result in any atrophic changes in rat testes (Hodge et al., 1952). Tullner and Edgcomb (1962) fed a 1% diet (1245.5 mg/kg/day[1]) for 33, 45 and 55 days to weanling rats, which resulted in testicular atrophy. Gellert et al. (1974) treated neonatal rats with 1 mg/kg/day (0.00061%[1]) methoxychlor on days two, three and four of life with no apparent effect on the testes or sperm motility at maturity. Dosing male rats with 100 and 200 mg/kg/day (0.11 and 0.22% respectively[1]) methoxychlor for 70 days resulted in testicular pathology (Bal and Mungkornkarn, 1977).

Dosing female rats with 100 and 200 mg/kg/day (0.11 and 0.22% respectively[1]) for 14 days prior to mating resulted in their inability to become pregnant (Bal and Mungkornkarn, 1977). Gellert et al. (1974), Hodge et al. (1950), and Hodge et al. (1952) did not report any changes in the female rats used in their studies.

The literature lacks reports of teratological tests performed with methoxychlor. This study examined the possibility, based on the gonadal toxicity observed in adult rats, that methoxychlor would have a teratological effect on fetal rats. A single dose of methoxychlor was administered to pregnant rats on the day of fetal gonadal sensitivity to teratogens. Dosage strength was the highest dose affecting mainly the reproductive system used in previous studies, 200 mg/kg/day,

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[1]Approximate values. See Table 1 and Appendices A and B.

with twice the dose used on the succeeding day on a second group of pregnant rats. The gonads of the fetuses, taken on day 20 of gestation, were compared to a control group of fetuses taken from control dams dosed only with a vehicle.

LITERATURE REVIEW

Methoxychlor

Methoxychlor is a chlorinated hydrocarbon with the chemical structure of 2,2-di-(p-methoxyphenyl)-1,1,1-trichlorethane (Hodge et al., 1952). It resists breakdown by heat, oxidation, and moisture under normal conditions, and is also resistant to ultraviolet light. Indoors it is stable indefinitely. Its dehydrochlorination rate is 29.5 ($K \times 10^5$; 1/min/mol; 30°C) (Brooks, 1974a).

As a pesticide, methoxychlor is used on crops, around domestic animals, and in controlling insect pests of man. On crops it is applied at the rate of 1 to 1.5 pounds per acre. It is used in the control of lice on hogs and cattle, and in dairy barns against horn-flies and houseflies. Methoxychlor has been effective in the control of vectors of diseases of man, such as plague, murine typhus, and epidemic typhus (Brooks, 1974a).

Methoxychlor was first applied in a dairy barn, under the trade name Marlate® (Brooks, 1974a). Methoxychlor was sparingly used because it was less toxic and cost more to produce and use than DDT. However, in 1946 DDT-resistant strains of houseflies appeared, and in 1951 DDT-resistant strains of the body louse and mosquitoes were found (Brooks, 1974b). With the recent banning of DDT use, the use of methoxychlor increased. It is preferred to other environmentally approved insecticides due to its relatively long residual action against many species of insects and its low toxicity to man and other warm blooded animals (Meister, 1977).

Reproductive System Related Toxicity Studies in the Rat

Studies have shown that methoxychlor does cause pathological changes in post-natal rat reproductive systems, although feed concentrations of less than 0.1% methoxychlor (less than 122.1 mg/kg/day[1]) for periods of two days to two years have no apparent effect on rats (Gellert et al., 1974; Hodge et al., 1950, 1952).

In a short-term toxicity study, Hodge et al. (1950) fed rats feed containing 0.0, 0.01, 0.1, 3.0, and 1.0% (0.0, 12.2, 122.1, 3663.6, and 1304.6 mg/kg/day respectively[1]) methoxychlor for 45 days, 30+ days for the latter. The 0.1, 1.0, and 3.0% diets all resulted in testicular atrophy and significant growth retardation. The 3.0% diet caused a general reduction of organ weight in relation to body weight. The testes had the greatest organ weight decrease, weighing an average of 2.51 g in the controls and 0.28 g in the 3.0% diet group. The ratio of testis weight to body weight for the 1.0 and 3.0% groups was 3 mg/kg, compared to 19 mg/kg for the controls. The testes showed general atrophy of the seminiferous tubules, which were lined with immature germ cells. Many primary spermatocytes showed necrosis.

In a chronic feeding study, Hodge et al. (1952) fed rats feed containing 0.0, 0.0025, 0.02, and 0.16% (0.0, 1.2, 9.7, and 77.0 mg/kg/day respectively for males[1]) methoxychlor for two years. No pathological changes occurred in any of the females. In the males,

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[1]Approximate values. See Table 1 and Appendices A and B.

only the highest dose resulted in any changes. These males had a significant decrease in body weight. The testes showed no significant change.

At these lower dose levels, there is a discrepancy between the results found by Hodge et al. (1950) and those found by Hodge et al. (1952) concerning the effect of methoxychlor on the testis. A 0.1% concentration for 45 days resulted in testicular changes (Hodge et al., 1950). However, a slightly higher concentration of 0.16% fed for a much longer period (two years) produced no testicular changes (Hodge et al., 1952). Although the same research group conducted both experiments, no explanation for the discrepancy was given.

Tullner and Edgcomb (1962) conducted paired feeding studies (controls received only as much feed as dosed rats consumed) of weanling rats using a control group and a group on feed containing 1% methoxychlor (up to 1245.5 mg/kg/day[1]). Two series were run, using rats at 37 to 41 g starting weight in one series and rats at 65 to 75 g in the other. The rats were maintained for periods of between 33 and 55 days. The testes, accessory sex glands, and kidneys of the dosed rats showed histopathological changes, while their livers, pancreata and adrenals did not. Spermatocytes had pyknotic nuclei and sudanophilic cytoplasmic granules. The dosed rats had an estimated seven- to fourteen-fold decrease in the number of interstitial cells. The kidneys showed an abnormally large number of small cystic tubules.

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[1]Approximate values. See Table 1 and Appendices A and B.

The testes, ventral prostates, and seminal vesicles of the dosed rats weighed less than 20% of the respective weights in the controls.

Bal and Mungkornkarn (1977) fed adult male rats 100 and 200 mg/kg/day (0.11 and 0.22% respectively[1]) methoxychlor for 70 days. The testes showed spermatogenesis arrested at the primary spermatocyte stage in the seminiferous tubules. Polyploidy and large multinucleate cells were found. Other cells showed fatty changes and degeneration with large degenerated cell bodies located toward the centers of the lumens.

In the same study Bal and Mungkornkarn (1977) fed adult female rats 100 and 200 mg/kg/day (0.11 and 0.22% respectively[1]) from 14 days prior to mating to day 20 of gestation. The females failed to become pregnant. Histological examination showed lack of corpora lutea in the ovaries. The granulosa cells of the follicles showed signs of degeneration indicating the follicles to be atretic.

Meister (1977) reports the LD_{50} for rats from a single oral dose of methoxychlor to be around 6000 mg/kg.

In male rats exposure to 1% or more of methoxychlor in feed consistently resulted in pathological changes of the testes. No pathological changes in the ovaries of female rats were produced by doses less than 100 mg/kg/day (Bal and Mungkornkarn, 1977; Gellert et al., 1974; Hodge et al., 1950, 1952; Tullner and Edgcomb, 1962).

These results are summarized in Table 1.

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[1]Approximate values. See Table 1 and Appendices A and B.

Table 1. Summary of effects of various doses of methoxychlor on the reproductive system of the rat

Age	Methoxychlor dose %	mg/kg/day	Time period
Weanling	0.00061[a]	1.0	3 days (days 1, 2 and 3 of life)
Weanling	0.0025	1.2[a]	2 years
Weanling	0.01	12.2[a]	45 days
Weanling	0.02	9.7[a]	2 years
Weanling	0.1	122.1[a]	45 days
Adult	0.11[a]	100.0	34-70 days
Weanling	0.16	77.0[a]	2 years
Adult	0.22[a]	200.0	34-70 days
Weanling	1.0	1245.5[a]	33-55 days
Weanling	1.0	1304.6[a]	30+ days
Weanling	3.0	3663.6[a]	45 days
Adult	17.4[a]	6000.0	Single dose

[a]Approximate values used for ease of comparison. See Appendices A and B.

Sites of observed changes, if any	Source
	Gellert et al., 1974
	Hodge et al., 1952
	Hodge et al., 1950
	Hodge et al., 1952
Testis, body weight	Hodge et al., 1950
Testis, ovary	Bal and Mungkornkarn, 1977
Body weight	Hodge et al., 1952
Testis, ovary	Bal and Mungkornkarn, 1977
Testis, kidney, accessory sex glands	Tullner and Edgcomb, 1962
Testis, body weight	Hodge et al., 1950
Testis, body weight	Hodge et al., 1950
LD_{50}	Meister, 1977

Reproductive System Related Studies in Other Species

Macklin and Ribelin (1971) examined the relationship between abortion and pesticide residues in fetuses. Nonlactating dairy cattle were dosed with 9.9 mg/kg/day of methoxychlor until parturition. Of the two cows used in the study, one had 0.57 ppm methoxychlor in her colostral milk, while the calf of the other had 0.44 ppm in its perirenal fat. Residue analysis found no relationship between pesticide levels and field abortion of calves. Residue analysis of human abortuses showed no relationship either.

Jackson et al. (1975) fed to rams feed containing 0, 178, or 873 ppm methoxychlor. Examinations of sperm made after 0, 3, 6, and 11 months of continuous intake showed an increase in the number of dead tailless spermatozoa at 3 and 11 months.

In a chronic feeding study, Hodge et al. (1952) maintained two dogs each on doses of 0, 20, 100, and 300 mg/kg/day for one year. No variance from normal organ weights was found. No histological evidence of tissue damage was found.

Tegeris et al. (1966) dosed six dogs and six swine each with 1, 2, and 4 g/kg/day methoxychlor for six months, with 12 of each species used as controls. All dosed dogs and swine lost weight; the dogs lost weight steadily, while the swine started to regain weight after eight weeks. Half of the dogs on 2 g/kg/day and all of the dogs on 4 g/kg/day developed abnormal behavior as well as signs of chlorinated hydrocarbon intoxication. The dogs had hyperplastic mammary glands and showed a

dose-dependent absence of normal adipose depot sites. The swine had hyperplastic and hypertrophic mammary glands and a general granularity of the kidneys. The 4 g/kg/day swine had grossly enlarged uteri.

In a related report Tegeris et al. (1965) also mentioned that the dogs on the 4 g/kg/day dose showed hemorrhage in the small intestine.

These results are summarized in Table 2.

Studies Not Relating to the Reproductive System

Numerous other studies and investigations have been conducted in addition to those reported on the reproductive system. These investigations have been mainly concerned with the rat, although mice, cattle, dogs, man, and agricultural applications have also been studied.

Lillie et al. (1947) fed DDT to rats at the doses of 0.0, 0.02, 0.05, and 0.1% for seven days. At the 0.1% level, the rats showed signs of fatty degeneration in the kidneys and adrenal medullae. In comparison rats fed 2-8 g/kg methoxychlor for the same period showed similar damage: coagulative necrosis of the liver, fatty degeneration in the heart and liver, and some interstitial and perivascular lymphocytic infiltration in the lungs. Thus a concentration of methoxychlor more than 15 times greater than that of DDT produced similar toxic results.

Kunze et al. (1950) fed weanling rats diets of 0.0, 25, 100, and 500 ppm methoxychlor for at least four weeks. The rats on the 500 ppm diet stored 30 ppm in their fat, of which only a minimal amount was in

Table 2. Summary of effects of various doses of methoxychlor on species other than the rat

Species	Methoxychlor dose	Time period	Sites of observed changes, if any	Source
Cow	9.9 mg/kg	Gestation +		Macklin and Ribelin, 1971
Dog	20.0 mg/kg 100.0 mg/kg 300.0 mg/kg	1 year		Hodge et al., 1952
	1000.0 mg/kg 2000.0 mg/kg 4000.0 mg/kg	6 months	Weight loss	Tegeris et al., 1966
Pig	1000.0 mg/kg 2000.0 mg/kg 4000.0 mg/kg	6 months	Weight loss, grossly enlarged uteri	Tegeris et al., 1966
Sheep	178 ppm 873 ppm	3 to 11 months	Increased number of dead tailless spermatozoa	Jackson et al., 1975

the liver or kidneys. Feeding the diets for more than four weeks did not increase the amount stored in the fat, although the 100 and 500 ppm diets did retard growth. After a four week withdrawal period, no traces of methoxychlor were found in the fat.

Deichman et al. (1967), having previously determined that diets of 2000 ppm methoxychlor were tumorigenic, fed rats diets of 1000 ppm methoxychlor for two years. He found no significant difference between the controls and the experimental rats in the number of rats afflicted, the locations of tumors, or the number of malignancies. There was also no significant difference between these two groups and a group fed DDT at 200 ppm in the diet for two years.

Imai and Coulston (1968) fed single doses of methoxychlor to rats: 400 mg/kg to a group on feed and water ad libitum, 400 mg/kg to a group after a 48-hour fast, and 1600 mg/kg for three days to a group on feed and water ad libitum. There were no significant differences in the jejunal mucosa of the three groups of rats. In all groups there was a noticeable distention of mitochondrial vesicles.

Kapoor (1970) measured the amount of methoxychlor stored in the fat of rats fed various levels of methoxychlor for four weeks. One ppm of methoxychlor was found in the fat of rats on a diet containing 100 ppm. Of the rats on a diet containing 500 ppm methoxychlor, the males stored 36 ppm and the females 17 ppm in their fat. After a two-week withdrawal period, no methoxychlor could be detected in the fat of the rats.

Phillips and Hatina (1972) examined the effect of methoxychlor on vitamin A storage in weanling rats and their offspring. Feeding diets of 100 ppm resulted in no difference in vitamin A storage between either generation and the controls.

Kapoor (1970) fed ^{3}H labeled methoxychlor to mice. Within 24 hours 98.3% of the label had been excreted, of which 88.5% was in the feces and 11.5% in the urine. Coats et al. (1974), in a similar experiment using mice, found that at 48 hours 99% of the label had been excreted, 90% in the feces and 10% in the urine.

Ely et al. (1953) fed dairy cows feed containing varying levels of methoxychlor. Levels of more than 447 ppm in the feed resulted in detectable levels of methoxychlor in milk. Moubry et al. (1968) monitored a dairy herd that showed a methoxychlor residue in its milk. Methoxychlor was detected in the feed of two cows, and this source was removed. In eight days the methoxychlor in one cow's milk decreased from 1.97 ppm to 0.95 ppm while the other's decreased from 1.52 ppm to 0.25 ppm in six days.

Tegeris et al. (1968) examined the ultrastructure of beagle dog small intestine after three months of feeding 2.5 g/kg/day of methoxychlor. Progressive destruction of the mitochondria and smooth endoplasmic reticulum occurred.

Stein et al. (1965) administered 0.5, 1.0, and 2.0 mg/kg/day for eight weeks to human volunteers. Biopsies of their fat, testes, bone marrow, livers, and small intestine, indicate a safety margin for methoxychlor in excess of 200 times the "maximum permissible limit."

(Neither the "maximum permissible limit" itself, nor the source of the figure was stated.)

Wallis and Carter (1959) collected field samples from various plants to which methoxychlor had been applied at the recommended rate of 1.75 pounds per acre as either an emulsion or wettable powder spray, or as a dust. All plants had acceptable residues of less than 14 ppm within seven days of the application. This also held true for the wettable powder spray applied at twice the recommended level.

In summary, methoxychlor is relatively nontoxic at exposures of less than 1000 mg/kg in all tested species, with the exception of its effect on the gonads. It has little cumulative effect. Once administration has ceased, methoxychlor will clear itself from a mammalian system in a relatively short period. Methoxychlor also clears rapidly from the environment when used correctly.

Differentiation and Growth of the Gonads

The male and female gonads develop from an indifferent gonadal ridge in the fetus. The sex of the fetus cannot be determined by examination of the gonads during the indifferent stage. The male gonad, the testis, differentiates first. Shortly before birth the gonads are histologically differentiable. Sex can be determined by histological examination, as well as by gross examination.

Indifferent stage

The first sign of the fetal reproductive system appears as an outgrowth on the medial surface of the mesonephros. These outgrowths

are the genital ridges which consist of an outer surface epithelium and an inner blastema (Arey, 1965; Gondos, 1974). Primitive germ cells migrate from the yolk sac entoderm and combine with the surface epithelium of the gonads (Arey, 1965; Gondos, 1974; Ham, 1974).

At this stage the germ cells are large and oval (Gondos, 1974). The germ cells, surrounded by some of the surface epithelium, migrate into the ridges, forming epithelial cords. There are now three distinct cells: germ cells, epithelial cells, and mesenchymal cells (Arey, 1965; Gondos, 1974; Ham, 1974).

The germ cells are surrounded by the epithelial cells of the cords and have an oval-shaped eccentric nucleus. The nucleus contains evenly-distributed chromatin and a prominent nucleolus. The cytoplasm is pale; it is characterized by the presence of small spherical mitochondria. There are irregular cytoplasmic projections, suggesting amoeboid movement (Gondos, 1974).

The epithelial cells, covering the indifferent gonad and surrounding the sex cords, are mesothelial in origin. They are elongate, with a dark-staining cytoplasm. The mitochondria are rod-shaped. The nucleus is irregularly shaped (Gondos, 1974).

The mesenchymal cells are within the gonadal stroma, which has developed from the blastema. The cells are elongate and loosely arranged. The cytoplasm lacks organelles associated with hormonal activity (Gondos, 1974).

Differentiation of the testis

The testis distinguishes itself from the indifferent gonad before the ovary does. This is marked by a separation produced by a layer of connective tissue between the surface epithelium and the underlying stroma and epithelial cells. The layer of loose connective tissue is the developing tunica albuginea (Arey, 1965; Ham, 1974). The epithelial cords, now testicular cords, become more distinct and converge toward the mesorchium, where they organize with other tubules. This is the primordia of the rete testis, which becomes continuous with the proximal ends of the testicular cords. The peripheral ends of the testicular cords join in arches. The main portion of the cords coil, forming the tubuli contorti, while the proximal ends of the cords remain straight, forming the tubuli recti. The tubuli recti connects to the rete testis, which connects to the mesonephric tubules, the primordial vasa efferentia (Arey, 1965; Ham, 1974).

Within the testis at this point, there are three main cell types: interstitial cells (of Leydig), supportive (Sertoli) cells, and gonocytes, formerly the germ cells. The latter two are found within the testicular cords. The interstitial cells develop within the stroma (Gondos, 1974).

The gonocytes migrate to the periphery of the cords. The cords look like testicular tubules, except that they lack lumens, which will not develop until puberty. The gonocytes are large round to oval cells, singly arranged. Their nuclei are large and spherical, containing nucleoli of various sizes. The chromatin granules are sparse and

evenly distributed within the nucleoli. There is incomplete division of the gonocytes, resulting in cytoplasmic bridges between pairs of gonocytes. After a period of proliferation, the gonocytes go through a period of degeneration. At all times before birth, the gonocytes are outnumbered by the supportive cells (Gondos, 1974).

The supportive cells are the primordia of the sustentacular (Sertoli) cells. They form the surface epithelium of the testicular cords, separated from the stroma by a basal lamina. Their nuclei are elongate, very irregular in shape, with prominent nucleoli. The cytoplasm is poorly defined (Gondos, 1974; Ham, 1974).

Up through the indifferent stage, the interstitial spaces consist of undifferentiated mesenchyme. Shortly after sexual differentiation the interstitial cells differentiate. These cells continue to differentiate for a time within the fetus, or sometimes within the neonate, the length of time depending on the species. The cells then regress and take on an appearance intermediate between that of interstitial cells and that of fibroblasts. Before regressing, the cells are clumped or individually located within the stromal network. Their nuclei are round to oval and pale-staining, and contain one or more nucleoli. The cytoplasm appears vacuolated and granular. Overall diameter of the cells may exceed 20 μm (Bloom and Fawcett, 1975; Gondos, 1974; Ham, 1974).

The tunica albuginea is part of the testicular stroma lying immediately beneath the surface epithelium. During the early stages

of development, it is quite cellular, consisting of fibroblasts and capillaries. Later its cellularity decreases, and the layer consists of primary dense extracellular collagen with some smooth muscle (Gondos, 1974).

The surface epithelium is of mesothelial origin. During the early stages of gonadal differentiation, the epithelium is pseudostratified to cuboidal. As the tunica albuginea starts developing, the surface epithelium becomes a simple cuboidal layer (Andersen and Simpson, 1973).

The male duct system takes over parts of the mesonephros. Some mesonephric tubules connect to the rete tubuli; the remainder of the former degenerate. The mesonephric tubules connect to the mesonephric duct, the former becoming vasa efferentia and the latter the epididymis. The vas deferens is formed from that portion of the duct that extends from the mesonephros to the urogenital sinus (Gier and Marion, 1970).

The linings of the lumens of the various ducts differ. Rete tubules are lined with nonciliated cuboidal to squamous epithelium. The vasa efferentia are lined with ciliated columnar to pseudostratified columnar epithelium. The epididymis is lined with ciliated high (tall) columnar to pseudostratified columnar epithelium. The epithelium of the vas deferens is pseudostratified columnar (Gier and Marion, 1970; Ham, 1974).

Differentiation of the ovary

In the female the indifferent gonad remains unchanged longer than in the male. The first signs of the developing ovary are irregular thickenings of the surface epithelium which project into the underlying loose connective tissue. The surface epithelium continues to proliferate as dispersed clumps of cells which continue to send projections into the subepithelial connective tissue. The thickenings and projections constitute the primary cortex of the ovary (Andersen and Simpson, 1973; Arey, 1965; Ham, 1974).

At this time the ovary consists of three zones. The outer is the primary cortex. Beneath this is a primitive tunica albuginea consisting of loose connective tissue. Centrally located is the core consisting of the large cellular mass of the undifferentiated gonad (Andersen and Simpson, 1973; Arey, 1965; Ham, 1974).

The surface epithelium extends strands into the core through the tunica albuginea. The strands maintain continuity with the surface epithelium. The strands appear to merge with the tunica albuginea, increasing the cellularity of the latter. Within these strands the primitive germ cells invade the ovary (Andersen and Simpson, 1973; Arey, 1965; Gondos, 1974; Ham, 1974).

Various tubules and associated structures appear. The rete ovarii appears as irregular cords and tubules within the ovarian core and hilus. From the rete ovarii, some cords or strands extend to the regressing mesonephros. The paramesonephric ducts develop laterally

to the mesonephric ducts, and grow caudad. The oviducts, which develop from the paramesonephric ducts, are lined with cuboidal epithelium and surrounded by layers of mesenchyme (Andersen and Simpson, 1973; Arey, 1965).

The cortical rim, formed by the epithelial ingrowths, increases in the connective tissue located between the epithelial cell clusters. The core becomes less distinct as the tunica albuginea is invaded by the cortical cells. Lobules are formed within the cortex by connective tissue and capillaries surrounding some of the subepithelial clusters. The rest of the clusters maintain continuity with the surface epithelium (Andersen and Simpson, 1973).

The cords and lobules become more distinct. Oogonia, from the primitive germ cells, and pregranulosa cells increase in number. The oogonia have large spherical nuclei and lightly-staining cytoplasm. The pregranulosa cells have slightly oblong nuclei and the cells themselves are smaller than the oogonia. The proliferation of oogonia and pregranulosa cells continues throughout gestation. Incomplete division of the oogonia results in intercellular bridges (Andersen and Simpson, 1973; Gondos, 1974).

The cortical lobules and cords become composed almost entirely of oogonia and pregranulosa cells. Numerous mitotic figures are visible, as well as pyknotic nuclei of regressing oogonia. As the dying oogonia are phagocytosed the remaining oogonia progress through leptotene, zygotene, pachytene, and diplotene of the first meiotic division (Andersen and Simpson, 1973; Gondos, 1974).

The connective tissue and blood vessels in the core increase forming the distinct medulla. The medulla and the tunica albuginea remain recognizable until birth (Andersen and Simpson, 1973; Gondos, 1974).

The surface epithelium is a derivative of the coelomic mesothelium. It is composed of columnar epithelial cells and germ cells during sex cord formation and oogenesis. After oogenesis the surface epithelium consists of a single layer varying from cuboidal to columnar. All germ cells have migrated inward out of the surface epithelium (Gondos, 1974; Ham, 1974).

The female genital ducts develop from the paramesonephric ducts (Mullerian ducts). These are located lateral to the mesonephric ducts within the same ridge. The posterior portion of the ridge rotates, joining the paramesonephric ducts medially. The fused posterior portion forms the uterus and vagina while the nonfused anterior portions form the uterine tubes (Arey, 1965).

As the genital ducts develop, their epithelial linings differentiate. In the adult the uterine horns are lined with a single layer of cuboidal epithelium. This epithelium consists of ciliated cells and secretory cells which alternate irregularly. The uterus is lined with columnar and pseudostratified columnar epithelium which contains simple tubular glands that open into the lumen. The cervix is covered with tall mucus-secreting columnar cells. The vagina is lined with stratified squamous epithelium which varies in the adult according to

the estrus cycle. When the various epithelia become distinguishable is not stated (Ham, 1974).

The adult female genital duct system is surrounded by varying layers of smooth muscle. The differentiation of the smooth muscle is completed in the postnatal period in most animals (Ham, 1974).

External sex identification

By birth the sex of a fetus can be determined fairly accurately by an external examination. In females the genital tubercle is located immediately ventral to the anus. In males the tubercle is midway between the umbilicus and the pubic arch (Andersen and Simpson, 1973). The rat fetus is similar to the dog fetus.

Gonadal critical period

The critical period for the rat kidney occurs on days 11, 12 and 13 (day 0 being the morning a sperm-positive vaginal smear is obtained from the dam). The critical period for the rat external genitalia occurs on days 15 and 16 (Tuchmann-Duplessis, 1975). (With day 1 as the morning of a sperm-positive vaginal smear, the critical period for the kidney occurs on days 12 through 14, and the critical period for the external genitalia on days 16 and 17). This indicates the critical period for the actual gonads to be approximately days 12 through 14.

The gonad develops as the kidney does, and starts taking over the mesonephric ducts after the fetal kidney is functional. Once the mesonephric ducts have been incorporated into the genital system, the external genitalia develop, after the gonad has differentiated.

MATERIALS AND METHODS

Rat Preparation

Fifty-five pregnancy-timed rats were obtained from Biolabs and housed individually in wire-bottom cages over sawdust. They were given Teklab® Mouse and Rat ration and water ad libitum. All the rats were pregnancy timed by overnight pairing with proven sires on December 30-31. Day one of gestation was designated as the day a sperm positive vaginal smear was obtained (December 31).

Rat Dosing

Technical grade methoxychlor, obtained from DuPont, was mixed with Wesson® corn oil to make a 5% solution.

The rats were divided into three groups with different dosing levels and dosing days, as follows:

Dose level of methoxychlor	Number of rats	Day
0 mg/kg	8	13
200 mg/kg	10	13
400 mg/kg	10	14

Because rats are nocturnal, dosing was done in the morning when they were more likely to have food in their stomachs. To simulate the probable route of human exposure to methoxychlor, the rats were dosed by gavage.

Preparation and Fixation of Rat Fetuses

The dams were sacrificed on day 21 of gestation by carbon dioxide

(CO_2) asphyxiation. The dams' weights (designated "before Cesarean') were taken and recorded.

A midventral incision from the sternum to the pubis was made to expose the viscera. The ovaries were located, lifted out, and cut; corpora lutea on the ovaries were counted; and then the ovaries put in 10% buffered neutral formalin. The uterus was then examined; the number of resorption sites and any abnormalities were recorded. After an incision was made into the uterine horns, all the fetuses were removed and their ammiotic sacs cut away. All fetuses were examined grossly for any deformations and viability, and then they were weighed as a litter. The ventral part of their abdominal walls were incised to allow better penetration by the preservative, and then the fetuses were stored in jars of 10% buffered neutral formalin. Each jar was labeled with the dam number, dosing regime, and date of sacrifice. The dams were individually reweighed and their weight recorded as "after Cesarean." (See Appendices C, D, and E.)

The ventral abdominal cavity walls were removed from one-third of the fetuses from each litter. Each fetus' sex was determined by external examination. The intestines, tail, and hind limbs were removed and each fetus was cut transversely in half at the pelvis of the most anterior kidney. Each posterior fetal portion was placed in imbedding rings, labeled with dam number, fetus sex, and intra-litter number, and run overnight through the following modified auto-technicon imbedding schedule (Luna, 1968):

70% ethyl alcohol	greater than 1 hour
80% ethyl alcohol	1.5 hours
95% ethyl alcohol	1.5 hours
95% ethyl alcohol	1.5 hours
100% ethyl alcohol	1.5 hours
100% ethyl alcohol	1.5 hours
100% ethyl alcohol	1.5 hours
Xylene	1.5 hours
Xylene	1.5 hours
Hot paraplast or wax	1 hour
Hot paraplast or wax	1 hour
Hot paraplast or wax	greater than 1 hour

In the morning each portion was cast in a paraffin cutting block for a microtome, with the anterior part of the fetal hind portion on the cutting surface. Each block was labeled with the dam number, sex of the fetus and the intralitter number. (See Appendix F.)

Sectioning, Mounting, and Staining

The blocks were trimmed so a section would contain more than epidermis and spinal cord. The blocks were sectioned, anterior to posterior, at 7 μm. In the region of the gonads, every eighth section was mounted. Otherwise, every thirtieth section was mounted. Selected sections were floated on a hot water bath of double distilled water (added to the bath were thymol, to retard bacterial growth, and gelatin, to aid the sections in adhering to the glass slides). The

sections were slipped onto glass slides and allowed to air dry. Each slide was labeled with the dam number, fetus sex, intralitter number, and slide sequence number.

The dried slides were arranged in open-bottomed racks and stained by a modified version of the standard hematoxylin and eosin staining procedure (Lynch, 1969):

Xylene 1	5 minutes
Xylene 2	5 minutes
100% ethyl alcohol	2 minutes
100% ethyl alcohol	2 minutes
95% ethyl alcohol	2 minutes
70% ethyl alcohol	2 minutes
Distilled water	2 minutes
Harris' hematoxylin[1]	8 minutes
Running tap water	2 minutes
Acid alcohol[1]	1 quick dip
Running tap water	3 minutes
Lithium carbonate[1]	1 minute
Running tap water	5 minutes
Distilled water	2 minutes
Working eosin[1]	2 minutes
95% ethyl alcohol	1 minute

[1]See Appendix G.

95% ethyl alcohol	1 minute
100% ethyl alcohol	1 minute
100% ethyl alcohol	1 minute
Xylene 1	2 minutes
Xylene 2	2 minutes
Xylene 3	storage of slides

Slides were stored in xylene (xylene 3) until coverslipped. A couple of drops of a clear resin were put on a xylene-wet slide, and a coverslip was lowered onto it. Care was used in applying the slip to prevent air bubbles from being trapped between the tissue section and the coverslip. The slides were dried at 40°C and stored upright in slide boxes until examined.

RESULTS

Male Fetuses

The testes from the male fetuses of the three groups differed in composition and maturity, both structural and cellular. They all contained the same cellular elements and structural units, but the amounts and ratios of the elements and units varied within each group.

All fetuses were checked for signs of post mortem changes to ensure that the observed variations were not due to post mortem autolysis. The condition of the epithelial linings of the ureters, dorsal aorta, inferior vena cava, and gut was examined, and no significant post mortem imbibition had occurred. All fetuses appeared to be at the same stage of post mortem changes at the time of fixation.

Bal and Mungkornkarn (1977) reported consistently finding large multinucleated cells in the testes of adult male rats after 70 days of dosing with 100 or 200 mg/kg of methoxychlor. Care was taken to detect similar abnormalities in the present study.

General description of male fetal testes

At low power (40X) the testis fills about a quarter of the field. At this level the tunica albuginea is visible. Within it are the testicular cords and stroma. The cords are distinct and are circular, long and straight, or coiled, depending on the angle and level of the section. The stroma varies, usually consisting of densely packed cells between the cords near the rete and periphery of the testis. The center

of the open stroma (that part not occupied by testicular cords) is more loosely packed. Located centrally in some of the testes, distal and caudal to the rete, is a large area of open stroma: a stromal space. This stromal space contains a faint matrix and few visible cells.

At medium power (100X) a testis fills most of the field. This indicates the diameter of the testes to range from 1300 to 1500 μm at their thickest cross section.

At high power (400X) the absence of lumens in the cords is apparent. The width of a cord covers about one-fifth of the field, indicating their diameters to be about 75 to 90 μm. The parenchyma of the testicular cords consists of two cell types: supporting cells and gonocytes.

The supporting cell is the predecessor of the sustentacular (Sertoli) cell. Its dark-purple-staining, irregular oval-to-rectangular nuclei line the periphery of the cord. The chromatin lines the nuclear membrane, and forms occasional nucleoli. The boundaries between the cytoplasm of adjoining supporting cells, or adjoining gonocytes, cannot be resolved with the light microscope.

The gonocytes, predecessors of the spermatogonia, are within the band of supporting cells. The round granular nuclei stain light purple and contain evenly distributed chromatin. The nucleus is sometimes surrounded by a thin band of pink granular cytoplasm. The average size of the gonocyte is about 20 μm.

Vacuoles are intermixed with the gonocytes within the cords. In some sections a ring of vacuoles surrounds a gonocyte. The diameter of the vacuoles ranges up to 25 μm. The vacuoles are more evident in the sections of long cords and in portions of cords distal to the rete. The vacuoles may be part of the cytoplasm of the gonocytes, the supporting cells, both, or neither.

The stroma is composed of differentiated fibroblasts, mesenchymal cells, interstitial cells, a matrix, and capillaries. Differentiated fibroblasts, mesenchymal cells, and interstitial cells are usually densely packed between the testicular cords near the rete and periphery of the testis. Centrally, the stroma is less dense.

The interstitial cells are found in clumps, strands, or situated individually. Their nuclei are centrally located, round or oval, and purple-staining. The cytoplasm of the interstitial cells is pink. It forms an irregular band around individual cells or clumps of interstitial cells.

The nuclei of the differentiated fibroblasts and mesenchymal cells stain dark purple. They have an irregular oval shape and are smaller than the nuclei of interstitial cells. The cytoplasm is indistinct.

Some testes have a stromal space.

The tunica albuginea appears to consist only of a thick layer of fibrous connective tissue, the layer of muscle present in the adult being indistinguishable with the light microscope. This is covered by a layer of simple squamous epithelium.

Testes of control male fetuses

The testes of the control male fetuses varied in appearance, in the number of testicular cords and their constituent elements, and in the amount of open stroma and its constituent cells. A description of testes from five of the ten male fetuses sectioned follows to illustrate this morphological variation; the fetuses were 1CMA, 2CMA, 5CMA, 6CMA, and 7CMA (See Appendix F for significance of designations).

1CMA Numerous vacuolated testicular cords are visible within the testis (Figure 1). There is little open stroma; what is present is dense with cells.

The gonocytes are scattered unevenly within the testicular cords (Figure 2). There are a few nucleoli present; the rest of the chromatin is evenly distributed within the nuclei. The cytoplasm of some of the gonocytes has distinct boundaries, while that of others is obscure.

Within the cords are many small to large vacuoles. The larger ones are more centrally located and measure up to 22 μm in diameter.

The interstitial cells are arranged in cords and clumps. There are some nucleoli. The rest of the chromatin lines the nuclear membranes.

2CMA The testis has few testicular cords and few cells in the open stroma (Figure 3). The cords are fairly dense. The stroma contains a few small clumps of interstitial cells. There is a stromal space.

The gonocytes are numerous and evenly distributed within the cords (Figure 4). The granular cytoplasm lacks any definite boundaries.

There are numerous small to medium sized vacuoles within the cords. The medium sized vacuoles are centrally located and are up to 9 μm in diameter.

The interstitial cells are found in a few scattered clumps within the open stroma. The chromatin is evenly distributed within the nuclei and forms an occasional nucleolus.

There are a few scattered differentiated fibroblasts and mesenchymal cells.

5CMA The stroma varies in density (Figure 5). The interstitial cells are packed between the testicular cords near the rete, and are scattered elsewhere.

Immature gonocytes pack the cords (Figure 6). The granular nuclei contain evenly distributed chromatin, which also forms individual or multiple nucleoli. Some boundaries of the granular cytoplasm are defined by the numerous vacuoles.

There are many small vacuoles surrounding many of the gonocytes. They are smaller than the gonocyte nuclei.

There are clumps of interstitial cells, and a few individual cells, in the stroma. The chromatin lines the nuclear membranes and forms some nucleoli.

6CMA The testis contains a moderate number of testicular cords, areas of open stroma, and scattered clumps of interstitial cells (Figure 7). The cords are fairly dense with no large visible vacuoles.

The gonocytes are numerous and evenly distributed within the cords (Figure 8). The granular cytoplasm has no definite boundaries. There are numerous small vacuoles scattered within the cords. They are smaller than the nuclei of the gonocytes.

The interstitial cells are scattered individually or in clumps. The chromatin lines the nuclear membranes and forms some nucleoli.

7CMA The testis contains a moderate number of testicular cords, a few large clumps of interstitial cells, and a lot of open stroma (Figure 9). The cords are fairly dense, without large vacuoles.

The gonocytes are evenly dispersed within the cords (Figure 10). There are a few mitotic figures. The nuclei are faint and granular; a few nucleoli are present. The cytoplasm is granular or vacuolated, and has indefinite boundaries.

There are numerous small vacuoles within the cords. They are evenly distributed and smaller than the gonocyte nuclei.

The spaces between the testicular cords are large. The open stroma within these spaces contains few cells. There are some large clumps of interstitial cells. Some nucleoli are present within the interstitial cells.

Testes of male fetuses exposed to 200 mg/kg methoxychlor

There was variety in the composition of the testes from the male fetuses of dams which had received a 200 mg/kg dose of methoxychlor on day 13 of gestation. The number of testicular cords and their composition varied. There was a variation in the density of the open

stroma and in the presence or absence of a stromal space. This difference appeared between the two testes within a fetus as well as among testes of different fetuses. To illustrate this morphological variation, five testes are described: two from the same male fetus, and three others from three different fetuses, out of a total of 20 sectioned male fetuses. The sections are from 5MC (left testis), 5MC (right testis), 6MD, 7MB, and 8MB (See Appendix F).

5MC (left testis) The testis contains numerous testicular cords, leaving little open stroma (Figure 11). The numerous interstitial cells are found in large clumps near the rete, and peripheral and central within the testis. There is a stromal space containing a few clumps of interstitial cells and numerous differentiated fibroblasts and mesenchymal cells.

The gonocytes are evenly distributed within the cords (Figure 12). The chromatin forms some individual and multiple nucleoli within some of the nuclei. The cytoplasm lacks any definite boundaries. There are many small to medium-sized vacuoles within the cords. Some of the larger vacuoles surround gonocyte nuclei.

The interstitial cells form large clumps and wide bands within the stroma. There are some nucleoli present within the interstitial cell nuclei. There are numerous differentiated fibroblasts and mesenchymal cells filling the remainder of the stroma. In some nuclei the chromatin is evenly distributed, while in others it lines the nuclear membrane. Some nucleoli are present.

5MC (right testis) This testis contains fewer testicular cords and interstitial cells, and has a sparser open stroma than the left testis from the same fetus (Figure 13). There are small scattered clumps of interstitial cells and a large stromal space.

The gonocytes are evenly distributed within the cords (Figure 14). There are some mitotic figures. The granular cytoplasm lacks any definite boundaries. There are numerous small vacuoles within the cords.

6MD The testis contains a moderate number of testicular cords (Figure 15). A few large vacuoles are visible within the cords. The open stroma is evenly distributed around the cords. The interstitial cells are unevenly distributed, being more numerous at the rete and in the larger areas of open stroma.

The testicular cords contain some faint and some prominent gonocytes (Figure 16). The nuclei of the faint gonocytes stain light purple and contain evenly distributed chromatin which forms some nucleoli. The cytoplasm of the faint gonocytes is granular, light pink, and lacking in definite boundaries. The nuclei of the prominent gonocytes stain a darker purple and contain evenly distributed chromatin. The cytoplasm is indistinct.

There are numerous vacuoles within the cords. The larger ones form rings around the more prominent gonocyte nuclei.

The interstitial cells are in distinct small clumps within the open stroma. The chromatin is evenly distributed within the nuclei and forms some nucleoli.

Some of the numerous differentiated fibroblasts and mesenchymal cells contain individual or multiple nucleoli.

7MB The testicular cords and interstitial cells are concentrated along the periphery of the testis (Figure 17). There are a moderate number of cords containing many visible vacuoles. Some of the vacuoles span the entire width of a cord. In the center of the testis is a large stromal space containing a few differentiated fibroblasts, cords, interstitial cells, and mesenchymal cells.

The central areas of the cords are fairly empty of gonocytes (Figure 18). Numerous randomly distributed vacuoles, up to 25 μm in diameter, fill the cords.

The gonocytes seem to be pushed to the sides of the cords by the vacuoles. There are individual and multiple nucleoli within some of the gonocyte nuclei. The cytoplasm is interrupted by the vacuoles.

The interstitial cells are in clumps along the periphery of the testis, and in small clumps or individually located within the central area. Some of the nuclei contain nucleoli. The fibroblasts and mesenchymal cells are numerous along the periphery of the testis, but sparse within the central area.

8MB The testis contains numerous testicular cords and interstitial cells (Figure 19). Both are evenly distributed. Many vacuoles are present in the cords.

The gonocytes are reduced in number (Figure 20). The nuclei appear to be pushed to the sides of the cords by the large vacuoles.

There are some individual and multiple nucleoli present within some of the gonocyte nuclei. The cytoplasm is indistinct.

Vacuoles of various sizes fill the central portions of the cords. The larger ones, up to 18 μm in diameter, are located centrally within the cords; the smaller ones peripherally.

The interstitial cells are scattered throughout the testis in numerous small clumps. The chromatin lines the nuclear membranes and forms some nucleoli. Numerous differentiated fibroblasts and mesenchymal cells fill the open stroma.

Testes of male fetuses exposed to 400 mg/kg methoxychlor

There was variety in the composition of the testes from the male fetuses of dams which had received a 400 mg/kg dose of methoxychlor on day 14 of gestation. The number of testicular cords and their composition varied. There was a variation in the amount and density of the open stroma, as well as in the presence or absence of a stromal space. To illustrate this morphological variation, five testes from different male fetuses, out of 14 which were sectioned, are described. The sections are from 11MA, 12MA, 14MA, 18MD, and 20 MA (See Appendix F).

11MA The testis contains a few scattered testicular cords and clumps of peripherally located interstitial cells (Figure 21). The cords are dense with gonocytes. The open stroma is sparsely filled.

There are numerous gonocytes filling the testicular cords (Figure 22). Some of the nuclei contain nucleoli. The granular cytoplasm lacks definite boundaries. There are numerous small to

medium-sized vacuoles in the cords. The medium-sized ones form rings around some of the centrally located gonocytes.

The interstitial cells form strands in the open stroma. The cytoplasm stains a brighter purple, and the nuclei a darker purple, than those in other testes. The differentiated fibroblasts and mesenchymal cells are few and scattered within the stroma.

12MA The testis contains a moderate number of testicular cords (Figure 23). The interstitial cells are scattered in small clumps. A large stromal space is located in the center of the testis.

There are fewer gonocytes than average; they are evenly spaced within the cords (Figure 24). Some nucleoli are present within the nuclei. The cytoplasm is indistinct.

Many vacuoles fill the cords. All the sizes of vacuoles are evenly distributed. The largest are 18 μm in diameter.

The interstitial cells are in small clumps scattered throughout the testis, with more located towards the periphery. Some nucleoli are present. The differentiated fibroblasts and mesenchymal cells are unevenly distributed within the stroma, fewer being in the stromal space than elsewhere.

14MA The testis contains numerous testicular cords (Figure 25). Some vacuoles are visible within the cords. The interstitial cells are numerous and scattered in various-sized clumps throughout the open stroma.

The number of gonocytes is low (Figure 26). Chromatin lines the nuclear membranes of some, and is evenly distributed within the nuclei

of others. Some nucleoli are present. The cytoplasm is indistinct.

Many vacuoles are present within the cords. The larger ones, up to 18 μm in diameter, surround some of the gonocytes.

There are numerous interstitial cells. The clumps are not as compact as in the previously-described fetuses. The nuclei contain some individual and multiple nucleoli.

The nuclei of the differentiated fibroblasts and mesenchymal cells are unevenly distributed. They are more numerous near the rete and periphery of the testis.

18MD The testis contains numerous testicular cords (Figure 27). Many moderate-sized vacuoles fill the central area of the cords. There are numerous interstitial cells, differentiated fibroblasts, and mesenchymal cells evenly distributed throughout the testis.

The cords contain only a few gonocytes which lie peripherally within the cords (Figure 28). Some nucleoli and mitotic figures are present. The cytoplasm is indistinct.

Numerous vacuoles fill the cords. Many are larger than the gonocytes. The larger vacuoles fill most of the central areas of the cords.

The interstitial cells are scattered in numerous small clumps. There are some nucleoli present.

20MA The testis contains fewer testicular cords than the other testes (Figure 29). The cords are dense with immature gonocytes. A moderate number of interstitial cells are concentrated near the rete and along the periphery of the testis.

The immature gonocytes are numerous and evenly distributed in the cords (Figure 30). There are some nucleoli present. The cytoplasm has some visible boundaries. What vacuoles are present are small and unevenly distributed around the gonocytes.

The interstitial cells are in small clumps. There are some nucleoli present.

The differentiated fibroblasts and mesenchymal cells are more numerous near the rete and periphery of the testis.

Male genital ducts

The genital duct systems, derivatives of the mesonephric ducts, of all the sectioned male fetuses of the three groups were examined. In all cases the ducts were patent.

Female Fetuses

The ovaries from the female fetuses of the three groups displayed variety in their structural composition and differentiation. They all contained the same cellular elements, but in varying amounts.

All fetuses were checked for signs of post mortem changes to ensure that the observed variations were not due to post mortem autolysis. The condition of the epithelial linings of the ureters, dorsal aorta, inferior vena cava, and gut were examined, and no significant post mortem imbibition had occurred. All fetuses appeared to be at the same stage of post mortem changes at the time of fixation.

Bal and Mungkornkarn (1977) reported consistently finding degenerated granulosa cells in the ovaries of adult female rats

after 34 days of dosing with 100 or 200 mg/kg of methoxychlor. Care was taken to detect similar abnormalities in the present study.

General description of female fetal ovaries

At low power (40X) the ovaries fill only a small part of the field. The ovary is bean-shaped. The stroma is uniform in appearance. Some thin cords, and varying numbers of the round nuclei of gonocytes, are visible. The hilus is composed of uniform mesenchyme.

At medium power (100X) the ovaries measure about 700 μm at their widest diameter. They are covered with a layer of simple cuboidal epithelium. Some similarity is seen between the cells forming the cords and those forming the surface epithelium.

At high power (400X) the types of individual cells can be determined. The ovary is composed of gonocytes, pregranulosa cells, differentiated fibroblasts, and mesenchymal cells.

The gonocytes vary considerably in appearance and numbers. The nuclei are round and contain evenly distributed chromatin. The gonocytes are generally grouped.

The pregranulosa cells form thin cords that course through and around clumps of gonocytes. The nuclei are irregular and contain chromatin which lines the nuclear membranes. The nuclei stain dark purple. The cytoplasm is indistinct.

Differentiated fibroblasts and mesenchymal cells lie within the cords formed by the pregranulosa cells. Their nuclei stain darker than those of the pregranulosa cells, and are more elongate.

Ovaries of female control fetuses

The ovaries of the female control fetuses varied considerably in the appearance of the cortex. The most noticeable variation was in the maturity of the gonocytes. Some ovaries were packed with immature gonocytes; others contained numerous degenerating nuclei surrounded by large vacuoles. Three ovaries from different fetuses, out of 13 which were sectioned, are described to illustrate this morphological variation: 1CFB, 2CFB, and 8CFB (See Appendix F).

1CFB Some surface epithelial cells are continuous with the pregranulosa cells within the ovary (Figure 31). The pregranulosa cells form a network of fine cords which enclose vacuolated lobes of gonocytes. The cords and gonocytes are evenly distributed within the ovary.

Purple to very dark purple degenerating gonocyte nuclei are enclosed by vacuoles and pregranulosa cells (Figure 32). The nuclei are surrounded by a thin band of irregular pink cytoplasm; the more pyknotic the nucleus, the more eosinophilic the cytoplasm. Three stages of gonocyte development are present: nondegenerating, degenerating, and pyknotic. The nondegenerating gonocyte nuclei are purple; the degenerating nuclei dark purple; and the pyknotic nuclei very dark purple, small, and round.

2CFB The ovary is packed with immature gonocytes (Figure 33). A few pyknotic nuclei are present. The thin cords are distinct and connect with the surface epithelium.

The gonocyte nuclei virtually exclude all other cell types from

the field (Figure 34). The immature irregularly-circular nuclei are surrounded by pink granular cytoplasm. There are a few large vacuoles (about 14 μm diameter) within some of the lobes of gonocytes. The pyknotic gonocyte nuclei are surrounded by a vacuole or vacuoles of the same dimension.

8CFB The ovary is filled with vacuoles (Figure 35). Numerous pyknotic gonocytes dot the ovary. The pregranulosa cells form cords which are more often continuous with the hilus than with the surface epithelium.

The pyknotic gonocyte nuclei are most prominent, but there are other nuclei which have not regressed (Figure 36). The pyknotic nuclei are surrounded by a thin band of eosinophilic cytoplasm and abut the larger vacuoles. The vacuoles range up to 40 μm in diameter.

The mature nondegenerating gonocyte nuclei are purple and closely associated with the thin cords of pregranulosa cells.

Ovaries of female fetuses exposed to 200 mg/kg methoxychlor

The structural variety of this group of ovaries was not as great as that of the controls. The main differences among the ovaries of this group were found in the relative prominence of the thin cords of pregranulosa cells and in the distribution of the gonocytes within the ovaries. To illustrate this morphological variation, ovaries from different female fetuses, out of 12 sectioned, are described: 2FB, 4FB, and 7FB (See Appendix F).

2FB Pregranulosa cells make up the bulk of the ovary although there are no distinct thin cords present (Figure 37). The cells form a layer beneath the surface epithelium and a central mass in the ovary. Between the two areas of pregranulosa cells are lobes and bands of vacuoles and pyknotic nuclei of gonocytes.

The degenerating nuclei of gonocytes are closely associated with clumps and bands of vacuoles (Figure 38). The nuclei are surrounded by a thin layer of eosinophilic granular cytoplasm. The mature gonocytes are surrounded by smaller vacuoles and closely associated with the pregranulosa cells. The cytoplasm of these gonocytes is light pink and indistinct.

4FB The pregranulosa cell cords are more definite (Figure 39). A layer of pregranulosa cells lies under the surface epithelium. The gonocytes and vacuoles are evenly distributed within the ovary.

There are only a few gonocytes which have pyknotic nuclei and eosinophilic cytoplasm (Figure 40). The cytoplasm of the other gonocytes is indistinct, and their nuclei lie close to the cords of pregranulosa cells.

7FB The ovary has distinct pregranulosa cords which extend from the surface epithelium to the hilus (Figure 41). The vacuoles and gonocytes form channels between the cords. The pyknotic gonocyte nuclei are more numerous near the hilus and surface epithelium.

The pyknotic gonocyte nuclei are surrounded by vacuoles and pregranulosa cells (Figure 42). The nondegenerating gonocytes abut upon other gonocytes and numerous pregranulosa cells.

Ovaries of female fetuses exposed to 400 mg/kg methoxychlor

The ovaries within this group ranged in condition from those which were packed with immature gonocytes to those which were packed with vacuoles. The distinctness of the pregranulosa cords varied. To illustrate this morphological variation, three ovaries from different female fetuses, out of 18 fetuses sectioned are described: 11FB, 18FF, and 20FC (See Appendix F).

11FB There are numerous gonocyte nuclei which appear to be surrounded by bands of light cytoplasm or rings of vacuoles (Figure 43). The advanced pyknotic nuclei lie to the side of large vacuoles. The pregranulosa cords are interrupted, forming strands and clumps.

The granular nuclei of the gonocytes vary from light purple to dark purple (Figure 44). The cytoplasm varies from granular pink to indistinct. The pregranulosa cells course around small lobes of gonocytes. The vacuoles are evenly distributed within the lobes.

18FF Large vacuoles compose the bulk of the ovary (Figure 45). The pregranulosa cell cords are broken. Pyknotic gonocyte nuclei are scattered within the vacuoles and clumps of pregranulosa cells.

Eosinophilic cytoplasm forms irregular bands around the pyknotic gonocyte nuclei (Figure 46). The nondegenerating gonocytes lack definite cytoplasm. Large vacuoles and chains of vacuoles separate gonocytes and pregranulosa cells. The pregranulosa cells are in numerous small clumps.

20FC The ovary is packed with immature gonocytes (Figure 47). Some cords of pregranulsa cords extend from the surface epithelium to

the hilus, while others are incomplete.

The gonocyte nuclei are granular and purple (Figure 48). The vacuoles are unevenly distributed, occasionally forming rings around gonocytes. The cords of pregranulosa cells divide the gonocytes into lobes.

Female genital ducts

The duct system (from the paramesonephric ducts) of all the sectioned female fetuses of the three groups were examined. In all cases the ducts were patent except at the point where they joined the urogenital sinus. In all cases the single duct was occluded.

Other Differences Among Dosage Groups

There were few differences among the three groups of fetuses. The average litter weights varied little, being 37.7 g with a standard deviation of 11.0 for the controls, 38.6 g with a standard deviation of 11.4 for the 200 mg/kg group, and 36.6 g with a standard deviation of 13.2 for the 400 mg/kg group. The average number of pups per litter showed a slight increase with dosage, being 10.1 pups with a standard deviation of 1.6 for the controls, 10.7 pups with a standard deviation of 3.2 for the 200 mg/kg group, and 11.1 pups with a standard deviation of 3.2 for the 400 mg/kg group. The average weight per pup varied little, being 3.6 g per pup with a standard deviation of 0.7 for the controls, 3.6 g per pup with a standard deviation of 0.3 for the 200 mg/kg group, and 3.3 g per pup with a standard deviation of 0.5 for

the 400 mg/kg group. These results, drawn from Appendices C, D, and E, are summarized in Table 3.

No gross abnormalities were noted in any of the fetuses at any of the dosing levels.

The uteri of all of the dams appeared normal. There were four resorption sites in three of the females of the 200 mg/kg group and five resorption sites in two of the females of the 400 mg/kg group (See Appendices C, D, and E).

Table 3. Summary of litter means

	Controls	200 mg/kg	400 mg/kg
Average litter weight (g)	37.7	38.6	36.6
Standard deviation	11.0	11.4	13.2
Average number of pups per litter	10.1	10.7	11.1
Standard deviation	1.6	3.2	3.2
Average weight per pup (g)	3.6	3.6	3.3
Standard deviation	0.7	0.3	0.5

DISCUSSION

Male Fetuses

On day 21 of gestation, the testes of male fetal rats lie within the pelvic cavity. The pair lie separately laterally or dorsolaterally on either side of the urinary bladder in the inguinal fossa. In a few specimens the testes abutted each other ventral to the urinary bladder.

The histological appearance of the testes varied within dosage groups and between the two testes of an individual fetus. The interstitial cells, numbers of testicular cords, and maturity of the developing gonocytes showed the most variation. In the fetuses from the control dams and from the dams dosed with 400 mg/kg methoxychlor, the gonocytes ranged from numerous but immature to mature with evidence of degeneration present. The testes from fetuses of dams dosed with 200 mg/kg methoxychlor had gonocytes which ranged from mature to mature with signs of degeneration.

The number and distribution of the interstitial cells varied with no apparent correlation between number and distribution. In some testes there were a few scattered clumps of interstitial cells, and in others there were large clumps and strands of the cells. Usually the interstitial cells were more numerous between the cords along the periphery and near the rete of the testes. The number of cells in the central area of the testes varied, as did the ovarall number of cells.

The number of apparent cords within the testes varied. In some the cords were few, coursing around the testis along its periphery,

just beneath the tunica albuginea. In others the cords followed the same course, but then involuted to fill the inner stroma. Cross sections of these testes exhibited a greater number of cords. Whether or not this constituted an increase in the number of the cords or just an increase in the length of the cords could not be determined.

The immature gonocytes had granular round nuclei with evenly distributed chromatin within the nuclei. The nuclei stained purple. The surrounding cytoplasm stained purple and was granular. A few small vacuoles were located between cells.

The mature gonocytes had granular nuclei which stained darker purple. The nuclei had evenly distributed chromatin. The vacuoles were more numerous and much larger. As vacuolation increased within the cords, the distribution of the gonocytes changed: they were more evenly distributed when small amounts of vacuoles were present, but tended to be located peripherally with increasing amounts of vacuoles.

Without extensive histochemical techniques or electron microscopy, the source of the vacuoles cannot be determined. They probably arise from the degeneration of gonocytes (Gondos, 1974), but could also result from the supporting cells, a combination of the two, or neither.

In all the male fetuses, the testicular cords lacked lumens, but the rete tubuli, vasa efferentia, ductus epididymis, and ductus deferens were patent. (The latter are connected with the urethra.) The patency of the genital duct system indicates that methoxychlor does not cause a change in that system which could lead to reproductive failure in the adult.

The variations in the appearance of the testes are probably not due to post mortem autolysis. The integrity of the epithelial linings of the ureters, dorsal aorta, inferior vena cava, and gut of all the fetuses were found to be similar. No signs of post mortem imbibition were found.

The variation in the developmental stage of the testes at the time of sacrifice indicates that the fetuses develop at different rates, making comparisons of this sort difficult. The variation in development may be increased by the variation in the time of conception, which may be up to 12 hours after overnight pairing with sires, and which cannot be detected by the use of sperm-positive vaginal smears to determine pregnancy.

The presence of immature gonocytes, degenerating gonocytes, and mitotic figures shows the developing testis to be an organ of continuing change in the fetus. It undergoes a continuous process of proliferation in the developing fetus, and in the postnatal animal as well (Ham, 1974).

Female Fetuses

On day 21 of gestation, the ovaries of female rat fetuses lie in the peritoneal cavity dorsomedial to or posterior to the kidneys.

The histological appearance of the ovaries varied within the three dosage groups. The amount of variance was similar within each group; the overall appearance of the three groups of ovaries was similar. The range was greater in the female fetuses from the

control dams and from the dams dosed with 400 mg/kg methoxychlor; it extended from an ovary packed with immature gonocytes to an ovary containing gonocytes with signs of degeneration. The fetuses from dams dosed with 200 mg/kg methoxychlor had ovaries that ranged from those containing normal gonocytes to those containing gonocytes with signs of degeneration.

The immature ovaries had cords of pregranulosa cells which extended from the surface epithelium to the hilus. These cords divided the ovary into lobes packed with large immature gonocytes. The gonocytes had granular round nuclei containing evenly distributed chromatin. A band of granular cytoplasm surrounded each nucleus. Both the nuclei and the cytoplasm stained purple.

The cords of pregranulosa cells were broken up and indistinct in the more mature ovaries. Within the scattered clumps of pregranulosa cells were lobules of vacuoles and gonocytes with dark-staining nuclei. The nuclei of the gonocytes were round and granular, but stained darker purple than those of immature ones. There were a few mitotic figures and a few pyknotic nuclei. The cytoplasm was often indistinct. Numerous vacuoles filled the lobes and partially surrounded the nuclei.

In all of the female fetuses, the uterine horns, uterus, and vagina were patent. In all cases there was an occlusion where the vagina joined the urogenital sinus. This indicates that methoxychlor did not cause a change in the genital duct system that might lead to reproductive failure in the adult.

The variations in development probably are not due to post mortem autolysis. The epithelial linings of the ureters, dorsal aorta, inferior vena cava, and gut of all the fetuses were found to be similar in integrity. No signs of post mortem imbibition were found.

The variation in the development of the ovaries in the female fetuses at the time of sacrifice indicates that the fetuses develop at different rates, making comparisons of this sort difficult. The variation may be increased by the variation in the time of conception, which may be up to 12 hours after overnight pairing with sires, and which cannot be detected using sperm-positive vaginal smears to determine pregnancy.

The presence of immature gonocytes, pyknotic nuclei, and mitotic figures demonstrates that the ovary is a dynamic organ. It undergoes a continuous process of change involving the proliferation and degeneration of gonocytes.

Teratogenic Effects

The results of this experiment indicate that a single oral dose of methoxychlor given to pregnant dams--200 mg/kg administered on day 13 of gestation, or 400 mg/kg on day 14--is not teratogenic in the fetal rat reproductive system. At this level, the methoxychlor did not cause any gross teratological changes either.

In testing a substance for teratogenicity, Canada (1975) lists three treatment schedules:

1. Single or multiple administration on certain days of gestation,
2. administration throughout gestation, and
3. administration prior to and during gestation.

Cook et al. (1969) further define procedures for teratogenic testing. They divide the testing into three main sections: teratology, fertility, and peri- and postnatal studies.

Teratological screening of substances for use in the United States should follow this procedure:

1. use at least two species;
2. use at least 20 pregnant female rodents per group and at least 10 pregnant female nonrodents per group;
3. use at least two dose levels:
   a. a large but sub-toxic dose (maximum tolerated dose),
   b. a small dose which is a multiple of the proposed therapeutic dose, and
   c. a control;
4. in the rat, administer from day six through day 15 of gestation; and
5. administer by the route of the most common exposure.

The procedures, established by Canada (1975) for the third test and by Cook et al. (1969) for fertility studies were followed by Bal and Mungkornkarn (1977).

The present experiment meets the requirements of the first test for teratological screening as set by Canada (1975). Bal and Mungkornkarn having satisfied the requirements for the third test, only the second test of continuous administration throughout pregnancy remains to be done before a final opinion can be given concerning the teratogenicity of methoxychlor, according to the methods of Canada (1975).

According to Cook et al. (1969), more tests need to be performed to evaluate the teratologic effect of methoxychlor, and peri- and postnatal studies must be performed, before a final opinion can be given concerning the teratogenicity of methoxychlor.

The significance of this study lies in its concentration at the microscopic level on the developing organs which methoxychlor damages when administered to adult rats. The test procedures outlined by Canada (1975) and Cook et al. (1969) employ only gross examinations of fetuses, and depend on peri- and postnatal studies to emphasize any microscopic damage through the appearance of dysfunction in, or death of, the offspring. If correlations could be established between the toxicity of a substance in adults and histological teratogenicity in fetuses, the time required for screening and approving substances for use could be reduced.

SUMMARY AND CONCLUSIONS

Male Fetuses

1. The variation in the histology of the testes of the control male fetuses was due to differences in the maturity of the developing testes.
2. The variation in the histology of the testes of male fetuses of dams exposed to 200 mg/kg methoxychlor on day 13 of gestation was due to differences in the maturity of the developing testes which were not caused by the prenatal exposure to methoxychlor.
3. The variation in the histology of the testes of male fetuses of dams exposed to 400 mg/kg methoxychlor on day 14 of gestation was due to differences in the maturity of the developing testes which were not caused by the prenatal exposure of methoxychlor.
4. The amount of variance was similar within each group; the overall appearance of the three groups of testes was similar. No signs of the abnormalities reported to result from chronic dosing (Bal and Mungkornkarn, 1977) were observed.

Female Fetuses

1. The variation in the histology of the ovaries of the control female fetuses was due to differences in the maturity of the developing ovaries.
2. The variations in the histology of the ovaries of female fetuses of dams exposed to 200 mg/kg methoxychlor on day 13 of gestation

was due to differences in the maturity of the developing ovaries which were not caused by the prenatal exposure to methoxychlor.

3. The variation in the histology of the ovaries of female fetuses of dams dosed with 400 mg/kg methoxychlor on day 14 of gestation was due to differences in the maturity of the developing ovaries which were not caused by the prenatal exposure to methoxychlor.
4. The amount of variance was similar within each group; the overall appearance of the three groups of ovaries was similar. No signs of the abnormalities reported to result from chronic dosing (Bal and Mungkornkarn, 1977) were observed.

Dosage Groups

1. There was a slight variation in average litter weight among the three groups:
   a. average litter weight for control litters was 37.7 g, with a standard deviation of 11.0;
   b. average litter weight for dams dosed with 200 mg/kg methoxychlor on day 13 of gestation was 38.6 g, with a standard deviation of 11.4;
   c. average litter weight for dams dosed with 400 mg/kg methoxychlor on day 14 of gestation was 36.6 g, with a standard deviation of 13.2.
2. The average number of pups per litter showed a slight increase with dosage strength:

a. average number of pups per litter for control dams was 10.1, with a standard deviation of 1.6;

b. average number of pups per litter for dams dosed with 200 mg/kg methoxychlor on day 13 of gestation was 10.7, with a standard deviation of 3.2;

c. average number of pups per litter for dams dosed with 400 mg/kg methoxychlor on day 14 of gestation was 11.1, with a standard deviation of 3.2.

3. The average weight per pup showed a slight decrease in the fetuses from dams dosed with 400 mg/kg methoxychlor on day 14 of gestation:

a. the average weight per pup from control dams was 3.6 g, with a standard deviation of 0.7;

b. the average weight per pup for fetuses of dams dosed with 200 mg/kg methoxychlor on day 13 of gestation was 3.6 g, with a standard deviation of 0.3;

c. the average weight per pup for fetuses of dams dosed with 400 mg/kg methoxychlor on day 14 of gestation was 3.3 g, with a standard deviation of 0.5.

4. There were more resorption sites in the uteri of the dams dosed with methoxychlor than in the uteri of the control dams:

a. there were no resorption sites in any of the uteri of control dams;

b. there were a total of four resorption sites in the uteri of three of the dams dosed with 200 mg/kg methoxychlor on day 13 of gestation;

c. there were a total of five resorption sites in the uteri of two of the dams dosed with 400 mg/kg methoxychlor on day 14 of gestation.

5. There were no gross deformities in any of the fetuses from any of the dams of any of the three dosing groups.

Teratogenicity and Methoxychlor

1. Previous experiments demonstrate that methoxychlor is not dangerous to vertebrates and the environment when properly used as a pesticide.
2. This experiment supports the contention that methoxychlor is not dangerous to vertebrates and the environment when properly used as a pesticide.
3. The final judgment on the teratogenic effects of methoxychlor on the fetal reproductive system should not be made until more comprehensive teratological experiments are performed.

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APPENDIX A: DOSAGE-CONCENTRATION CONVERSION

From % in feed to mg/kg/day:

$$\frac{\%\ \text{methoxychlor in feed}}{100} \quad \frac{(\text{g food eaten/day})}{(\text{kg body weight})} \quad \frac{(1000\ \text{mg})}{1\ \text{g}} = \text{mg/kg/day}$$

From mg/kg/day to % in feed:

$$100 \quad \frac{(\text{mg/kg/day})\ (1\ \text{g})}{(1000\ \text{mg})} \quad \frac{(\text{kg body weight})}{(\text{g food eaten/day})} = \%\ \text{methoxychlor in feed}$$

From ppm in feed to % in feed:

1 ppm = 0.0001%

Information concerning rats' body weight and grams of food eaten per day will be found in Appendix B. The intake of food for female rats during gestation used in Tables 1 and 2 has been calculated by comparing the average daily feed for female rats in gestation (19 g) to the listed weights at 44, 96, and 350 days. The weights corresponding to these days bracket the starting weights of the females used in this study.

APPENDIX B: WEIGHT GAINS AND FEED INTAKE BY AGE IN LABORATORY RATS[1]

	Growing rats					Adult rats	
						Maintenance	Gestation
Male rats' age (days)	23	33	42	53	108	350	
Body weight (g)	55	110	165	220	385	550	
Average daily feed (g)	9	15	18	21	20	19	
Female rats' age (days)	19	26	35	44	96	350	
Body weight (g)	32	65	98	130	228	325	
Average daily feed (g)	-	10	14	15	16	13	19

[1]Adapted from National Academy of Sciences, 1972.

APPENDIX C: DAM AND LITTER RECORD: CONTROLS

Gestation day 1: 12-31-76

	Female rat number							
	1C	2C	3C	4C	5C	6C	7C	8C
Dam weights (g) during gestation								
Day 13	216	249	201	256	241	225	248	241
Day 14								
B.C.[1]	301	335	211	340		296	336	336
A.C.[2]	238	281	NP[3]	292	274	251	283	278
Litter weight (g)	43	44	-	37	15	34	47	44
Pups per litter	11	11	-	10	7	10	12	10
Number of resorption sites	0	0	0	0	0	0	0	0
Number of corpora lutea	14	13	-	11	13	23	17	11
Litter ave. (g/pup)	3.9	4.0	-	3.7	2.14	3.4	3.9	4.4

[1]B.C.: before Cesarean, day 21.

[2]A.C.: after Cesarean, day 21.

[3]NP: not pregnant.

APPENDIX D: DAM AND LITTER RECORD: 200 mg/kg METHOXYCHLOR GROUP

Gestation day 1: 12-31-76

	Female rat number									
	1	2	3	4	5	6	7	8	9	10
Dam weights (g) during gestation										
Day 13	267	278	254	237	256	245	245	286	248	263
Day 14										
B.C.[1]	362	340	356	314	340	344	328	406	343	366
A.C.[2]	305	317	287	282	293	289	280	340	308	318
Litter weight (g)	43	20	48	23	35	48	44	55	30	40
Pups per litter	13	5	12	7	10	12	12	16	8	12
Number of resorption sites	0	0	0	1	0	2	0	0	1	0
Number of corpora lutea	14	18	22	19	22	14	19	24	13	18
Litter ave. (g/pup)	3.3	4.0	4.0	3.28	3.5	4.0	3.67	3.44	3.75	3.33

[1] B.C.: before Cesarean, day 21.

[2] A.C.: after Cesarean, day 21.

APPENDIX E: DAM AND LITTER RECORD: 400 mg/kg METHOXYCHLOR GROUP

Gestation day 1: 12-31-76

	Female rat number									
	11	12	13	14	15	16	17	18	19	20
Dam weights (g) during gestation										
Day 13										
Day 14	247	258	214	262	286	273	250	241	270	244
B.C.[1]	310	318	213	345	325	351	290	372	352	328
A.C.[2]	274	277	NP[3]	286	268	303	270	302	290	287
Litter weight (g)	24	33	-	45	45	36	14	55	49	28
Pups per litter	9	10	-	13	12	11	4	15	13	13
Number of resorption sites	0	0	0	1	0	0	4	0	0	0
Number of corpora lutea lutea	11	13	-	14	18	20	15	16	15	13
Litter ave. (g/pup)	2.67	3.3	-	3.46	3.75	3.27	3.5	3.67	3.77	2.15

[1]B.C.: before Cesarean, day 21.

[2]A.C.: after Cesarean, day 21.

[3]NP: not pregnant.

APPENDIX F: SECTIONING RECORD

Key: Dam number / Fetus sex[1] / Intralitter number

1C M A

Dam number	Male fetuses sectioned	Female fetuses sectioned
1C	1CMA, 1CMB	1CFA, 1CFB, 1CFC
2C	2CMA, 2CMB	2CFA, 2CFB
4C	4CMA	4CFA
5C	5CMA	5CFA
6C	6CMA, 6CMC	6CFA
7C	7CMA	7CFA, 7CFB, 7CFC
8C	8CMA	8CFA, 8CFB
1	1MA, 1MC	1FA
2		2FA, 2FB
3	3MA, 3MB, 3MC	3FA
4		4FA
5	5MA, 5MB, 5MC	
6	6MA, 6MB, 6MC, 6MD	
7	7MA, 7MB	7FA, 7FB
8	8MA, 8MB, 8MC	8FA, 8FB
9	9MA	9FA, 9FB
10	10MA, 10MB	10FA
11	11MA	11FA, 11FB
12	12MA	12FA, 12FB
14	14MA, 14MB, 14MC	14FA
15		15FA, 15FB, 15FC, 15FD, 15FE
16	16MA, 16MB	16FA, 16FC
17	17MA	
18	18MB, 18MC, 18MD	18FF
19	19MA, 19MB	19FA, 19FB
20	20MA	20FA, 20FB, 20FC

[1]Sex: M = male, F = female.

APPENDIX G: CHEMICAL SOLUTIONS

Acid alcohol (Luna, 1968)

70% ethyl alcohol	1000 ml
Hydrochloric acid, concentrated	10 ml

10% buffered neutral formalin (Lynch et al., 1969)

Distilled water	900 ml
Sodium phosphate monobasic	
(NaH_2PO_4), anhydrous	3.5 g
Sodium phosphate dibasic	
(Na_2HPO_4), anhydrous	6.5 g
Commercial (37-40%) formaldehyde	100 ml

Eosin (Luna, 1968)

Stock eosin	
Eosin V	1 g
Distilled water	100 ml
Stock phloxine	
Phloxine B	1 g
Distilled water	100 ml
Working eosin	
Stock eosin	50 ml
Stock phloxine	5 ml
95% ethyl alcohol	390 ml
Glacial acetic acid	.5 ml

Harris' hematoxylin (Luna, 1968)

Hematoxylin crystals	5 g
95% ethyl alcohol	50 ml
Aluminum ammonium sulfate or aluminum potassium sulfate	100 g
Distilled water	1000 ml
Mercuric oxide	2.5 g

Lithium carbonate (Luna, 1968)

Lithium carbonate	1 g
Distilled water	100 ml

APPENDIX H: PHOTOGRAPHS

Legend for Figures

(Note: hematoxylin and eosin staining used throughout.)

A. Capillary
B. Epithelium, simple cuboidal
C. Epithelium, simple squamous
D. Fibroblast, nucleus of
E. Gonocyte, cytoplasm of
F. Gonocyte, immature
G. Gonocyte, nondegenerating
H. Gonocyte, nucleus of
I. Gonocyte, pyknotic
J. Gonocytes, lobes composed of
K. Hilus
L. Interstitial cell, cytoplasm of
M. Interstitial cell, nucleus of
N. Matrix
O. Mesenchymal cell, nucleus of
P. Mitotic figure
Q. Nucleoli, multiple
R. Nucleolus
S. Pregranulosa cell, nucleus of
T. Pregranulosa cells, cords of
U. Stroma, open
V. Stromal space
W. Supporting cell, nucleus of
X. Testicular cord
Y. Testis, central area of
Z. Testis, periphery of
1. Testis, region of rete of
2. Vacuole

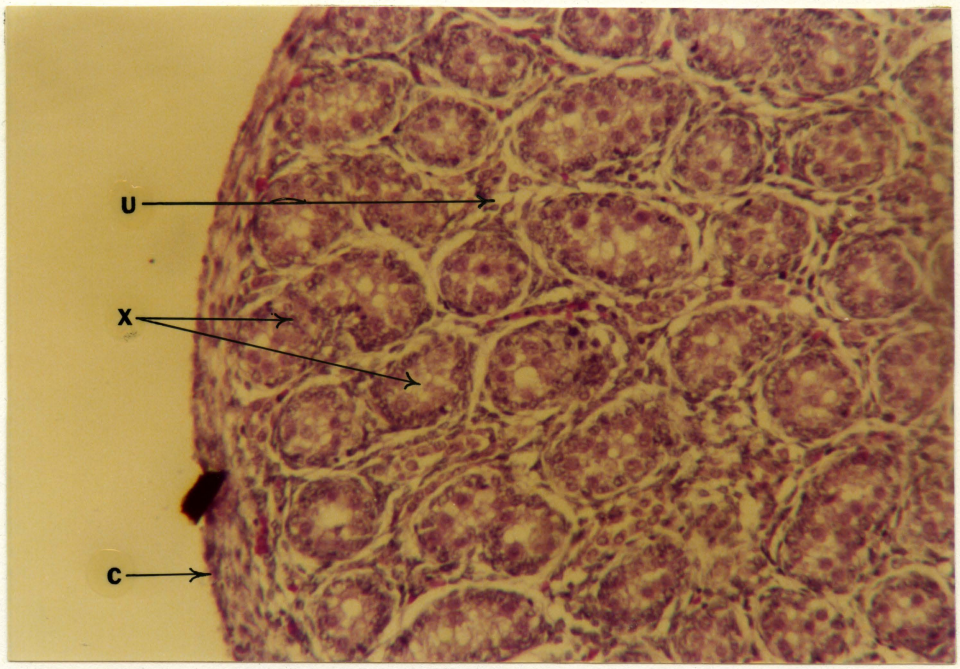

Figure 1. Testis from 1CMA. Magnified 175 times

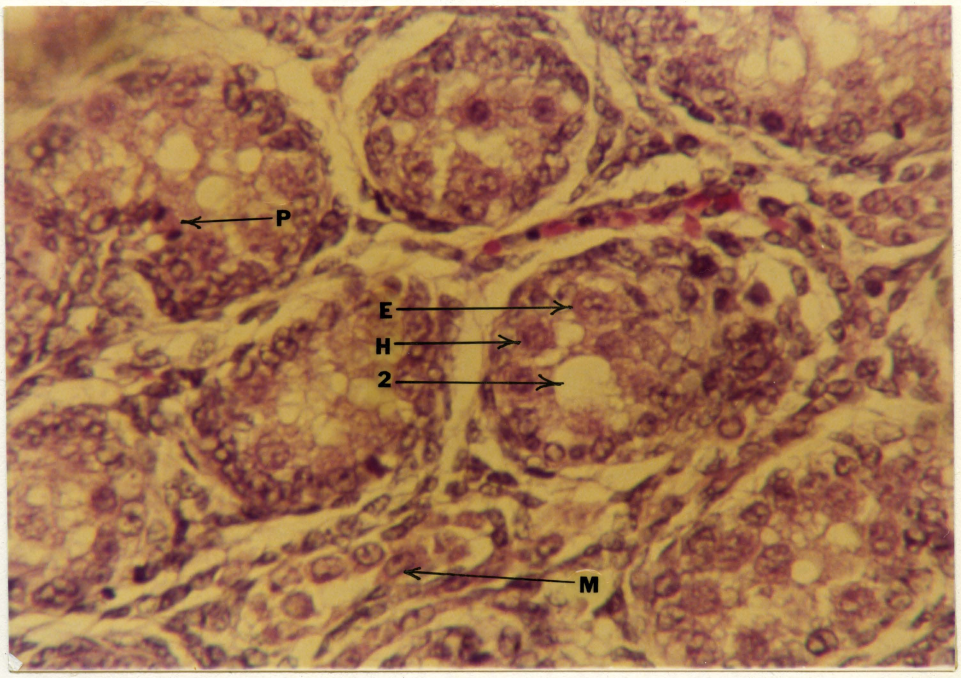

Figure 2. Testis from 1CMA. Magnified 430 times

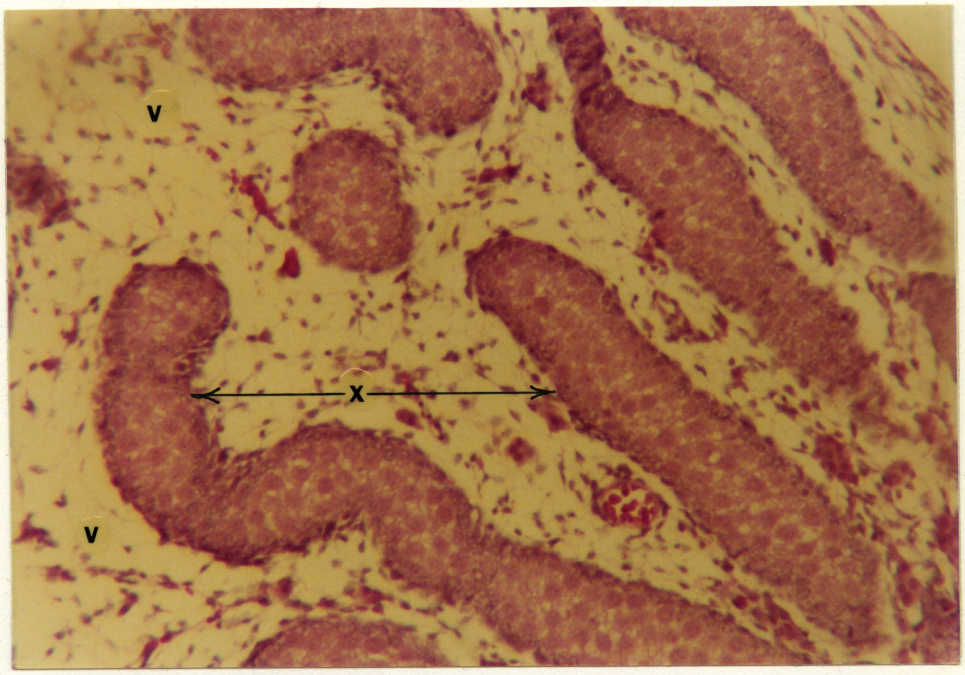

Figure 3. Testis from 2CMA. Magnified 175 times

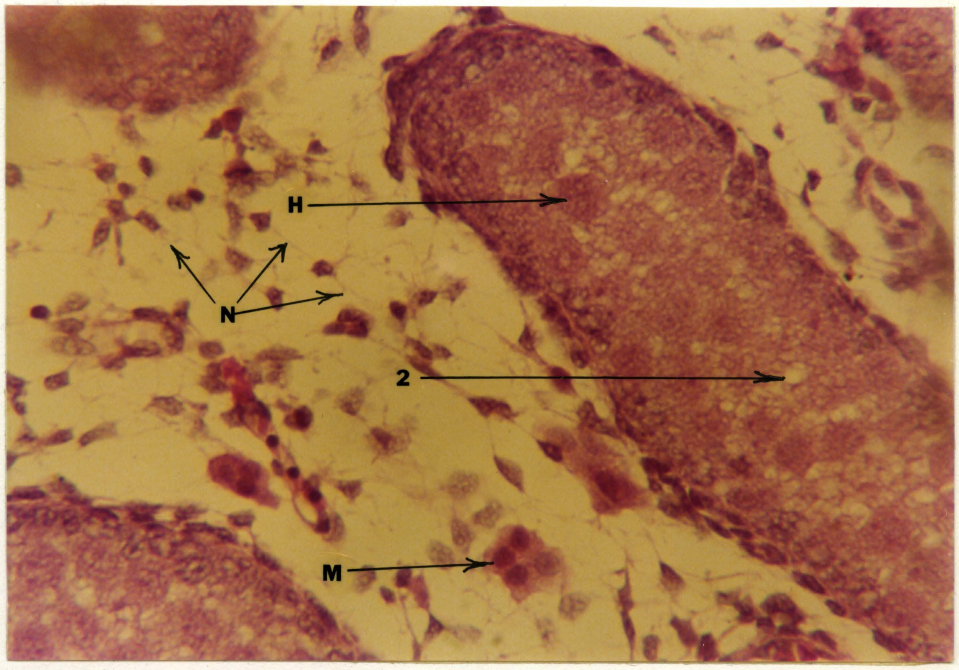

Figure 4. Testis from 2CMA. Magnified 430 times

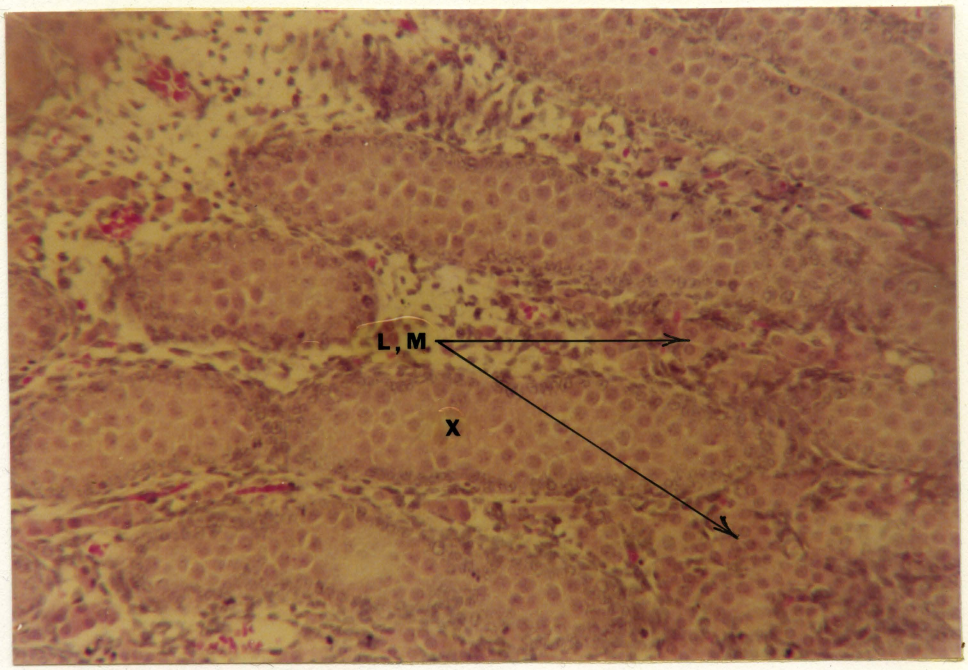

Figure 5. Testis from 5CMA. Magnified 175 times

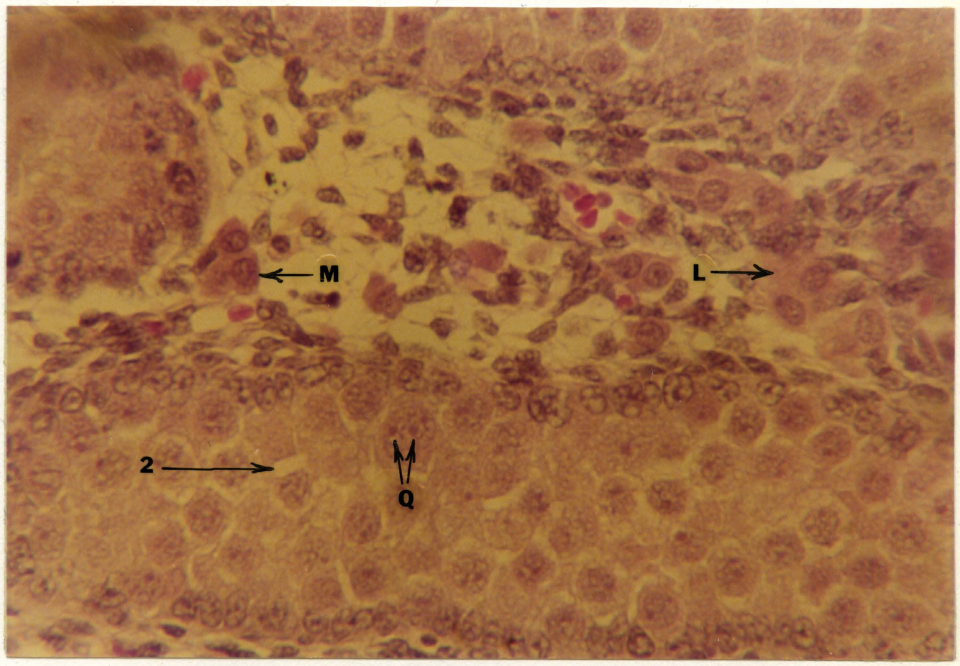

Figure 6. Testis from 5CMA. Magnified 430 times

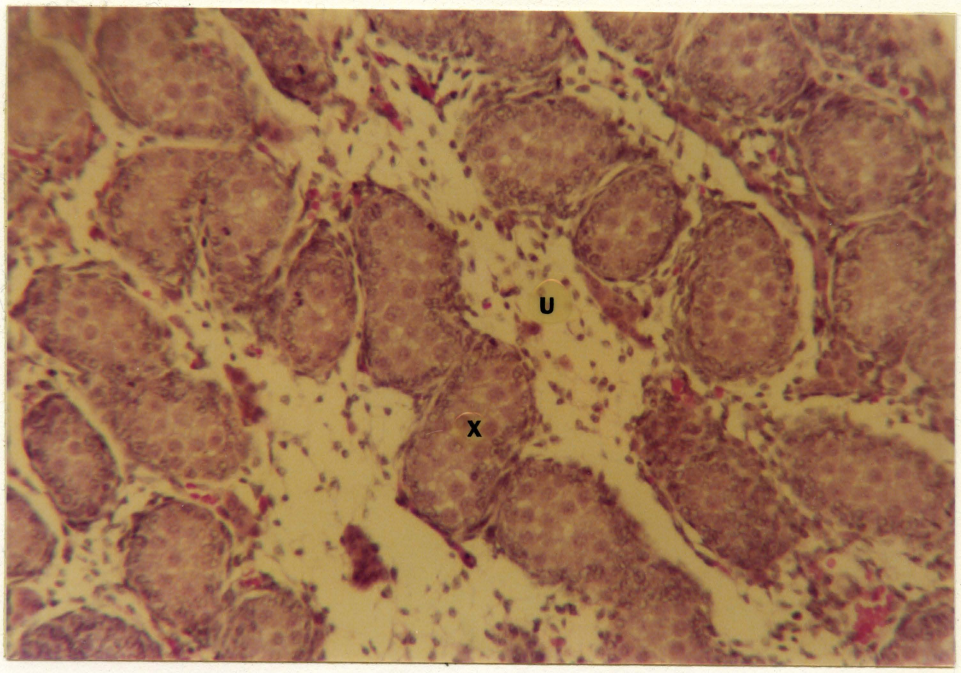

Figure 7. Testis from 6CMA. Magnified 175 times

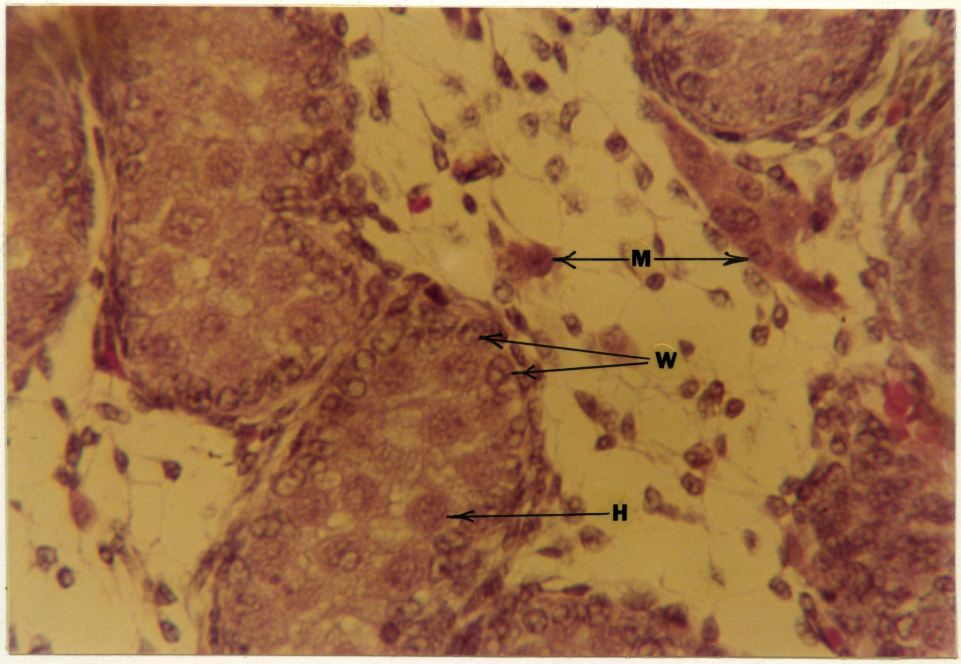

Figure 8. Testis from 6CMA. Magnified 430 times

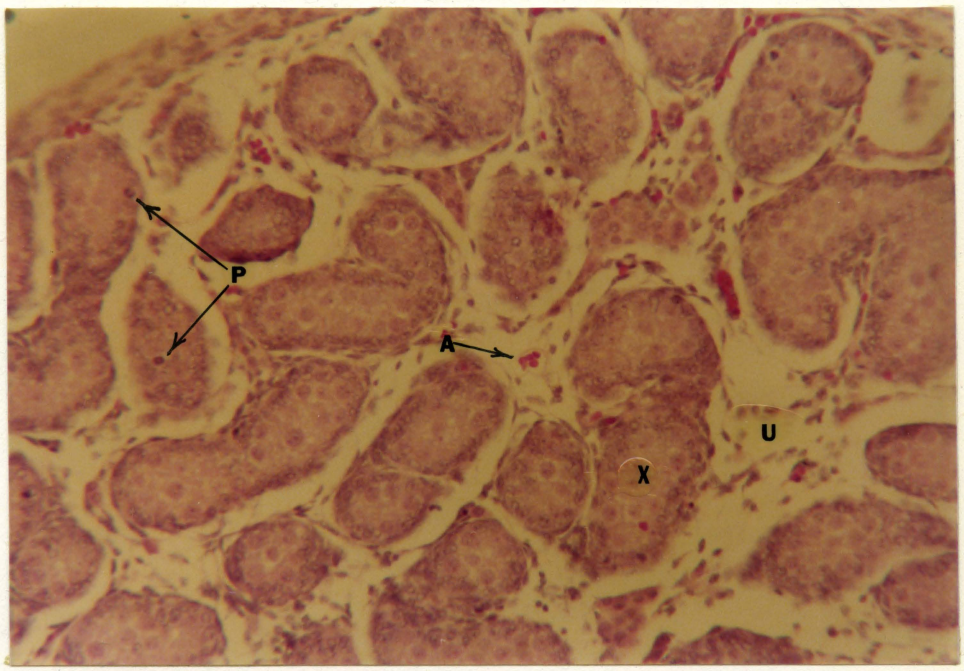

Figure 9. Testis from 7CMA. Magnified 175 times

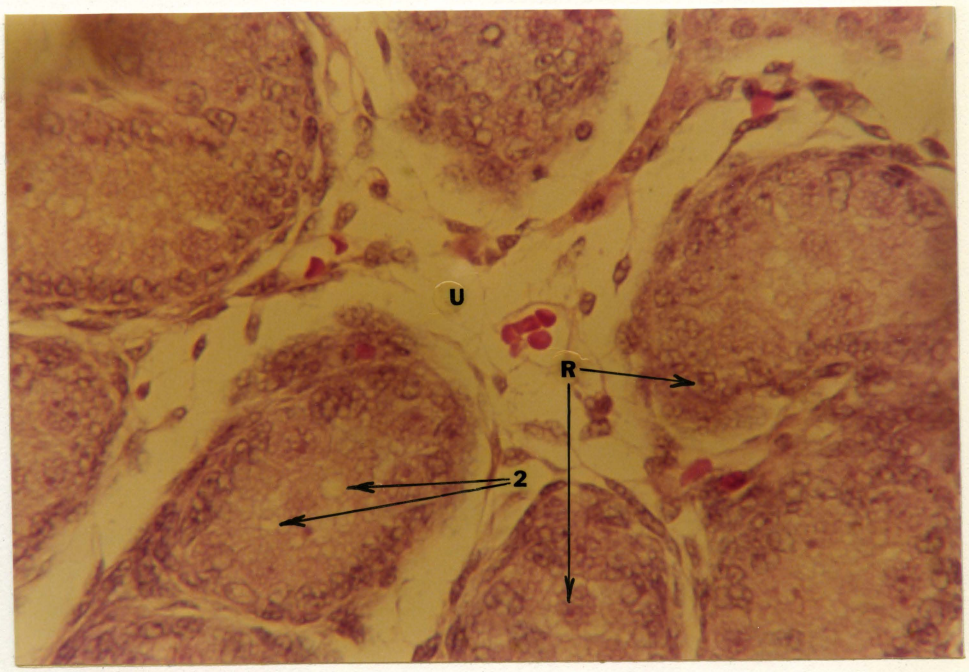

Figure 10. Testis from 7CMA. Magnified 430 times

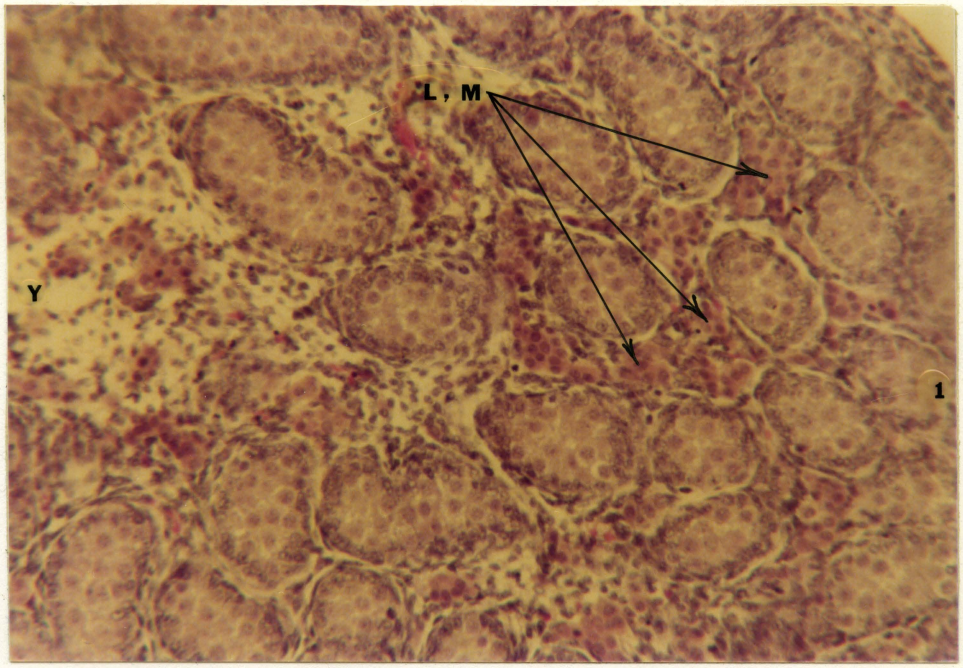

Figure 11. Left testis from 5MC. Magnified 175 times

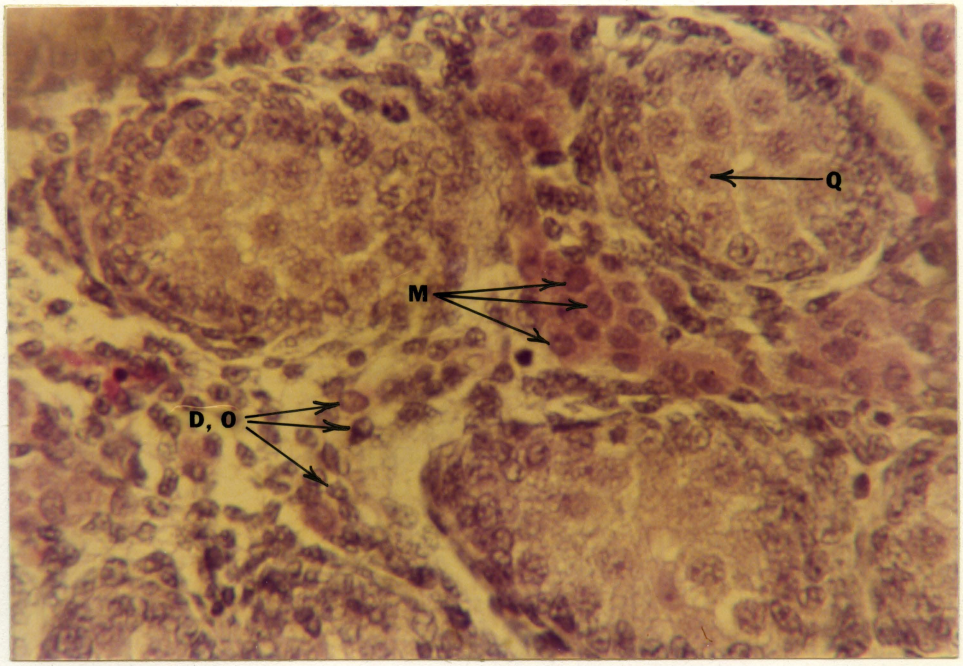

Figure 12. Left testis from 5MC. Magnified 430 times

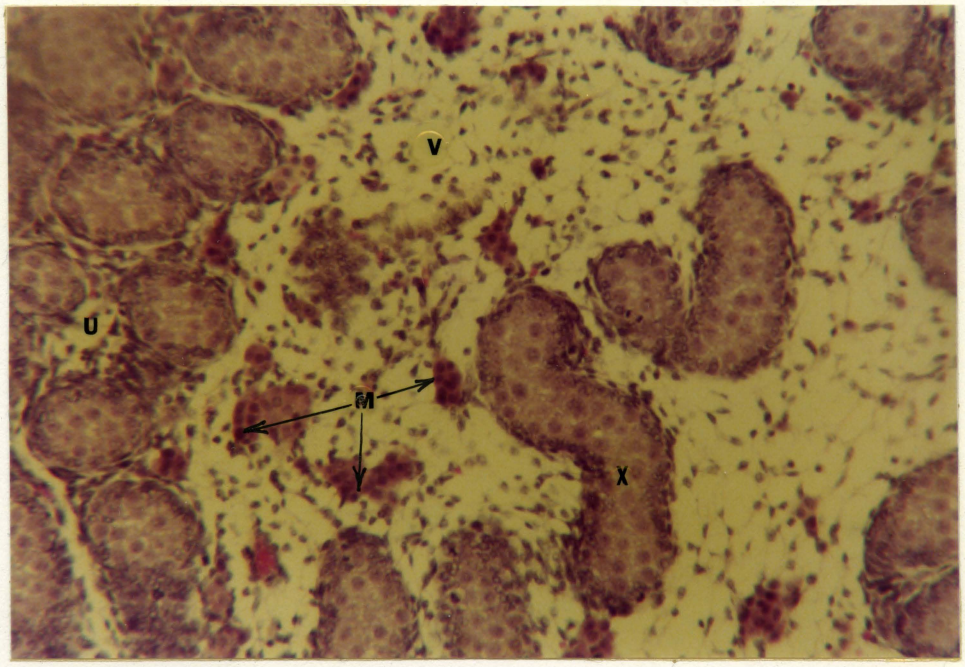

Figure 13. Right testis from 5MC. Magnified 175 times

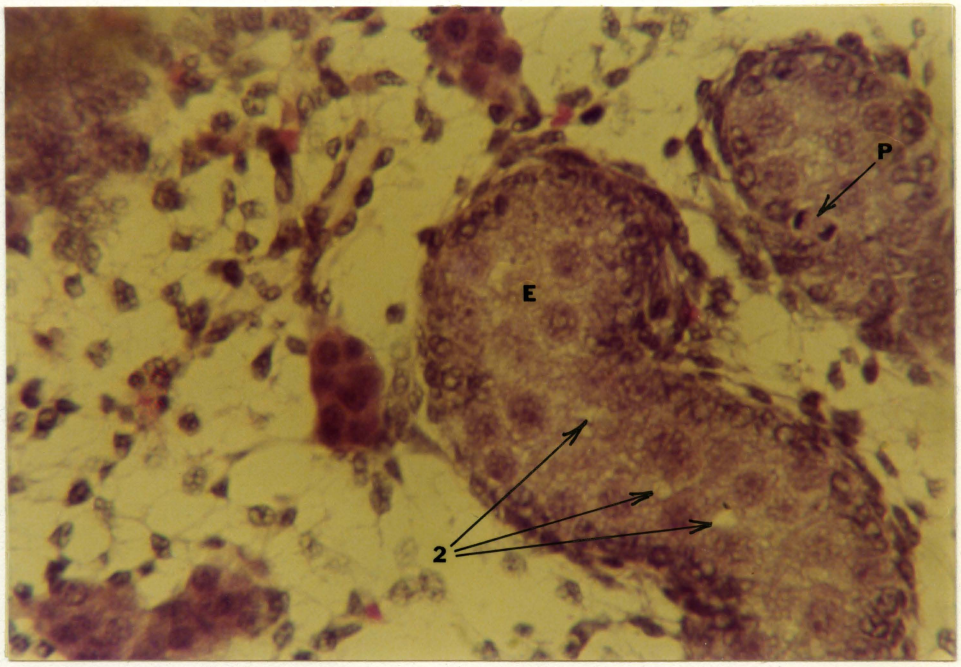

Figure 14. Right testis from 5MC. Magnified 430 times

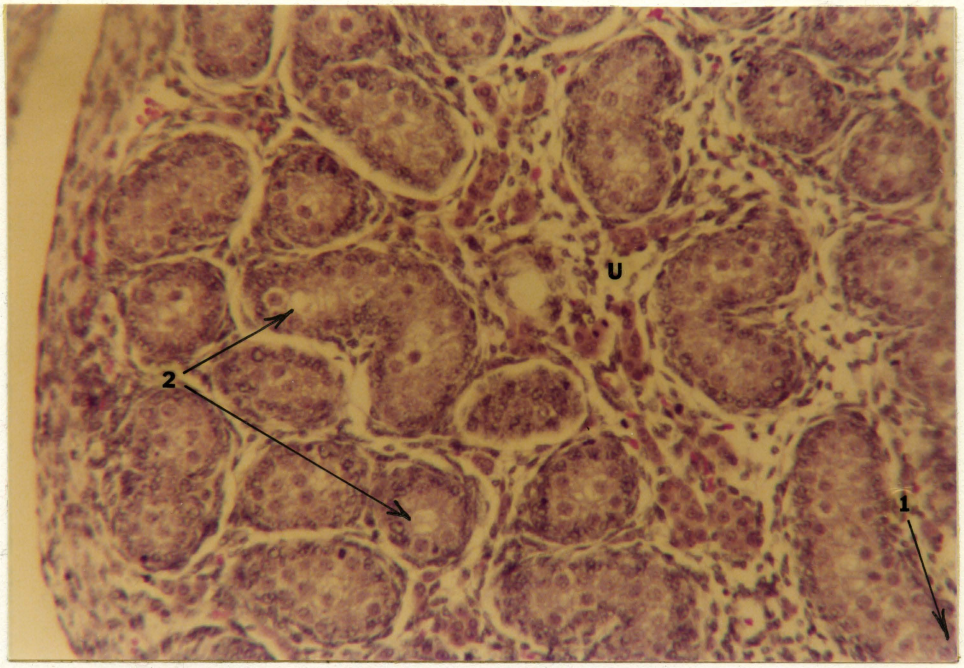

Figure 15. Testis from 6MD. Magnified 175 times

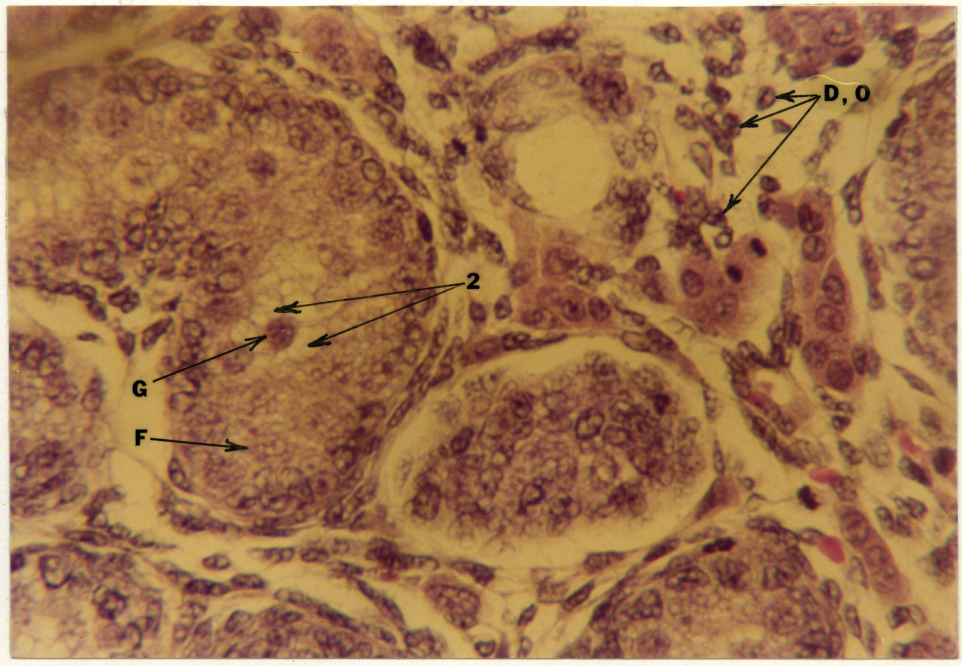

Figure 16. Testis from 6MD. Magnified 430 times

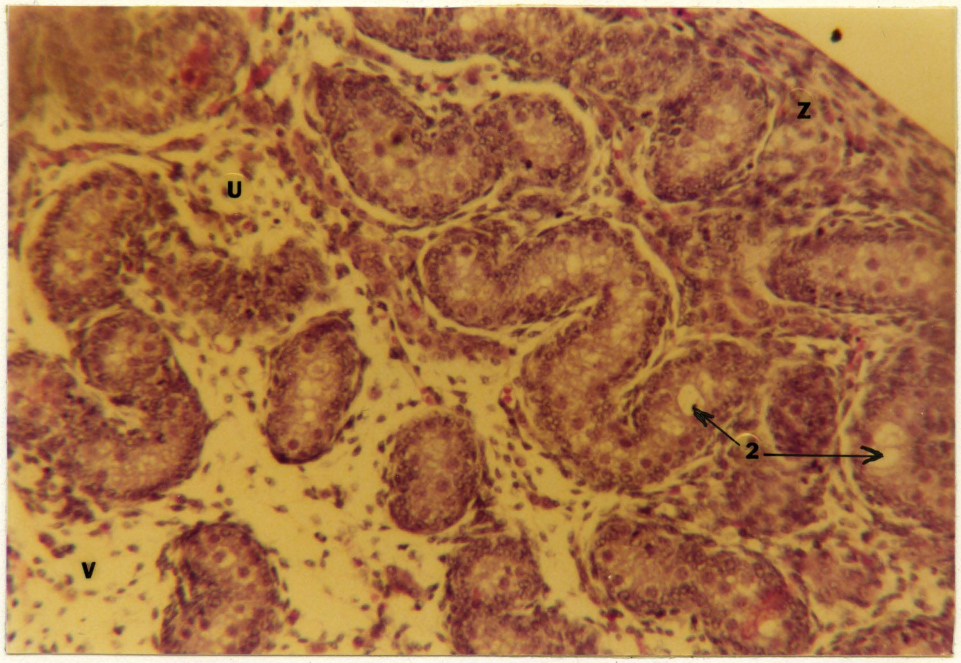

Figure 17. Testis from 7MB. Magnified 175 times

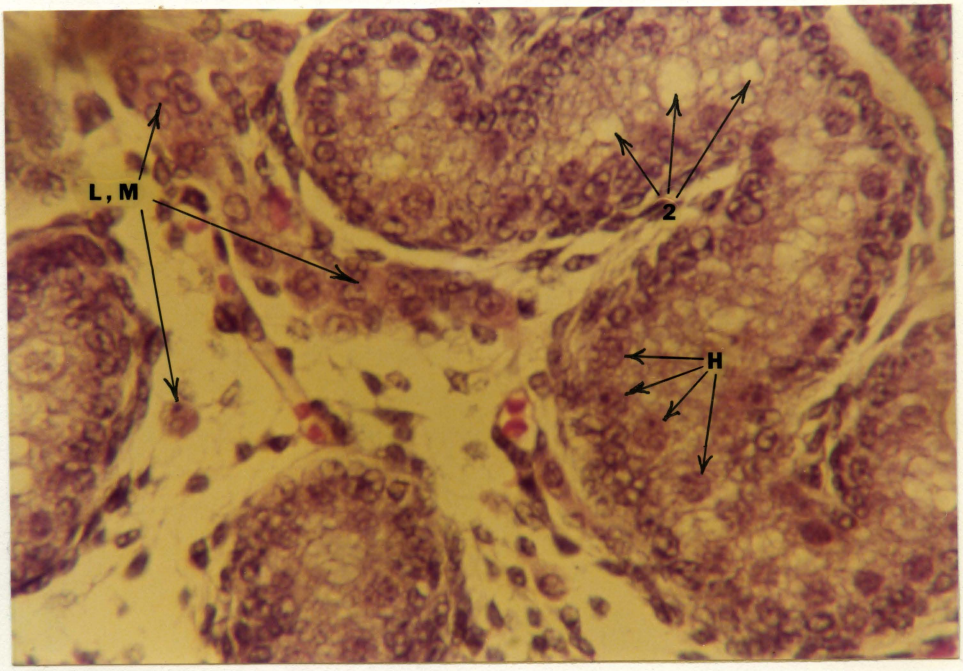

Figure 18. Testis from 7MB. Magnified 430 times

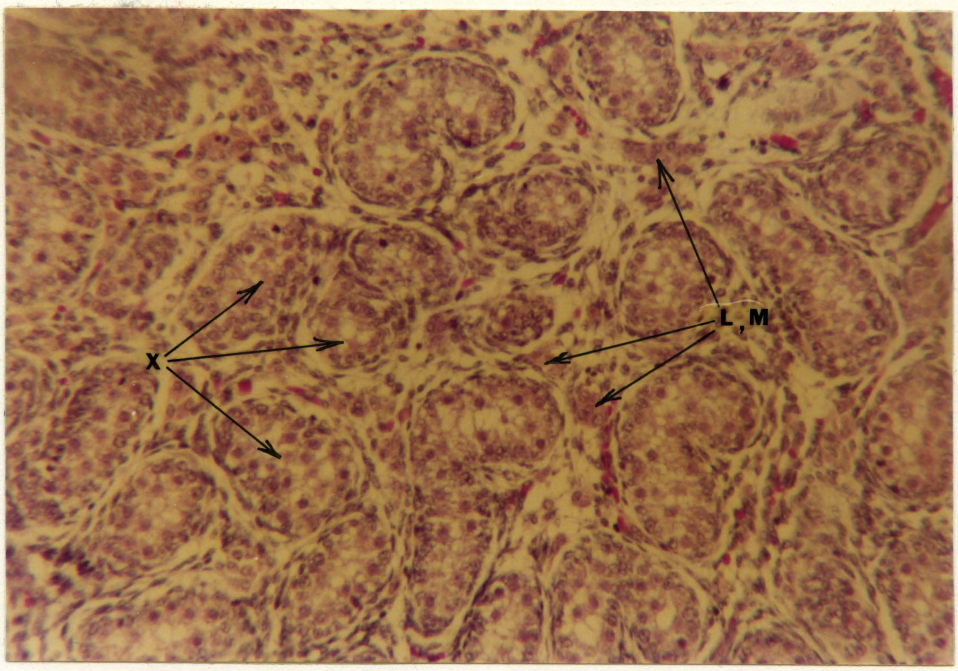

Figure 19. Testis from 8MB. Magnified 175 times

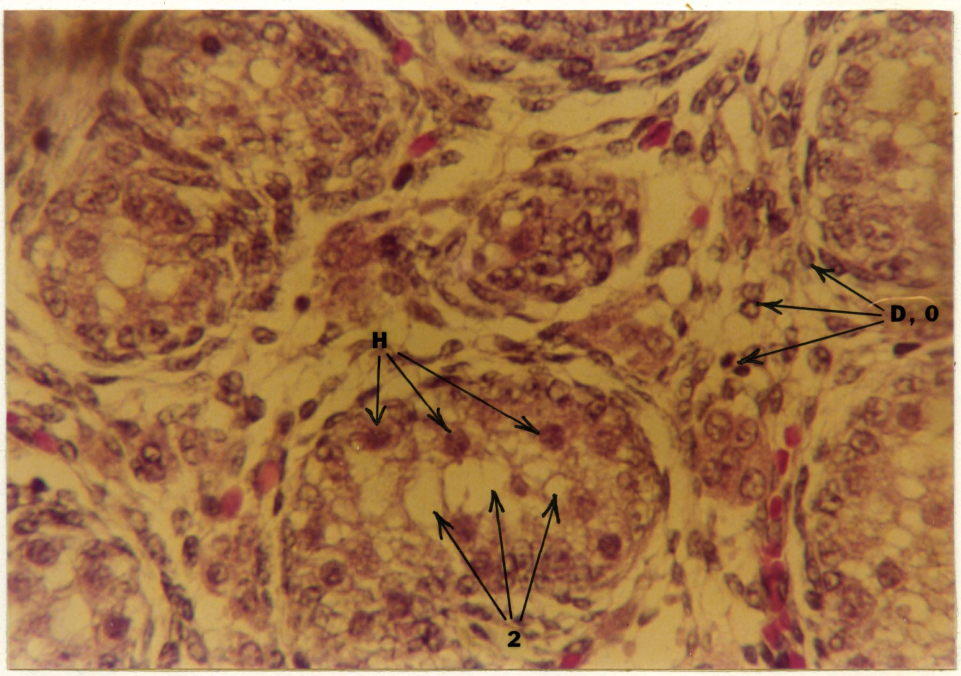

Figure 20. Testis from 8MB. Magnified 430 times

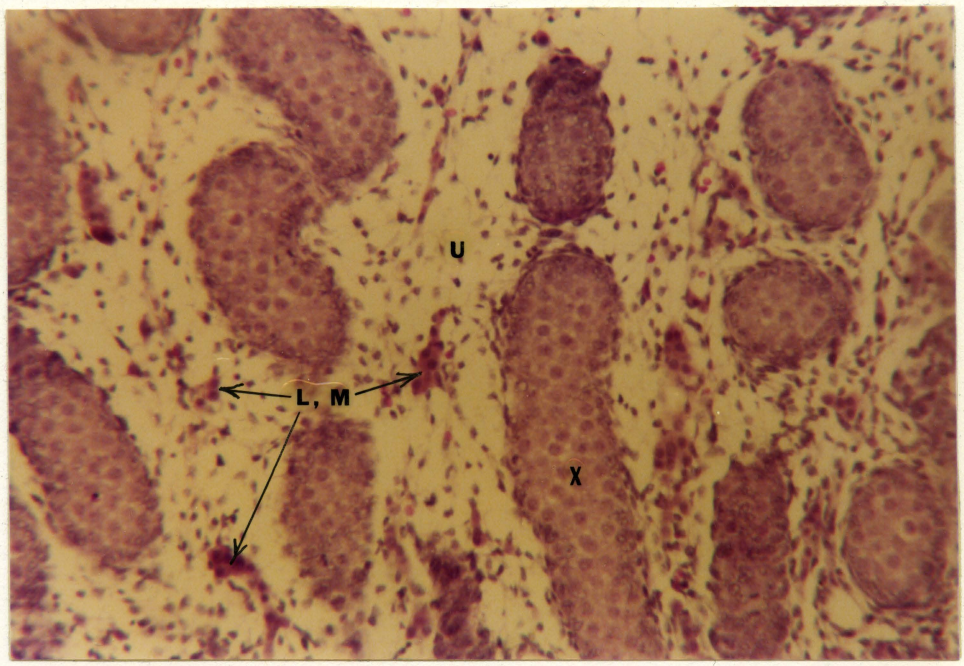

Figure 21. Testis from 11MA. Magnified 175 times

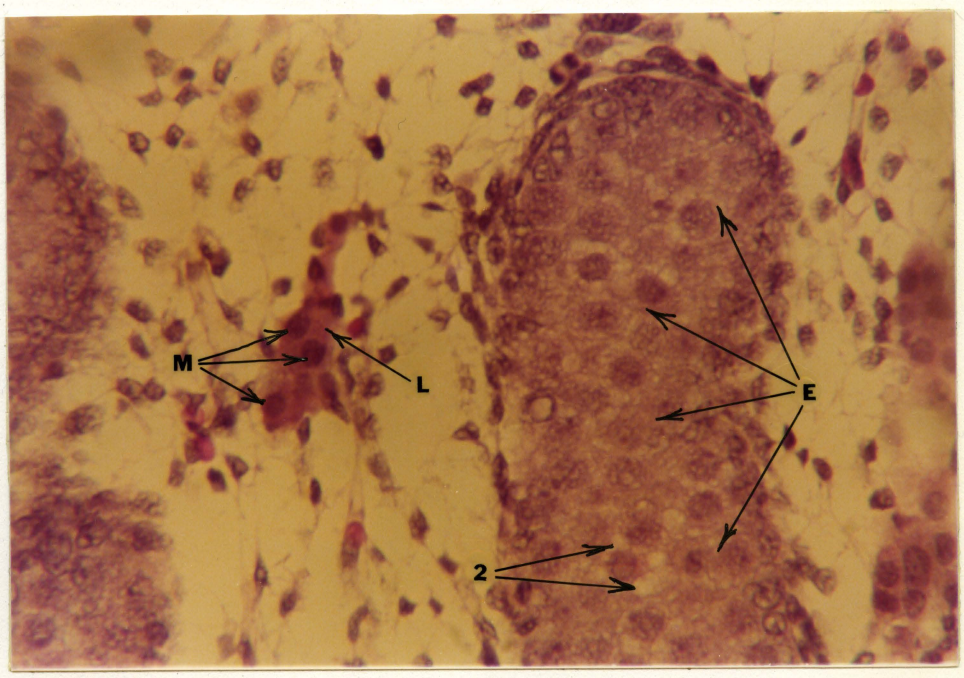

Figure 22. Testis from 11MA. Magnified 430 times

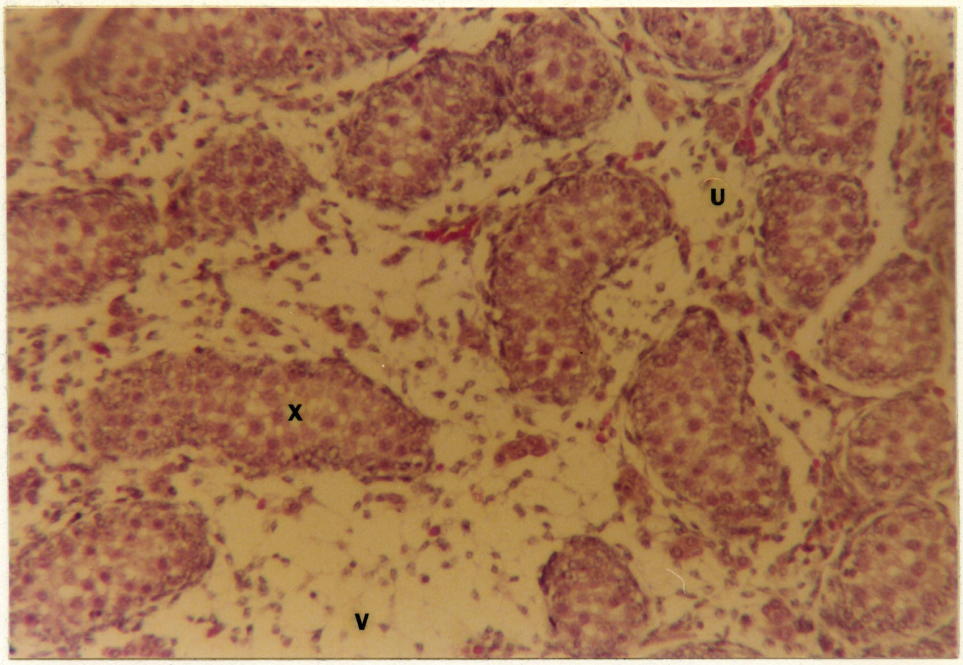

Figure 23. Testis from 12MA. Magnified 175 times

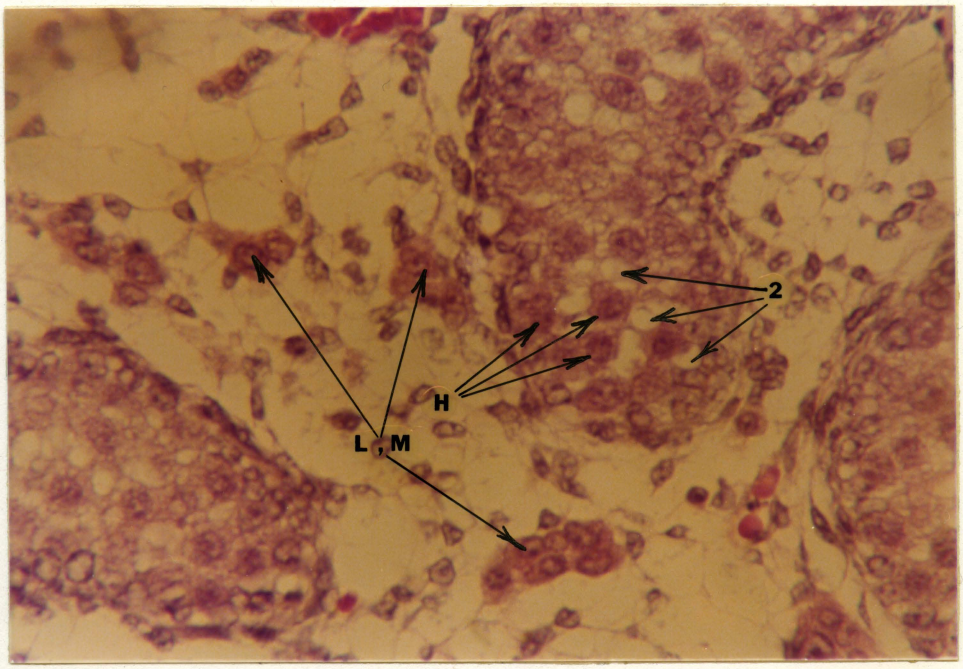

Figure 24. Testis from 12MA. Magnified 430 times

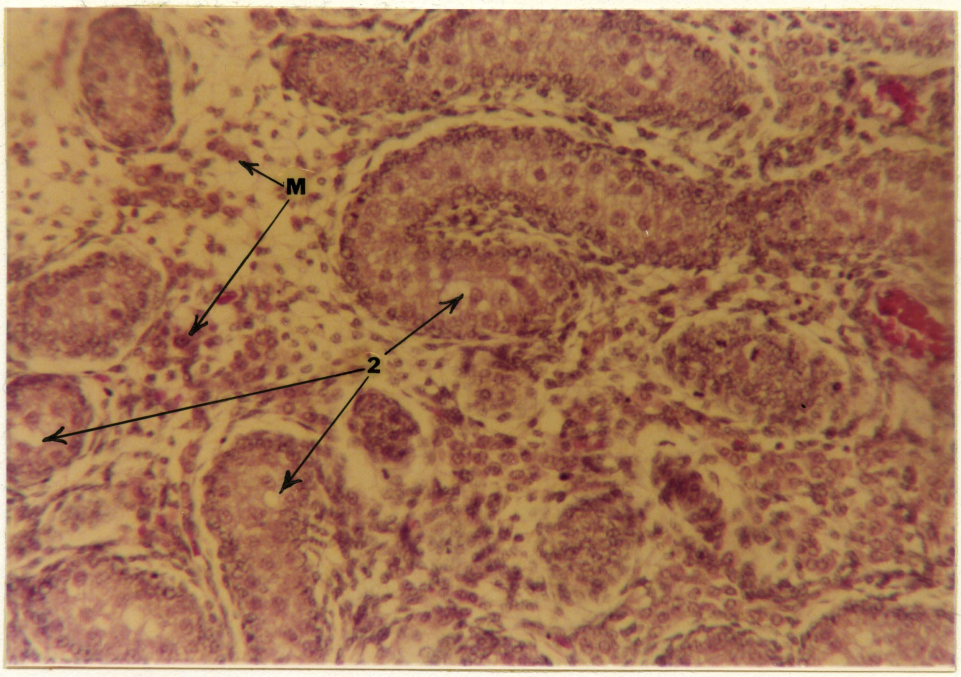

Figure 25. Testis from 14MA. Magnified 175 times

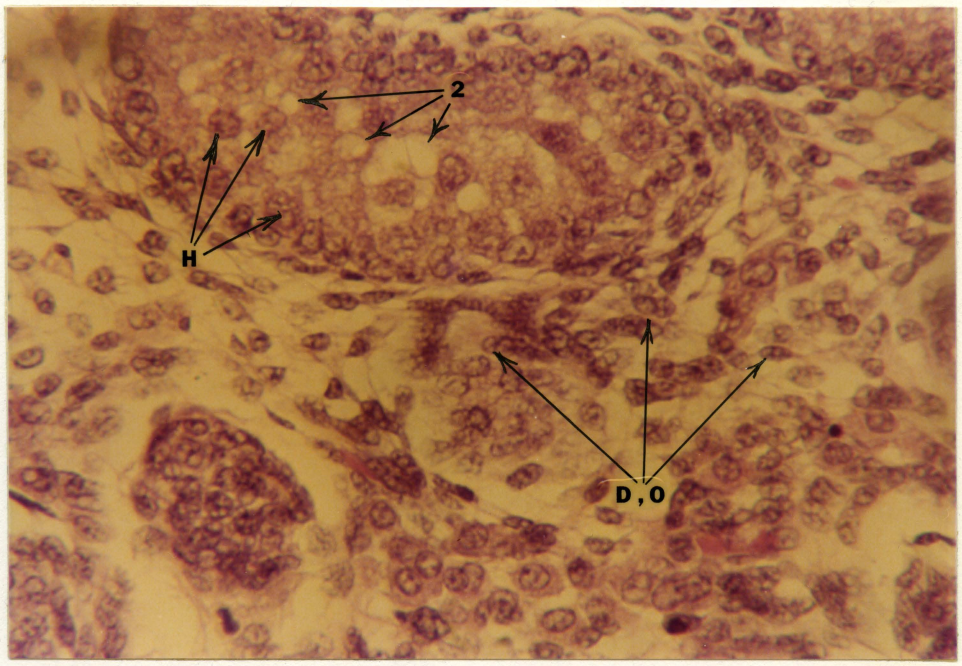

Figure 26. Testis from 14MA. Magnified 430 times

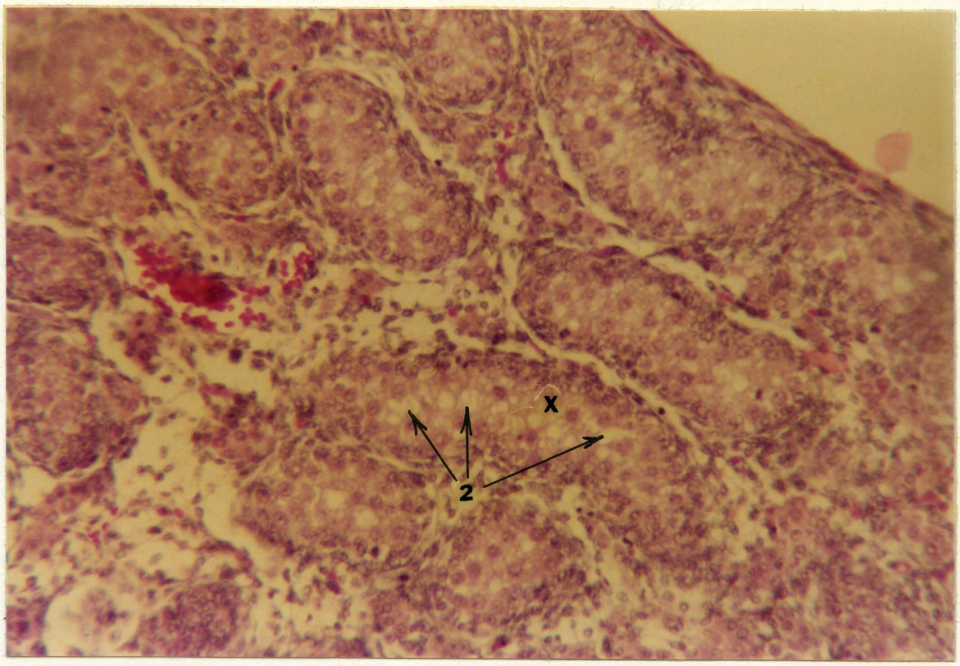

Figure 27. Testis from 18MD. Magnified 175 times

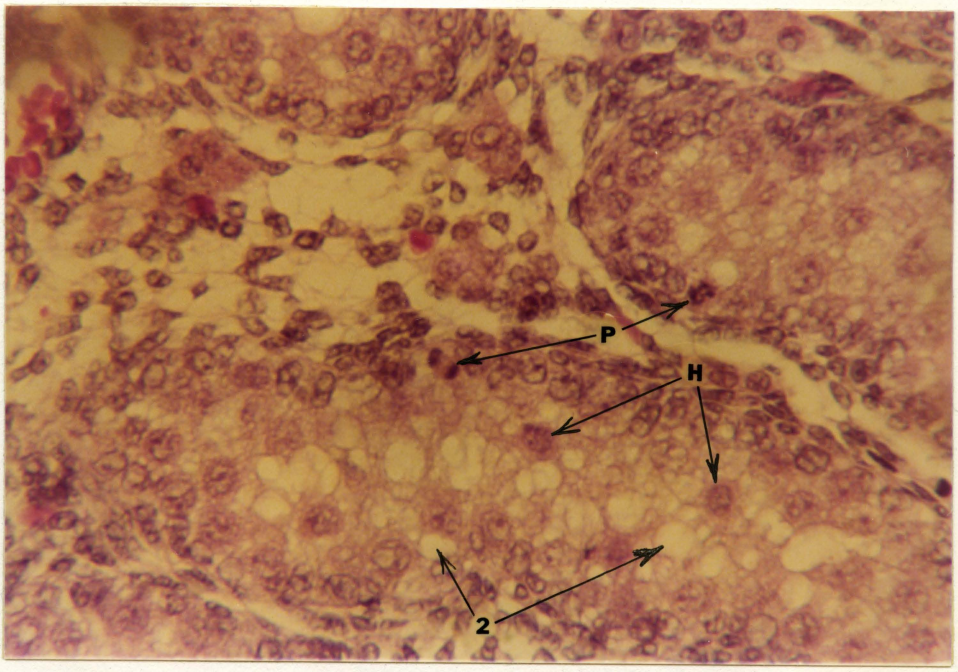

Figure 28. Testis from 18MD. Magnified 430 times

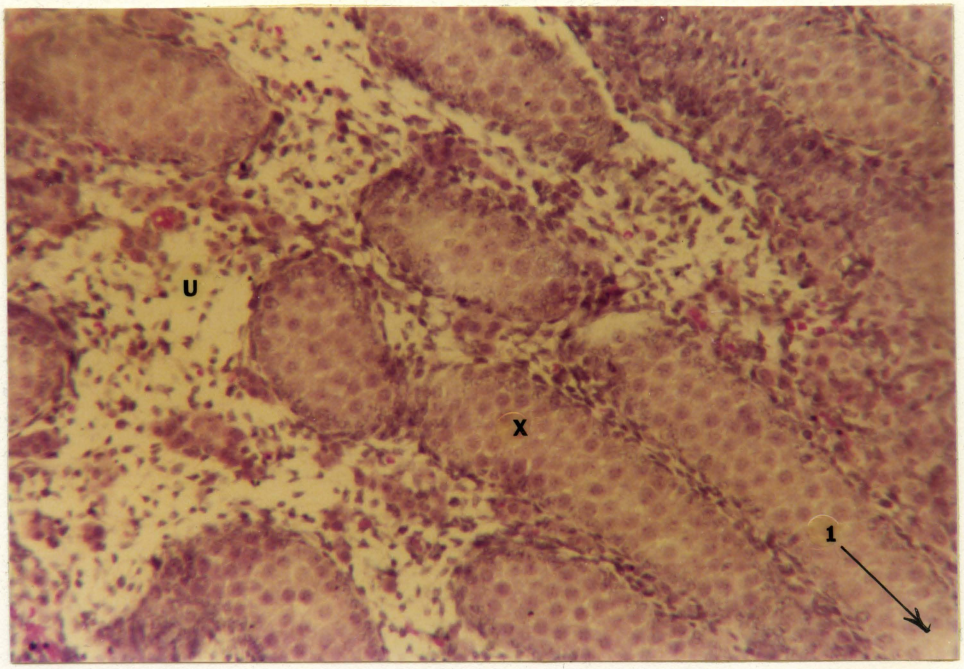

Figure 29. Testis from 20MA. Magnified 175 times

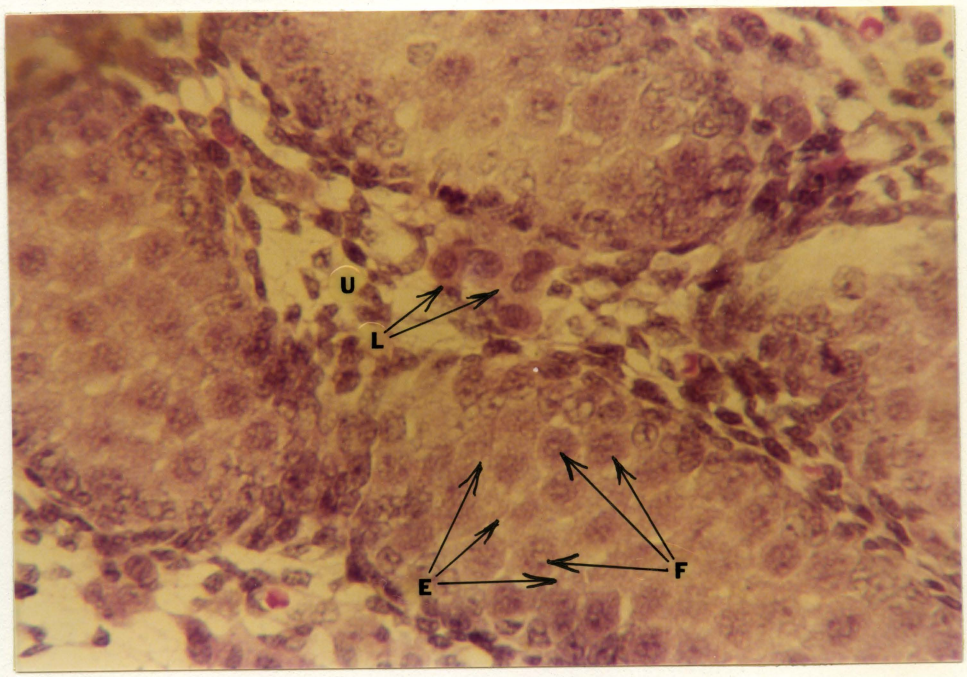

Figure 30. Testis from 20MA. Magnified 430 times

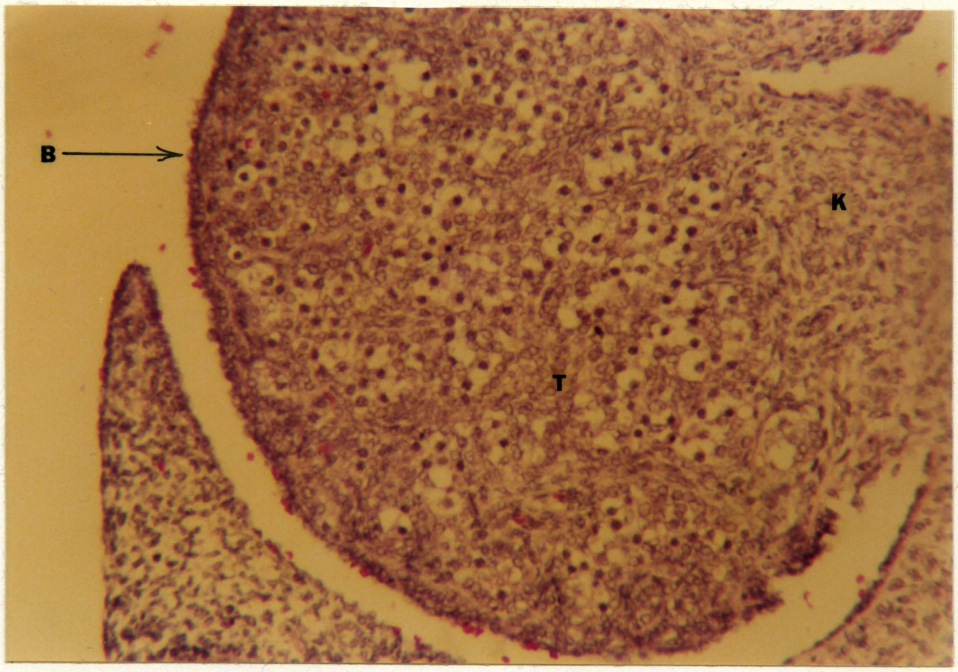

Figure 31. Ovary from 1CFB. Magnified 175 times

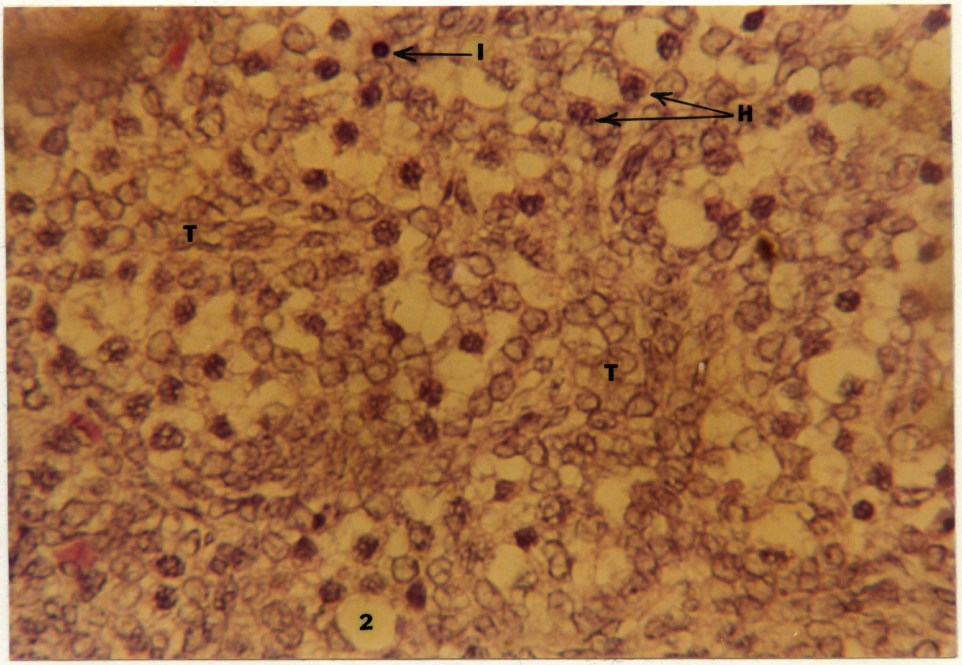

Figure 32. Ovary from 1CFB. Magnified 430 times

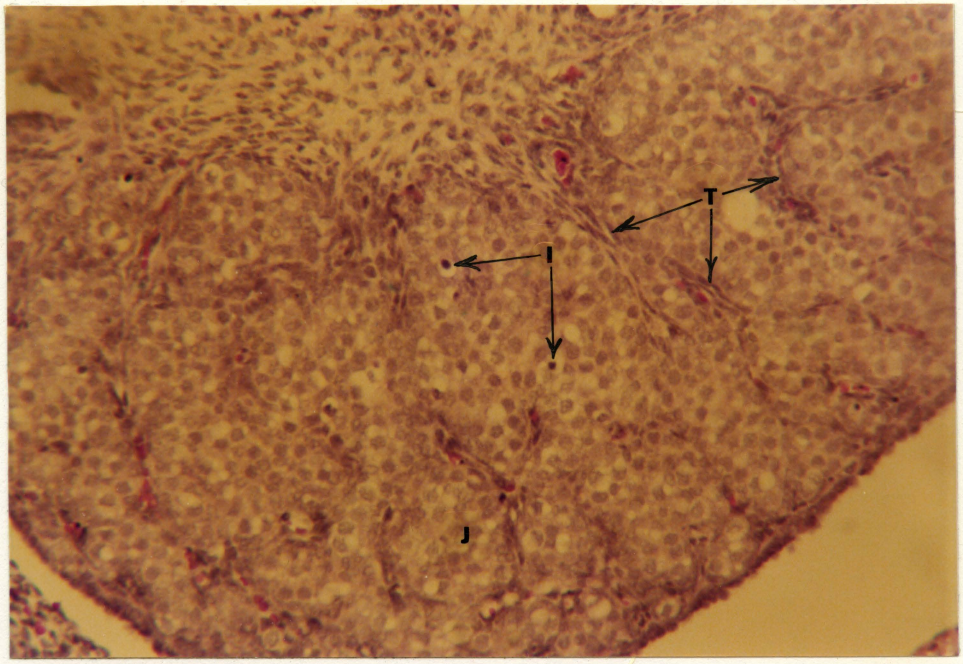

Figure 33. Ovary from 2CFB. Magnified 175 times

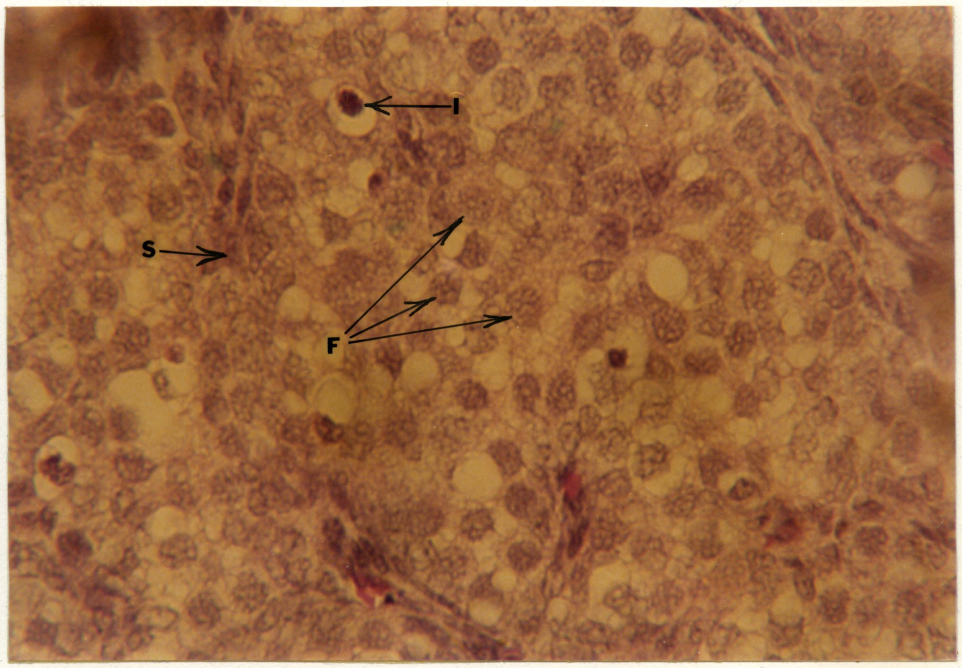

Figure 34. Ovary from 2CFB. Magnified 430 times

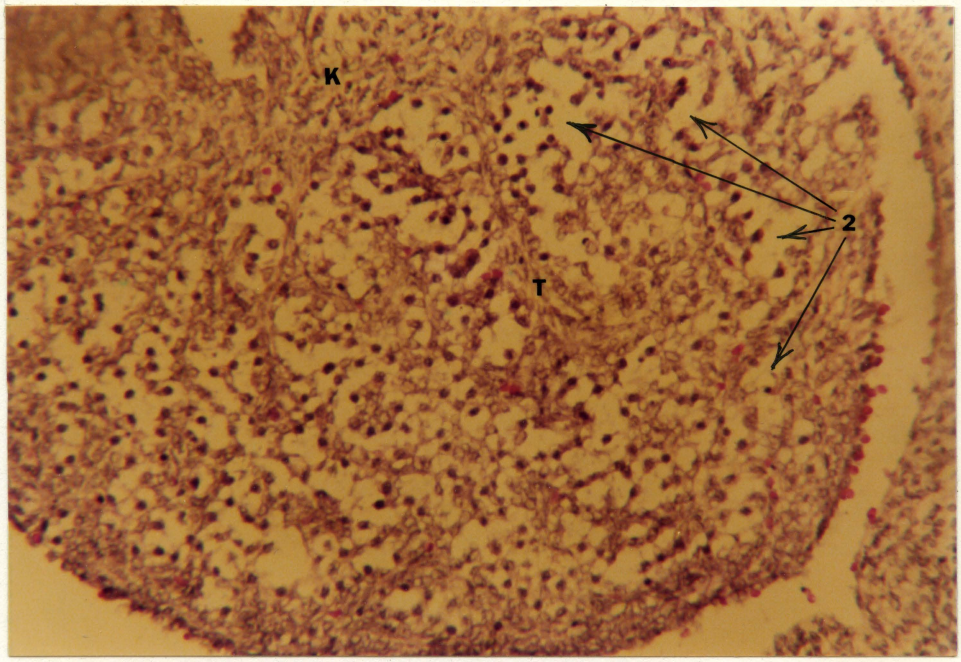

Figure 35. Ovary from 8CFB. Magnified 175 times

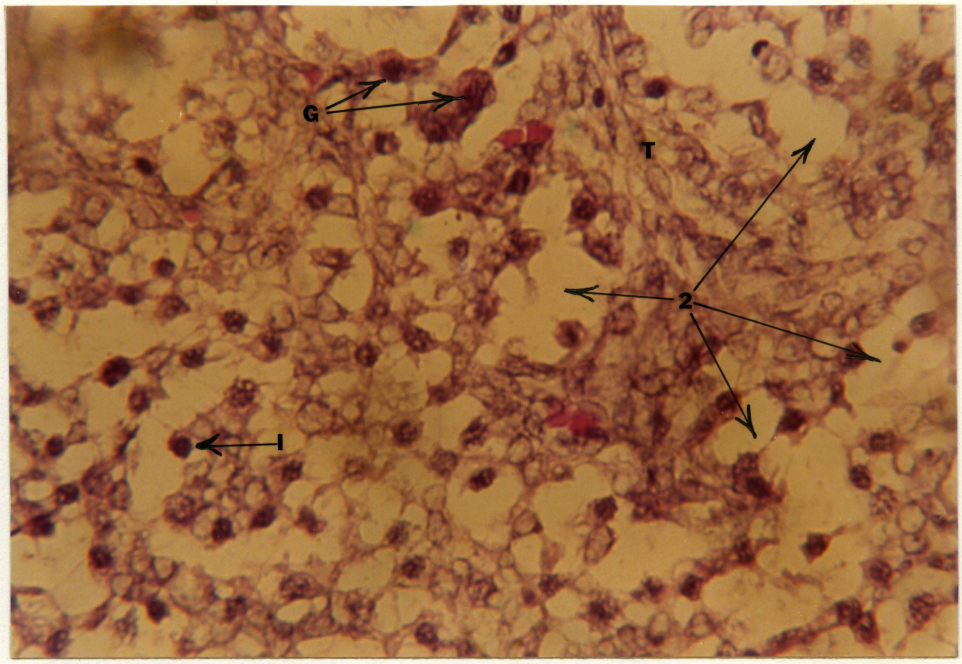

Figure 36. Ovary from 8CFB. Magnified 430 times

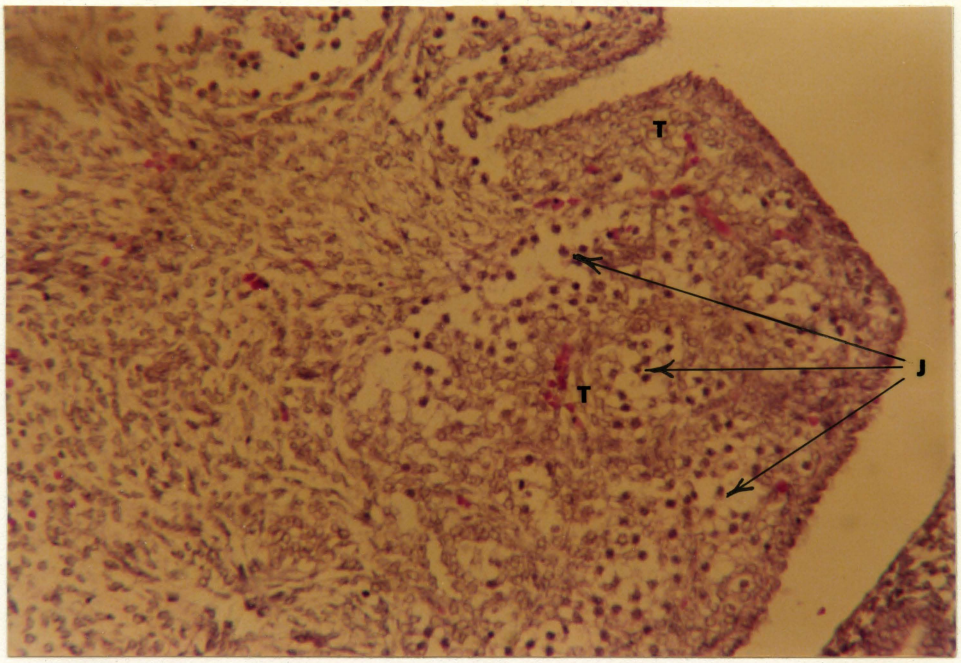

Figure 37. Ovary from 2FB. Magnified 175 times

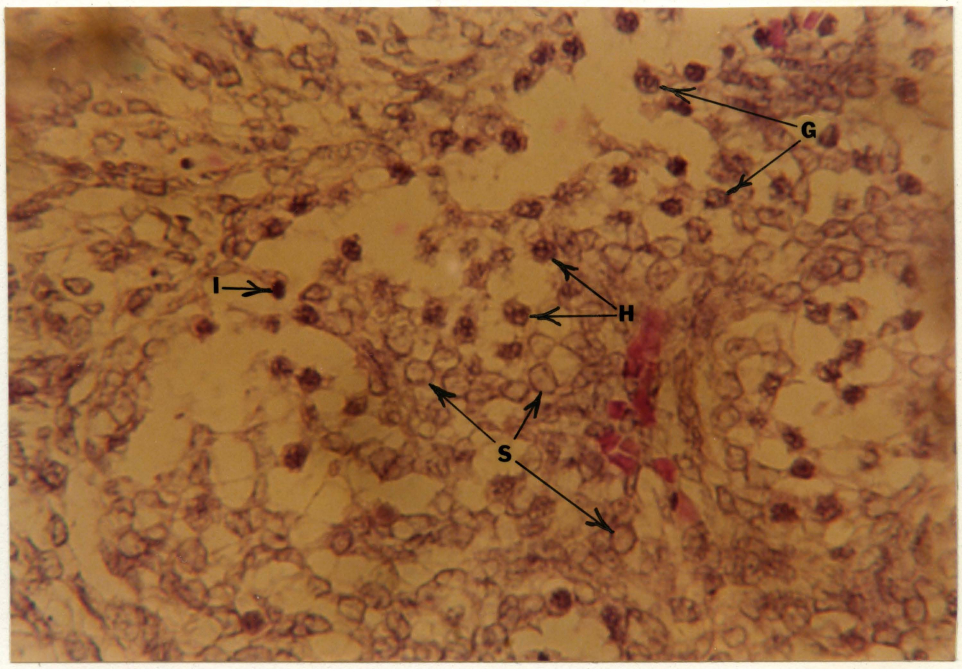

Figure 38. Ovary from 2FB. Magnified 430 times

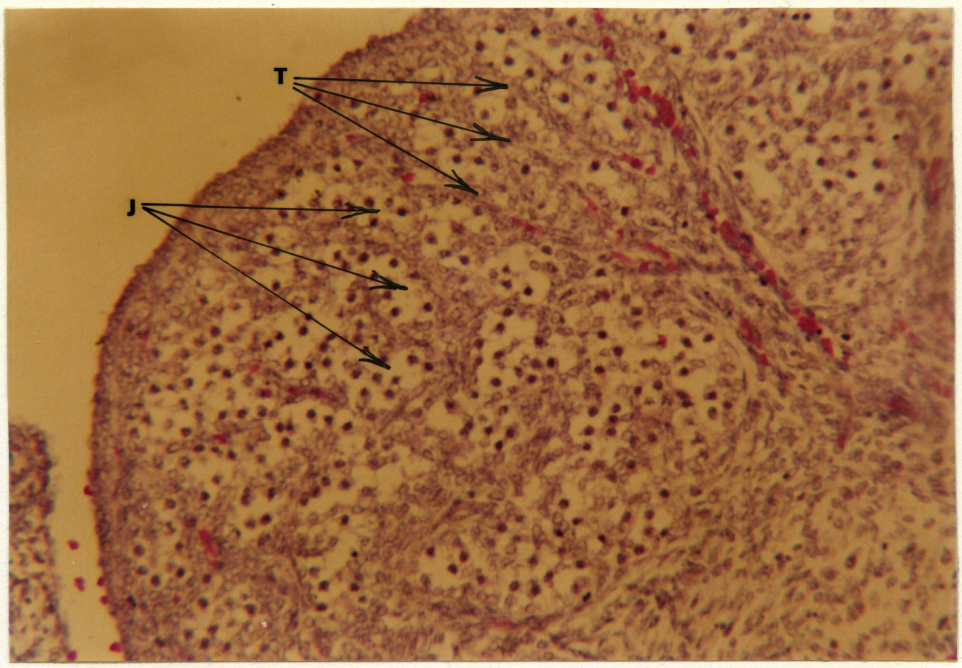

Figure 39. Ovary from 4FB. Magnified 175 times

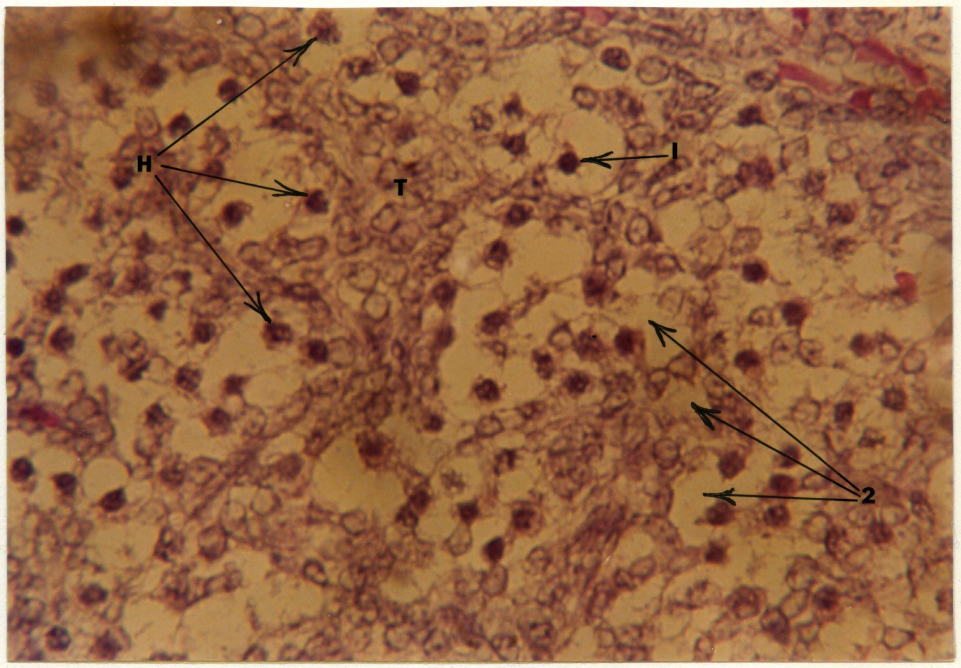

Figure 40. Ovary from 4FB. Magnified 430 times

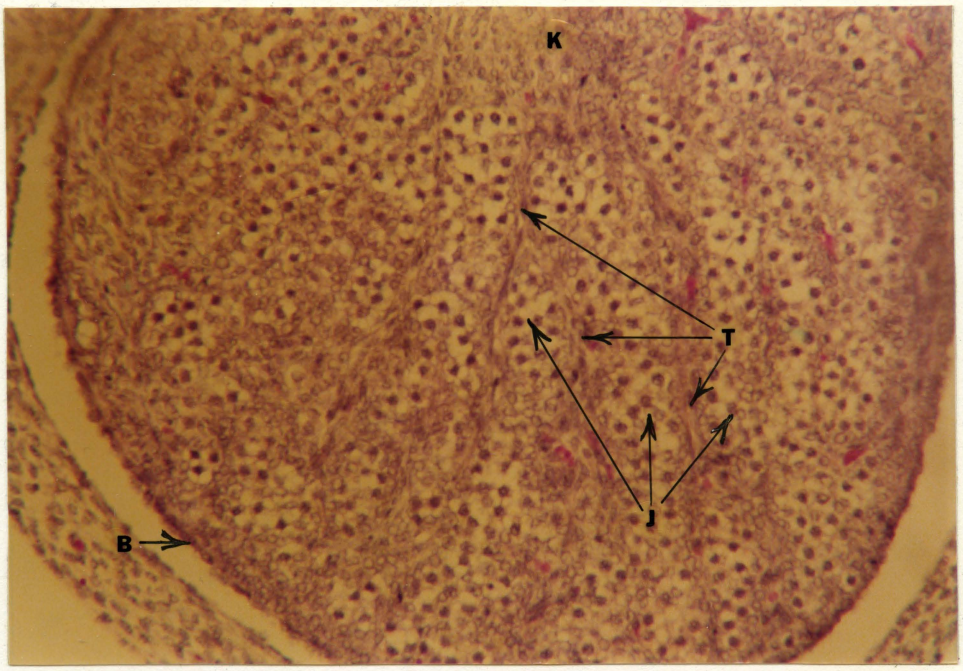

Figure 41. Ovary from 7FB. Magnified 175 times

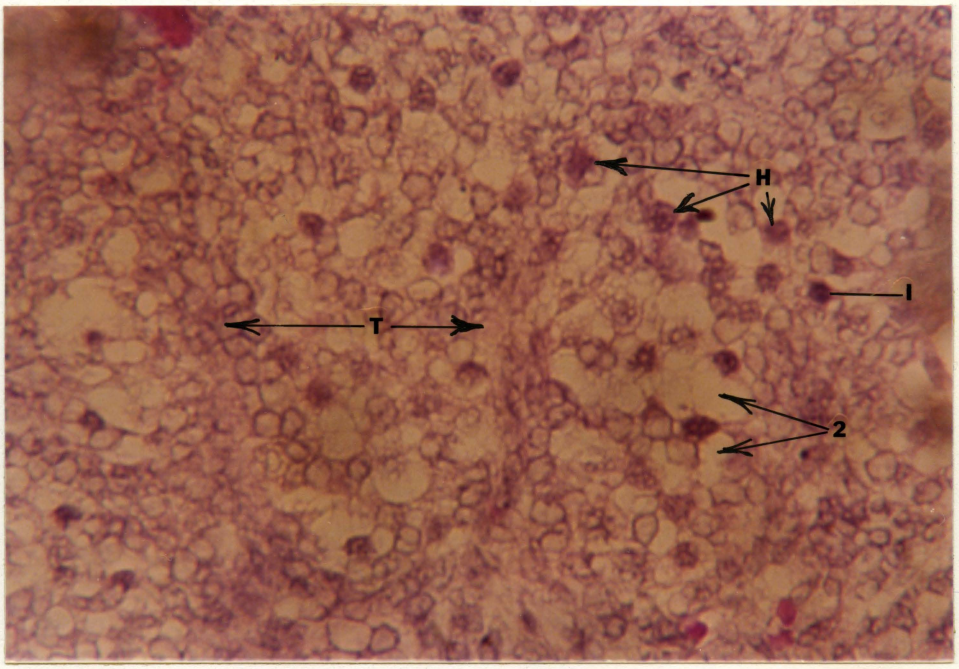

Figure 42. Ovary from 7FB. Magnified 430 times

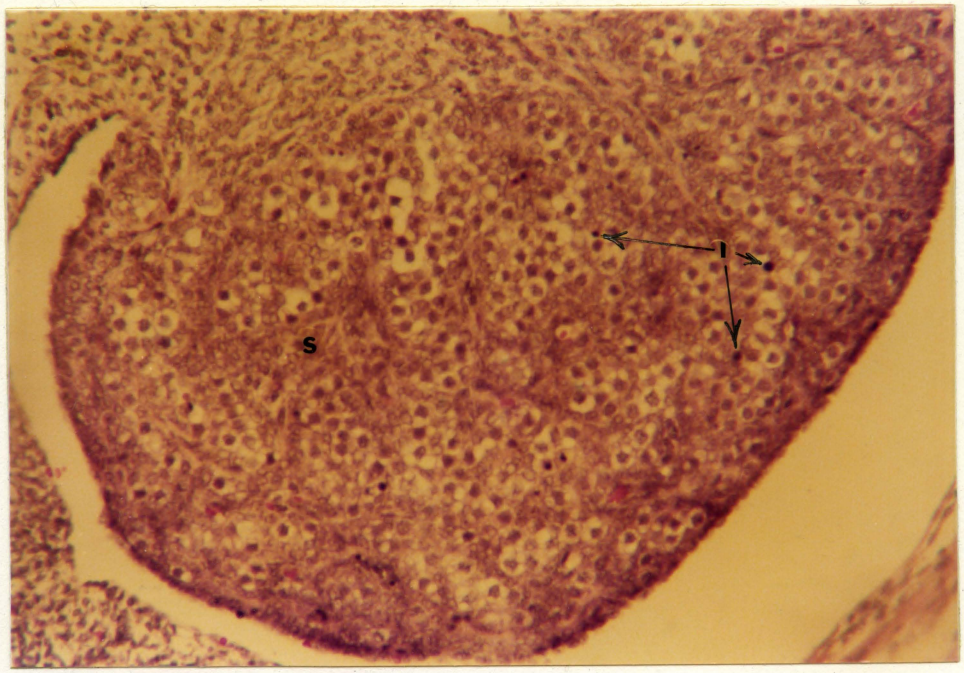

Figure 43. Ovary from 11FB. Magnified 175 times

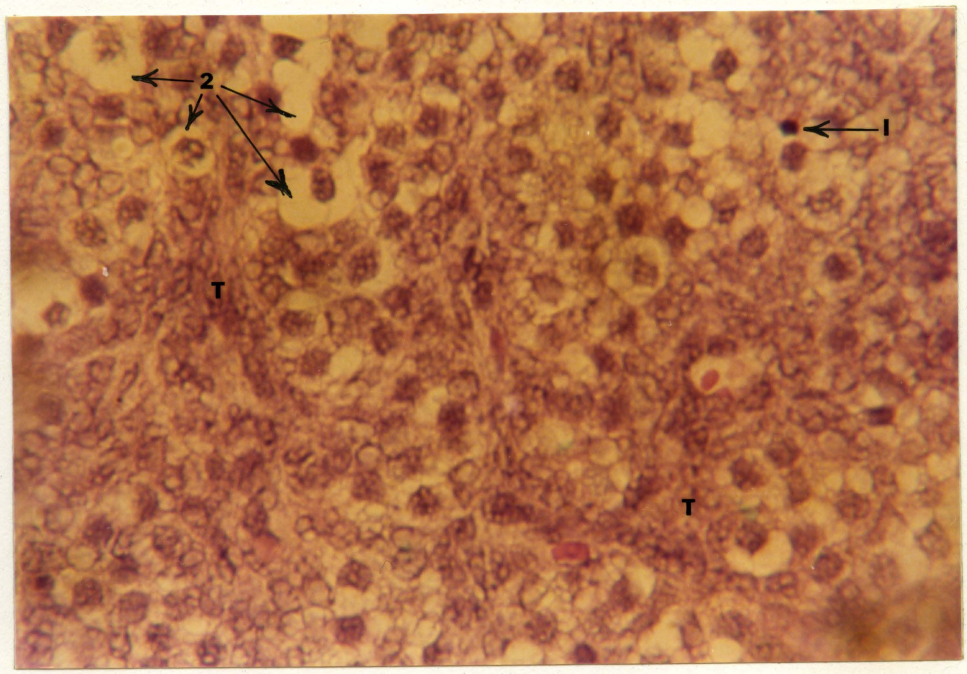

Figure 44. Ovary from 11FB. Magnified 430 times

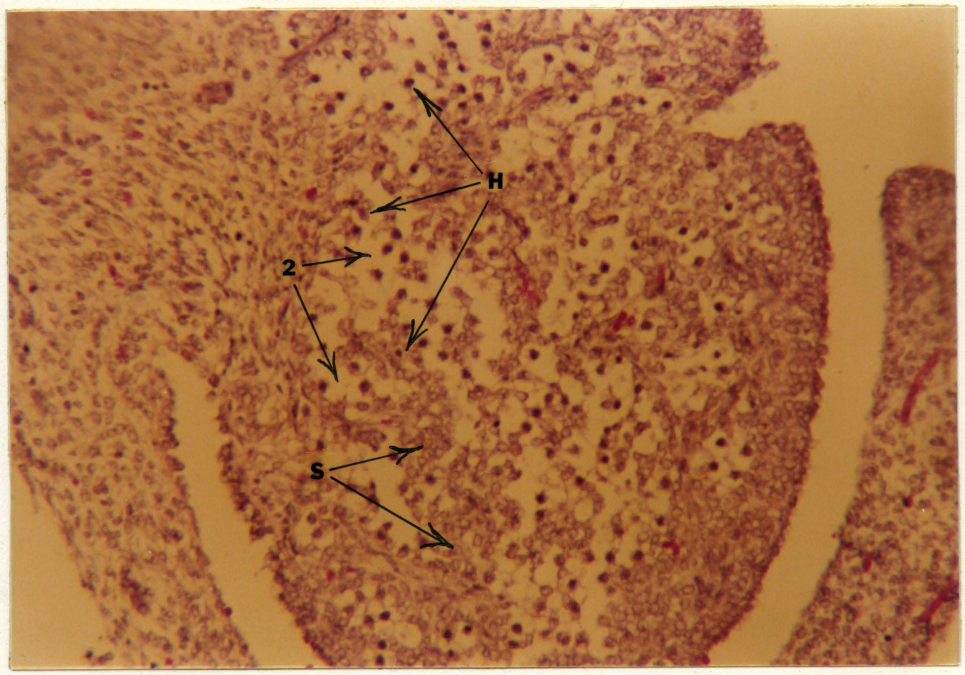

Figure 45. Ovary from 18FF. Magnified 175 times

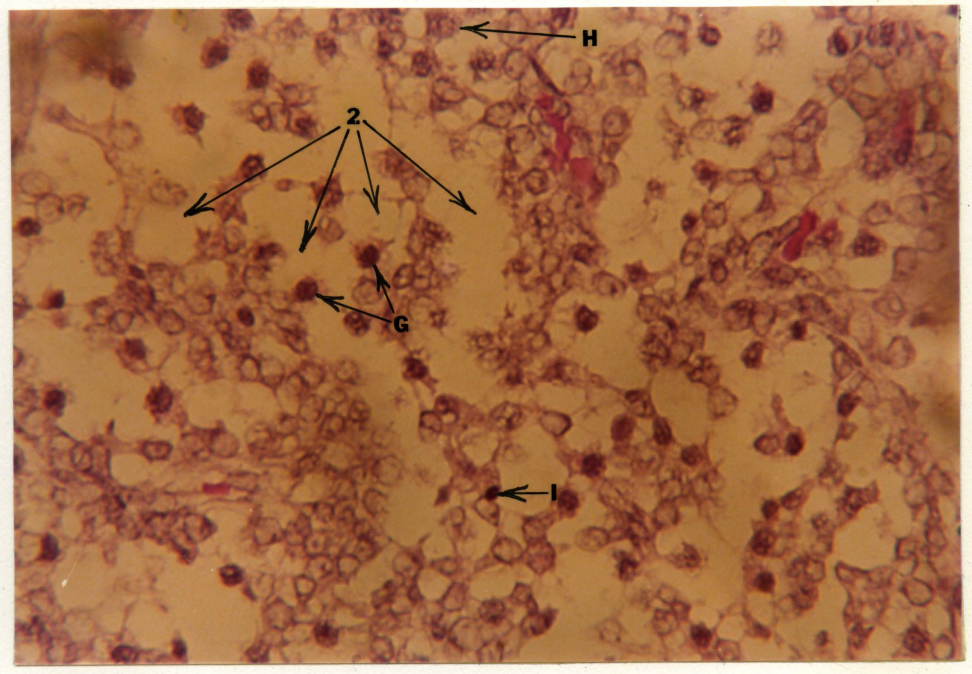

Figure 46. Ovary from 18FF. Magnified 430 times

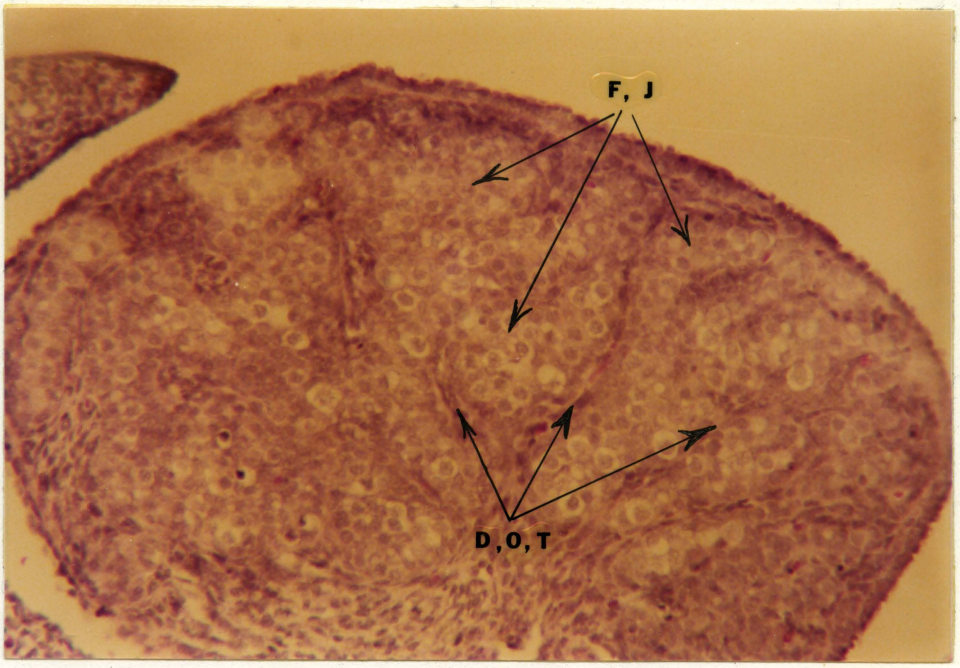

Figure 47. Ovary from 20FC. Magnified 175 times

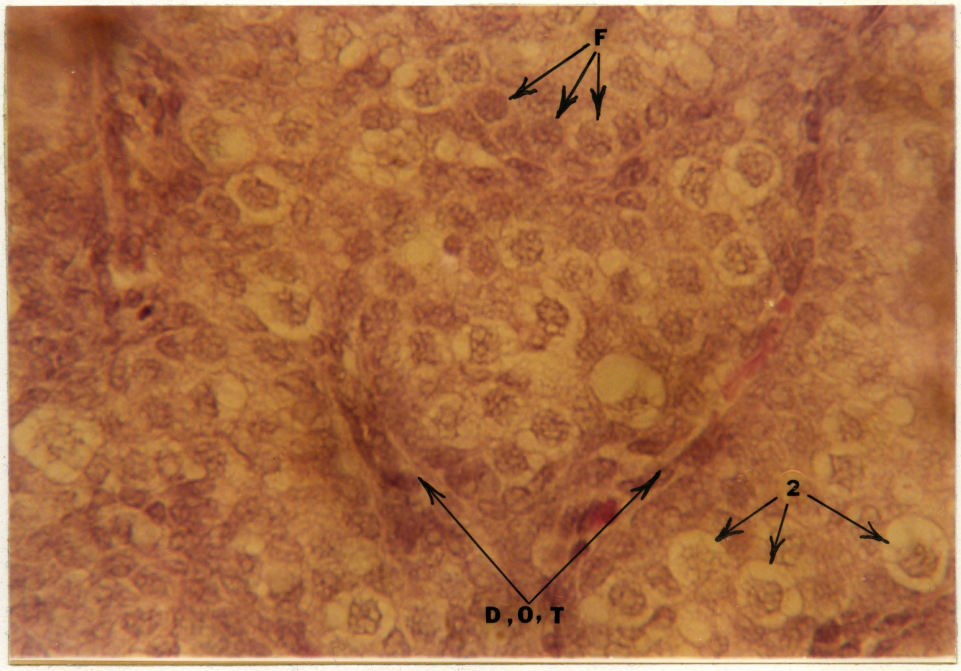

Figure 48. Ovary from 20FC. Magnified 430 times