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Effects of Ovine Pituitary FSH on the Embryonic Gonads of the Leghorn Chick During the Early Incubation

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EFFECTS OF OVINE PITUITARY FSH ON THE EMBRYONIC GONADS OF THE LEGHORN CHICK DURING THE EARLY INCUBATION

by

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BIOGRAPHY

Pham Duc Tuan was born in Hanam, North Vietnam, on December 28, 1938. He left his family and the Communist North Vietnam in 1954 to live as a refugee in Saigon, South Vietnam.

He was graduated from Saigon Chu Van An Lycee in September 1958. He attended the University of Saigon as a part time student in 1959 and at the same time taught Vietnamese Literature in Vinh Long Public High School, South Vietnam. He was a full time teacher of the above school in 1960. In 1961, he received a scholarship from St. Edward's University, Austin, Texas and was awarded the degree of Bachelor of Science in July 1963.

In August 1963, he moved to Chicago, Illinois, and began work at Alexian Brothers Hospital as a clinical laboratory assistant. He continued in this capacity until October, 1964. The following year, he left the United States of America for Strasbourg, France where he married the former Nguyen Bach Tuyet.

In November 1965, he began his graduate study in the Department of Anatomy, Loyola University Stritch School of Medicine, Chicago, Illinois. Currently, he is Research Assistant in the Department of Anatomy.

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ABSTRACT

The embryonic gonads of Leghorn chicks younger than 11 days of incubation were found to be effected by ovine pituitary FSH, however, this effect was not exerted on the formation or sexual differentiation of the gonads. In the presence of exogenous FSH, the gonads form and differentiate normally into either ovaries or testes.

The ovarian cortex of experimental embryos showed little deviation from the normal pattern but the medulla of the left ovary and of the rudimentary right gonad, as well as the testes, were significantly hypertrophied. This hypertrophy appeared to be mostly due to the distention of medullary tubules, in the female gonads, and to the larger size of the seminiferous tubules in male gonads.

The extent of the effect of FSH appeared to be dependent on the time of the initial injection, the dose of hormone administered and the duration of treatment. The ideal time for the initial injection was found to be the second day of incubation, that is, about one day before the gonads begin to form. The best overall results were obtained where the dose of hormone administered was gradually increased with the advance in development. The longer such treatment lasted the greater the effect.

Our experiments did not permit us to determine whether these effects on the gonads are permanent or whether the gonads would ultimately revert to their chronological age. A further question of importance and interest is whether the treatment carried out in these experiments will hasten or

delay maturity or lead to sterility.

Although the pituitary gonadotrophins of the chick have been found to differ qualitatively from those of mammals they are not species specific.

INTRODUCTION

Extensive work has been done in the last few decades to elucidate the inter-relations of pituitary hormones and gonadal activities. Various species from classes ranging from amphibians to mammals have been used in numerous investigations designed to illustrate the functional aspects of the hypophyseal-gonadal interaction.

A variety of methods have been repeatly employed which can be grouped into two categories: 1) The elimination of the pituitary gland before the onset of sexual differentiation by means of decapitation or X-ray, and 2) The transplantation and/or administration of hypophyseal material before and after the differentiation of sex.

The classical method of hypophysectomy applied by Smith ('16) on amphibian larvae belongs to the first category. It was, however, criticized by many investigators who argued that removal of the whole head of a developing embryo might have a greater effect on the development of the embryonic gonads than the removal of the pituitary gland itself.

Fugo ('40), using a modification of Smith's approach, surgically removed only one portion of the head which he believed to contain the future hypophysis. This partial decapitation definitely has an advantage over total removal of the head, while avoiding the objections raised to Smith's work.

Finer and more advanced methods have been used recently to avoid the neural effects following total or partial decapitation on the formation of the gonads. Wolff and Stoll ('37), Raynaud and Frilley ('47), Wells ('47),

and Jost ('47) have irradiated the hypophyseal region of mammalian embryos including rats, mice, and rabbits.

Although working with different species and with different methods, these investigators arrived at a similar conclusion for amphibian, avian, and mammalian classes: namely, that in vertebrates, the hypophysis plays no role in the primary differentiation of sex. It is, however, indispensable for the continuation of growth and full development of the gonads. The critical time at which the hypophysis begins to exert its great influence on the gonads has been set for the chick at between the 12th and 13th day of incubation (Fugo '40) and for the rat at between the 22nd and 24th day of gestation (Jost '47).

The method of transplantation and/or administration of hypophyseal material into experimental animals has been practiced by a greater number of investigators. These newer investigations followed hypophysectomy as a method of choice. Accepting the fact that the formation and sexual differentiation of gonads are independent of the hypophysis, these authors concentrated their efforts on studying the effects of exogenous gonadotropins on the morphology and function of the developing and mature gonads. The injection of pituitary extract into experimental animals has been utilized by Riddle ('31), Schockaert ('31), and Domm and Van Dyke ('32).

When the products of the pituitary glands were extracted and purified, individual gonadotropic hormones such as FSH and LH were generally used by Smith et al. ('34), Breneman ('36), Greep ('37), Taber ('48), Taber et al. ('58), and, recently Opel and Nalbandov ('61). Pregnant Mare Serum (PMS) and human chorionic gonadotropins have also been widely used.

The administration of either crude pituitary extract or purified gonado-

tropic hormones led to the same conclusions: 1) That the hypophysis maintains growth and development of the gonads following the period of direct genetic control. 2) That the addition of pituitary hormones accelerates the development of primary and secondary sex organs.

In short, the basic question of the hypophyseal-gonadal interaction has neared solution within the past 50 years. It would be interesting however, to learn whether or not the differentiation of the gonads could be affected by an adequate dose of pituitary gonadotropic hormones given at some critical time during the onset of their development. The direction of such effects, if any, would also be of particular significance. These questions, them, form the basis of the present investigation.

LITERATURE REVIEW

The fact that the formation of embryonic gonads is under genetic control and as such, independent of the influence of the hypophysis, has been demonstrated in many species of animals with a variety of methods.

The problem was first given attention by Smith ('16) who used frogs and toads to demonstrate that the removal of the pituitary gland resulted in profound testicular atrophy. It was suspected that the endocrine activities of amphibians, a class of cold-blooded vertebrates, might differ from those of birds and mammals. For this reason, amphibian species have been replaced by the chick in many investigations.

Riddle ('31), one of the early pioneers, was interested in the effects of various pituitary hormones on the weight of avian gonads. He found that the administration of ten daily doses of 0.4 cc of FSH and/or LH into immature doves and pigeons increased the weight of the testes from 500 to 2200% of those of the control birds. An increase in the weight of treated ovaries was less pronounced and varied from one-fourth to one-third of the increase in testicular weights.

Schockaert ('31) using a similar approach, prepared an anterior lobe extract of beef pituitary in saline and injected it into three-week old ducks. This author, too, observed an increase in the weight of the testes and ovaries after five or six injections during a two-week period. Microscopically, the tubules of treated testes were about three times larger than those of controls. A definite onset of spermatogonial activity in the tubules and a

precocious development of treated birds have also been described.

The more complicated and extensive works of Domm ('31), and Domm and Van Dyke ('32) have confirmed Schockaert's findings. Domm implanted homeoplastic hypophyseal material into domestic fowl and, later, Domm and Van Dyke injected pituitary hebin (powdered sheep pituitary) into immature brown Leghorn chicks from 21 to 47 days old. In the latter case, each bird received daily doses of from four to 32 rat units for a period of from 14 to 36 days. Following the treatment, drastic changes relating to the size, shape, and structure of the primary and secondary sex organs were meticulously recorded. Macroscopically, the size of the head furnishings in treated males was found to be double that in the controls and in treated females more than double that in the controls. On autopsy, the left ovaries, testes, oviducts and Wolffian ducts from treated birds all revealed hypertrophy. Microscopically, the testicular tubules were described as larger and more advanced in spermatogonial activity. The cortex of left ovaries was described as low, frequently discontinuous and the medullary portion, greatly hypertrophied, consisted of scattered cords and distended tubules.

The relation between gonadal hypertrophy and precocious development of the head furnishings in birds following pituitary hebin treatment was illustrated in Domm's further experiments. Into sinistrally ovariectomized and/or unilaterally and bilaterally castrated brown Leghorns of 28 and 36 days of age, Domm injected a daily dose of hebin with concentrations ranging from ten to 20 rat units for a duration of 21 and 22 days. The results of this investigation indicated that the hypertrophy of the gonads of sinistrally ovariectomized and unilaterally castrated chicks accompanied the changes in the

secondary sex organs. The precocious development of the head furnishings, oviduct, and Wolffian ducts, following hebin treatment could be interpreted on the basis of an increase in the secretion of estrogen and androgen by the ovarian cortex and medulla, respectively, in response to the stimulation of the injected pituitary hormone. These results were significant in that they illustrated not only the structural changes but also functional activities of a gonad subjected to the influence of heteroplastic pituitary hormones.

In 1934, Smith, Engle, and Tyndale, working with mammals, injected urinary-FSH from post-menopausal and ovariectomized women, into hypophysectomized six-day old, male and female rats. Between 14 and 25 days following treatment they observed a proliferation of germ cells in the males and a maturation of ova with follicular formation in the females. However, they found no change in the interstitial cells or in body growth.

Asmundson and Wolfe ('35) working along similar lines, injected pregnant Mare Serum (PMS), into white Leghorn chicks ranging from 42 to 91 days of age. The treated animals were sacrificed at ages from 63 to 115 days. A pronounced increase in size of testes, ovaries, oviducts and combs were reported but they found no indication of ovulation or spermatogenesis.

When the effects of the hypophysis and its products on the primary and secondary sex organs were understood, investigators became interested in the effect of individual hypophyseal hormones on the various structures of the gonads. Advanced methods of purification helped to differentiate pituitary gonadotropins into FSH and LH prior to 1936.

Breneman ('36) made daily injections of FSH and LH into five day-old, post-hatch, white Leghorn chicks. A dose of 0.2 cc was given subcutanously

for a duration of ten days. Twenty-four hours after the last injection, the birds were autopsied. The weight of the testes and ovaries in chicks treated with FSH was as much as 300% greater than that of the controls. A smaller increase in the weight of the testes and ovaries was observed in chicks treated with LH. This investigation owes its significance to the fact that the same concentrations of FSH and LH did not influence the growth of the gonads to the same degree. These interesting results with the use of individual hormones attracted more workers into the field.

Greep ('37) treated immature and adult male rats, monkeys and pigeons with either FSH or LH. FSH was found to be responsible for the increase in size in the seminiferous tubules while LH affected only the interstitial cells of the testes. Greep's results, therefore, lent support to the notion of a dual gonadotropic function for the pituitary gland.

Taber ('48) injected FSH, LH, and PMS, in doses of two and three rat units, into female brown Leghorn chicks from one day to five and a half months in age and extended the treatment from two to 51 days. At autopsy, the hypertrophy of the left ovaries, oviducts, and combs was found to vary with the particular hormone used. Histological examination revealed that PMS, LH, and FSH affected neither the ovarian cortex nor follicular growth but seemed to exert their influence on the ovarian medulla. Their potencies varied from PMS, the greatest, to LH, the least.

The results reported by Greep and Taber, combined with Domm's discoveries supported the theory that the medullary portion of ovaries and the tubular portion of testes had the same structural components prior to sexual differentiation. The problem of primary sexual differentiation and its genetic con-

trol was continued until the early 1940's when it received special attention.

Partial decapitation of white Leghorn chick embryos of three days of incubation was performed by Fugo ('40). This author confirmed previous reports that the hypophysis had no influence on the early morphological differentiation of the gonads. In hypophysectomized chick embryos, the primary sex organs formed and differentiated normally. The continuation of their growth and full development, however, could not be maintained beyond the 13th day of incubation.

With a slight modification of Fugo's technique, Jost and Cologne ('49) transplanted the gonads of rat fetuses into intact adults of the same species. The interstitial cells of the transplanted testes were observed to increase in size. When the transplantation was made into an hypophysectomized adult, the embryonic gonads underwent atrophy from the 16th to the 21st day following the operation. They concluded that gonadotropins from the adult host probably control the growth of the gonads.

One of the important structures of the gonads, the primordial germ cells, however, seemed to escape the attention of most investigators who had repeatedly studied the interaction of the pituitary gland and the gonads. Few observations have been reported concerning changes in the primordial germ cells following hypophysectomy, transplantation, and/or the administration of gonadotropins.

Foote ('55) was probably one of the first investigators to report on the effect of hypophysectomy on the primordial germ cells of the gonads. This author surgically decapitated 141 hamster fetuses of 12 days and 15 hours of gestation. At 51 hours following the operation, he recovered 22 of them

alive. Macroscopically, the gonads of the decapitated fetuses did not vary appreciably in size. In the male, the Wolffian ducts developed normally beyond the 13th day of age. They regressed, however, on the 13th hour of the 13th day, or, almost 24 hours following decapitation. In females, the Mullerian ducts did not attain the size of those in the controls. Macroscopically, the interstitial cells and, particularly, the primordial germ cells, were observed to be smaller in size and fewer in number than in controls.

Foote's observations received support from Raynaud ('50). The latter destroyed the pituitary glands of rat and field-mouse fetuses by X-ray prior to sexual differentiation and found that while the formation and differentiation of the embryonic gonads followed their normal course and developed into either testes or ovaries, the number and size of the primordial germ cells was greatly diminished.

It would be of great interest to know whether or not the hypophysis and its hormones are responsible for the gradual disappearance in the glycogen content of the primordial germ cells. These cells were reported to lose their glycogen granules when they reached the gonads following migration (Clawson and Domm, '63).

The investigation of the early function of the pituitary gland in white Leghorn chick embryos also contributed to an understanding of the interrelation of the hypophysis and the reproductive system. By means of aldehydefuchsin (AF) and periodic acid Schiff (PAS) stains, Phillips ('62) was able to follow the formation and early function of the pituitary gland in the chick. This gland was found to come into existance on the third day of incubation. This is approximately the same time that the gonads begin to form.

The hypophyseal cells were found to increase greatly in number on the fifth day. The dorsal pouch separates from the ventral pouch on the seventh day. The apparent function of the earliest hypophyseal hormone, the thyroid stimulating hormone (TSH), however, did not occur until the thirteenth day of incubation, as indicated by the degranulation of its AF and PAS-positive cells and the presence of AF and PAS material in the veins near the hypophysis.

A final problem remained to be solved before any definite conclusion could be drawn. This was the question of whether or not pituitary gonadotropins from one species are capable of exerting their influence in another species. This problem was partially solved by Das and Nalbandov ('55) and Taber et al. ('58). These authors treated immature fowl with pituitary extracts from chicks and mammals. The effects however, were not the same Das and Nalbandov reported that follicular growth in chicks of 45 days occurred 20 days after treatment with chick pituitary powder. Follicular growth, however, did not follow treatment with mammalian gonadotropins at this age. The authors concluded that in addition to FSH and LH, the chick pituitary preparation was composed of something other than FSH and LH.

A review of the various aspects of the problem has encouraged us to begin our investigation with a study of the effects of ovine pituitary FSH on the development and differentiation of chick gonads particularly during the first half of incubation.

MATERIALS AND METHODS

A single pituitary hormone, (FSH)[1] was employed in the present investigation.

The eggs used in our experiments were from two stocks. The brown Leghorn eggs were supplied from a purebred colony maintained at the laboratory by Dr. Domm and the white Leghorn eggs were obtained from a reliable source near Chicago.

The hormone was in the form of a dry powder sealed in a small vial. Each vial contained 10 mg of FSH powder. The hormone was prepared for injection by adding 1 cc of a 0.75% saline solution to the vial with the aid of a syringe. The mixture was well shaken and allowed to stand at room temperature for at least half an hour. It was shaken again before each injection. Once in solution, the hormone was kept in a refrigerator for succeeding injections in order to avoid any possible denaturation of the hormone caused by high temperatures. The hormone was put into solution just before it was to be used and each vial of solution was usually used up in a few days time.

All eggs were candled just prior to the initial injection in order to eliminate the infertile ones and to determine the injection site.

Since all injections took place in the evening, we were able to candle and locate the blastodisc in the white Leghorn eggs before incubation. The experiments were divided into two series in accordance with the particular

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[1]The pituitary hormone, NIH-FSH-S-3 ovine, was liberally supplied to Dr. L. V. Domm by the Endocrine Study Section, National Institutes of Health, Bethesda, Md.

aspect of the problem under investigation.

The first series was designed to study the effects of exogenous FSH on the formation and differentiation of the gonads. A selected number of white Leghorn eggs received an initial injection immediately before they were incubated. Prior to this injection, the eggs were candled to locate the position of the blastodisc. A small hole was made through the egg shell and membrane with the aid of a pin vice just above the blastodisc. A measured amount of the FSH solution was then dropped onto the albumin by means of a $\frac{1}{4}$ cc tuberculin syringe, scale 1/100 cc. Considerable care was taken to avoid disturbing the developing embryo. The hole was sealed with Scotch tape and the eggs returned to the incubator and incubated at 100°F. The injections were repeated every other day. The embryos were sacrificed at either 24 or 48 hours after the last injection which corresponded to the end of the fourth, fifth, sixth, seventh, eighth, nineth, tenth, and 11th days of incubation.

Eggs used in the second series of experiments were incubated prior to the initial injection. These were candled at the end of the fifth day of incubation and all infertile eggs and those with dead embryos were eliminated. A small hole was then made through the egg shell and membrane at a site, usually between two visible blood vessels in the area vasculosa and a selected amount of the FSH in solution dropped onto the vitelline membrane and the opening subsequently sealed with Scotch tape. Injections were repeated daily or every other day depending on the total amount of hormone to be administered. The embryos were sacrificed either at the end of the 11th or the 18th day of incubation.

The dose per injection in the two series varied from 0.2 to 2.5 mg and

the number of injections ranged from one to six. The controls in each of the experimental series were either maintained intact or treated with 0.75% saline solution in amounts equal to those of the hormone injections. Both treated and control embryos were staged, photographed, and dissected with the aid of a stereoscopic microscope and camera. Pictures of whole embryos and intact gonads were used for gross comparisons.

Both treated and control gonads were fixed in Gendre's fluid and kept at a temperature of 20°F for two or three days. They were embedded in paraffin and sectioned at 6u. Serial sections of the gonads were carefully mounted in the same manner and were treated with PAS in order to demonstrate the glycogen content of primordial germ cells, or with Mallory's triple stain to reveal the connective tissue of ovaries and testes. Cross sections of testes and ovaries of both treated and control embryos were photographed in order to facilitate comparisons.

RESULTS

Generalization

One hundred and ninety six white Leghorn and 140 brown Leghorn embryos were studied in the present investigation. Out of the 196 white Leghorn eggs, 102 were treated with FSH, 14 with saline solution, and 80 kept intact. The distribution of the 140 brown Leghorn embryos was as follows: 102 were treated with FSH, eight with saline solution, and 30 kept intact. The saline treated and intact embryos served as controls. Ninety FSH-treated and 92 control embryos of different ages of incubation were collected (table 1).

Treatment and Fatality

The mortality observed in our experiments was higher than that reported in previous reports. The death rate among all of the treated embryos was 50% and that of the controls 18%.

The mortality rate was almost constant for the controls but varied greatly among the treated embryos. The high death rate of treated embryos was probably not due to the manner of injection (technique) but rather to a number of factors such as dose of hormone, time of injection, and frequency of injection.

Embryos from the first to the fifth day of incubation could not tolerate a dose of more than 0.4 mg of FSH / injection. However, in order to increase the area available for a larger dose, we either opened a small window on the egg shell of embryos of 24 hours incubation, or punctured the air sac of the unincubated egg. In both cases, we found that the embryos could survive

total doses of 1.5 and 2.5 mg of hormone. We suspected that the hormone may have been absorbed by the egg yolk rather than by the blastodisc or the embryo proper. After the sixth day of incubation, the embryos were found to tolerate a dose as high as 1.0 mg / injection.

The injection age was also a critical factor. In our experiments, the most lethal period for injection extended from the third to the sixth day of incubation.

The death rate increased with an increase in the frequency of the injections. This had been reported by Domm in the early 30's. An increase in the injection frequency or dose during the critical period could bring the death rate to a maximum. In one of our experiments, we selected 15 embryos of five days of incubation and treated them with a dose of 0.2 mg of hormone / injection for three consecutive days, that is from the fifth through the seventh day of incubation. Twenty four hours after the third injection, all 15 embryos were dead. It may be true that during the period of sexual differentiation, the embryos were more sensitive to the exogenous hormone and could not tolerate a large dose of FSH.

Treatment and Weight of Eggs

In eight of our 22 experiments, the treated and control eggs were weighed before incubation and immediately before sacrifice. The difference between these two weights during the first half of the incubation period was not the same for the treated as for the control eggs. The treated eggs were observed to lose more weight. This loss became even in the latter half of incubation (table 11).

In most of our experiments, the embryos older than five days of incuba-

tion were also carefully weighed before autopsy. These weights were found to support the notion that large eggs give rise to large embryos and that large embryos, in turn, give rise large gonads (table 12).

The purpose of this correlation was to evaluate the effects of FSH on the treated gonads when these gonads were compared grossly with those of the controls, especially when dealing with the gonads of embryos younger than 11 days of incubation.

Embryos of Four Days Incubation

Three embryos of the white Leghorn stock were initially treated after 12 hours of incubation. A second injection was made two days later. The dose for each of these injections was 0.2 mg. The total amount of FSH injected into each embryo was 0.4 mg. Two intact embryos of the same age of incubation served as controls. The embryos were sacrificed at the end of the fourth day of incubation.

With the aid of a stereographic microscope, both treated and control embryos were staged according to the Hamburger-Hamilton method. Their stages were not uniform but ranged from stage 21 to stage 23. No apparent effect was detected in the treated embryos. On autopsy, no gonads could be found (table 2).

Histological examination revealed a slight thickening of the germinal epithelium in the gonadal regions of all treated and control embryos. A smooth increase in thickness of the germinal epithelium could be detected at about the origin of the vitelline arteries. The left and right germinal epithelia were not equally thick. That on the left side was thicker and consisted of four or five layers of peritoneal cells. That on the right side

consisted of only two or three layers of the same type of cells. The cranial half of the germinal epithelium on both sides contained no primordial germ cells but only peritoneal cells. The nuclei of these cells were either round or oval. The presence of primordial germ cells was observed in the caudal half of the germinal epithelium. These were either embedded among the peritoneal cells or located at the stromal side of the germinal epithelium. In the former case, the proliferation of the primary sex cords of the gonads was still remote while in the latter this proliferation seemed to be under way. The total number of primordial germ cells in the gonadal region differed from one embryo to another, varying from 25 to 87, and the total number of these cells counted on the left side was more than twice that found on the right side. This was true for both the treated and the control embryos. They were easily recognized because of their cytoplasmic PAS-positive material and also because of the size and shape of their nuclei. The primordial germ cell of this stage of incubation has a thin PAS-positive membrane, a mostly clear cytoplasm, and a slightly oval nucleus. This nucleus is eccentrically located and lies close to one side of the cell membrane. It is in this portion that the PAS-positive material of the cytoplasm appears.

The stromal area between the germinal epithelium and the mesonephros consisted of loose connective tissue and scattered, irregularly shaped cells.

The differences between the treated and the control embryos were apparently in the mitotic activity of the germinal epithelial cells and in the location of the primordial germ cells.

In serial sections of the gonadal region, a peritoneal cell (or cells) was frequently found in the process of division, on both sides of the treated

embryos. Mitotic activity was less frequently observed in the control embryos and escaped notice among the primordial germ cells. The position of the primordial germ cells, however, caught our attention. In all three treated embryos, clusters of three, four or five PGCs were located on the stromal side of the germinal epithelium. The position of the PGCs in the treated embryos may indicate an early phase of sex-cord proliferation which usually occurs at five and a half to six days of incubation (Swift '16). This clustering arrangement was not observed in the two control embryos.

Embryos of Five Days Incubation

Three treated and three control embryos were collected at the end of the fifth day of incubation. The treated embryos of this group received two doses of 0.2 mg each of FSH solution. The initial injection was given at 12 hours of incubation and the second, two days later. The total FSH administered was 0.4 mg.

Cross examination revealed no apparent change in the treated group. Their developmental stages were approximately the same, i.e. stage 25. On autopsy, no gonads could be seen, and the whole embryo was fixed (table 3).

Histologically, the genital ridge of both treated and control embryos was seen to be well formed. The ridge consisted of a mass of peritoneal cells and a U-shaped germinal epithelium containing PGCs. It clearly protruded into the abdominal cavity. The germinal epithelium of the five day old embryos was thicker and more compact than that of four day old embryos with a greater thickness occurring on the left side in both groups. On both sides, it was separated from the rest of the genital ridge by strands of connective tissue stained brightly red with the PAS technique. The germinal epithelium

had the same thickness in both treated and control embryos but differed in appearance. It curved smoothly in control embryos but was crenated in the treated embryos. The medullary portions of treated and control embryos were approximately equal in size but differed in structural detail. The medullary cells were diffuse and disorganized in gonads from control embryos. In treated embryos the medullary mass was divided into clusters of five or six cells surrounded by connective tissue.

The mitotic activity of the somatic cells was greater in the treated embryos, especially in the gonadal region. Mitoses among primordial germ cells were rare. The shape and glycogen content of the PGCs at this stage were still the same as in the four day embryos. The PGCs were slightly reduced in size that is, from 13 and 12u to 10u but they were definitely increased in number, and most of them were located at the stromal side of the germinal epithelium. The increase in the number of PGCs was greater on the left side.

Embryos of Six Days Incubation

Nine treated and seven control embryos were collected at the end of the sixth day of incubation. Each treated embryo received a total of either 0.5 mg or 0.6 mg of FSH and the treatment lasted for a period of five and a half or six days. Their stages varied from one embryo to another with a similar variation in each group. Macroscopic examination revealed no change in the treated embryos. The gonads could be easily seen following the removal of the abdominal contents. The lengths of the gonads were measured with the aid of a stereoscopic microscops. The shortest gonads measured approximately 1 mm in length and belonged to the youngest embryo. The longest gonads of the

group were about 3 mm. In seven of the treated embryos, the left and right gonads had the same length and width and were thought to be male. The other three treated embryos had unequal sized gonads, those on the left side being longer and broader than those on the right. These embryos were considered to be females. The sex of the embryos could therefore, be, grossly recognized by the end of the sixth day of incubation (table 4).

These gross differences in the gonads could not be taken as an indication of the effects of FSH on the gonads of a treated embryo but rather a reflection of the particular developmental stage of the embryo. The sexing of embryos based on stained, serial sections was also difficult. Similarly, the thickness of the germinal epithelium could not be used as a basis for the determination of sex at this stage.

The proliferation of the primary sex cords had occurred after six days of incubation. These were well defined on the left side by a strand of connective tissue stained red with PAS. The cords were more compact in the gonads of control embryos where little connective tissue was found. They tended to have a rather loose arrangement in the gonads of the treated embryos.

The primordial germ cells were located not only in the germinal epithelium but also in the sex cords. Their glycogen content was further reduced and so they were recognized principally by the size of their nuclei and poor outlines. Mitotic activity among the PGCs was not observed.

Embryos of Seven Days Incubation

Six treated and six control embryos were collected at the end of the seventh day of incubation. The treated embryos received a total of 0.6 mg

of FSH and the duration of treatment was seven days. Their stages varied from 30 to 32. Their body weights ranged from 340 mg to 870 mg.

The gonads of both treated and control embryos had increased greatly in size. The average lenth of the gonads was about 2.5 mm. The widths of the gonads were still too small to be measured accurately (table 5).

Microscopic examination revealed that the germinal epithelium of the right gonads was substantially reduced in thickness. In most cases, it consisted of a single layer of either cuboidal or squamous cells. On the left, it differed from one gonad to another and varied from one portion to another portion of the same gonad. Its appearance could be used to distinguish one sex from the other. A germinal epithelium with four or five layers of peritoneal cells was found in the left ovaries. It consisted of only one or two layers of cuboidal cells in left testes. No significant effect attributable to the FSH treatment was observed in this portion of the treated embryos.

The sex cords were well formed in both right and left gonads and even distended in the left gonads. The number and size of the distended cords was greater in the treated embryos.

The distribution of PGCs differed from the ovaries to the testes. In the ovaries, a great number of these cells were located on the medullary side of the germinal epithelium. A greater number of them were scattered throughout the sex cords. In the testes, only a small number of PGCs appeared in the thin germinal epithelium while most of these cells were embedded in the sex cords. The size of these cells was only slightly reduced but their glycogen content was greatly diminished.

Cell divisions were found here and there in somatic cells but they did

not predominate in the gonads of treated embryos.

Embryos of Eight Days Incubation

Two treated and two control embryos were studied. The treated embryos received a cumulative dose of 0.8 mg of FSH during a period of seven and a half days. Macroscopic examinations of the treated and control embryos were compared as follows:

Animal	Stage	Weight of embryo in mg	Left gonad Length in mm	Left gonad Width in mm	Right gonad Length in mm	Right gonad Width in mm	Sex
20 I*	34	1050	2.8	0.8	2.2	0.4	♀
21 I	32	880	2.8	0.6	2.5	0.5	♂
22 C**	34	1060	2.5	0.6	2.5	0.5	♂
23 C	30	870	2.2	0.7	1.7	0.4	♀

The above data clearly illustrates the difficulty in drawing conclusions regarding macroscopic change due to FSH treatment without considering the developmental stage and body weight of the embryos along with the length and width of the gonads.

In this experiment, treated and control embryos of the same stage were observed to have similar body weights. Unfortunately, these embryos belonged to different sexes so no accurate macroscopic comparison could be made.

Microscopically, the left gonad of both sexes was larger than the right. Ovaries and testes were easily distinguished. The differentiation of sex had definitely been completed at this period.

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*I treated embryos

**C control embryos

Under high power, the left ovaries of both treated and control embryos could be grossly divided into three regions: The outermost part which consisted of germinal epithelial cells and a large number of PGCs was compact and well arranged and stained deeply with PAS; the middle region was occupied by cells of star-shaped nuclei. These cells were diffused throughout and irregularly arranged. This portion was a prominent feature in the ovaries of treated embryos of this stage and comprised the largest portion of the gonad. No PGCs were found in this region; The innermost part was located between the middle portion of the gonad and the mesonephros. Here, the medullary cords were well defined with PGCs scattered within them.

The right ovaries consisted of only two portions. The germinal epithelium was reduced to a single layer of either squamous or cuboid cells and the rest of the gonad was occupied by sex cords. The cords were outlined by strands of connective tissue and were similar to the medullary portion of the left ovaries.

The right testes could be easily distinguished from those on the left side. In cross sections, the left testis was larger in size. Its germinal epithelium consisted of two or three layers of peritoneal cells on the medial side. The germinal epithelium of the right testis consisted of only a single layer of squamous cells.

The seminiferous tubules were well developed. In longitudinal sections, they were made up of two parallel layers of cuboidal cells. In cross sections, the tubules were circular with four or five cells. They were separated from the interstitial cells by a thin membrane. Little connective tissue was found in the control testes.

The PGCs were situated among the supporting cells of the tubules. A round, large nucleus was still their chief identifying characteristic. Their cytoplasm appeared empty. The cell membrane stained slightly with PAS. The nucleus was eccentrically located along one side of the cell membrane. The section of cell membrane in contact with the nucleus was stained a bright red. No glycogen granules could be seen in the PGCs at this stage of incubation.

Embryos of Nine Days Incubation

Eight treated and eight control embryos were collected at the end of the ninth day of incubation. Each treated embryo had received from 0.8 mg to 1.4 mg of FSH during a period ranging from three to eight and a half days (table 7)

Macroscopically, no effects or apparent change could be observed either in the treated embryos as a whole or in the gonads. Microscopic study, however, revealed effects of FSH on the gonads.

A. Treated and Control Ovaries

The germinal epithelium of nine-day old ovaries showed pronounced variation. This epithelium from the left ovaries of treated embryos ranged between ten and 20 micra in thickness. It contained a great number of PGCs along the medial side. The secondary tunica albuginea was discontinuous and incompletely separated the germinal epithelium from the secondary sex cords. The sex cords were almost round in cross sections and their diameters reached 40 micra in places. The cortex was separated from the medulla by cells of the primary tunica albuginea.

The medulla could be artificially divided into two parts. The superficial portion was solid. Its cords were well defined and were irregularly

arranged. The central reticular portion consisted of distended tubules demarcated by connective tissue. The lumina of the distended tubules had diameters of about 35 micra in cross sections and these were lined either by peritoneal or PGCs. The PGCs were more numerous in the reticular portion than in the superficial part.

The germinal epithelium of the right rudimentary gonads from treated embryos, was just a thin strand of squamous cells and was loosely separated from the medulla. The latter consisted of structures of two types, solid cords and distended tubules. The cords were not well defined but the distended tubules were clearly demarcated and were lined with an average of 12 cuboid cells. The diameters of the distended tubules ranged from ten to 25 micra. The primitive cortex was still attached to the germinal epithelium.

The germinal epithelium of the left ovaries from control embryos was thicker than that from treated embryos and measured from 10 to 25 micra in thickness. It was solid and tightly packed with either cuboidal or columnar cells. The cortex diffusely connected the germinal epithelium to the medulla. The latter contained both solid cords and distended tubules but the lumina of the distended tubules were not as large as those in the treated embryos. Also the tubules were less numerous in the control embryos.

B. Treated and Control Testes

No significant effect could be detected in the testes of treated embryos at this stage of incubation. Macroscopically, the slight difference in the size of testes could not be definitely attributed to the effect of treatment. Microscopically, very little, if any, effect was seen in the tubules. However, more intertubular material was observed in the left testes

of treated embryos than in the left testes of control embryos.

Embryos of Ten and 11 Days Incubation

Many experiments were undertaken and a great number of treated embryos were collected following ten and 11 days of incubation (table 8,9). Attention was focussed on these ages because, macroscopically, the size of treated and control gonads could be accurately measured, and , microscopically, definite structures within the gonads could be meticulously compared, and, last but not least, the duration of treatment was long enough to bring about significant effects on the gonads of treated embryos.

In an effort to avoid repetition, a description of the effects observed in the gonads of ten and 11 day old embryos will be presented together and in somewhat greater detail.

A. Effects of FSH on the Ovaries

A striking effect was observed in embryos which were treated before the formation of the gonads and which had received a fairly high dose of FSH.

Macroscopic examination revealed a noticeable increase in the size of left ovaries of treated embryos. They were both longer, and, on the whole, broader than those of control embryos. The right rudiments of treated embryos had increased only in width. Their lengths measured 2.4 mm and were the same as those of the controls at this stage. In treated embryos both left and right gonads had a honeycomb appearance under the stereoscopic microscope. This appearance was in contrast with the smooth surface observed in ovaries from control embryos (figs, 1,2).

Microscopically, the structures of left and right ovaries of treated embryos revealed definite effects of FSH (figs. 9,10).

In cross sections, the left ovaries from treated embryos were mushroom-shaped with a great number of vacuoles. Their radii, starting from the ovarian artery, were approximately one and a half times those found in control ovaries. The germinal epithelium was two or three cell-layers thick and consisted of peritoneal cells and primordial germ cells. The nuclei of the peritoneal cells were either round or oval and the cells were irregularly arranged. The PGCs were situated on the medullary side of the germinal epithelium and their nuclei were eccentrally located.

The cortex was in an advanced stage of development. However, it was not completely separated from the germinal epithelium by the secondary tunica albuginea. In most areas of the sections, the secondary sex cords were tenuously attached to the germinal epithelium and only rarely were they separated from the latter by a thin layer of connective tissue. The size of these cords differed from region to region of the cortex. In the cranial and caudal portions of the ovaries, where they were most numerous, the diameter of the cords reached 40u and their invagination into the medulla was as deep as 65u. Also, in these regions, the cords were well outlined by connective tissue. They were compact and contained cells of different size. The PGCs were rarely seen in the cords but oogonia were frequently encountered.

In the middle portion of the left ovaries, from the treated embryos, the secondary sex cords thinned out. Their diameters became smaller and their invaginations shorter than in the cranial and caudal portions. Many cords were found to be detached from the germinal epithelium.

In short, the cortices of left ovaries from treated embryos were less developed than those from control embryos. The superficial medulla from

treated embryos consisted of an equal number of solid cords and distended tubules. In the middle portion of left treated ovaries, the number of distended tubules was so great that these tubules even invaded the domain of the cortex and reached the germinal epithelium. In cross section the diameters of their lumina measured 50 micra and were lined with either cuboidal or squamous cells. These cells were situated far from one another and were connected by strands of connective tissue. The solid cords were not well outlined by a basement membrane but formed a mass of disorganized cells (figs. 11,12).

Few primordial germ cells were found in the superficial medulla.

The reticular portion of the medulla occupied about one third of the left ovaries and consisted of only distended tubules. The diameters of these tubules reached 100u and they were lined by strands of squamous cells. Clusters of two or three primordial germ cells were observed within the walls of the tubules.

The germinal epithelium of the right rudimentary gonad consisted of a single layer of squamous cells. It rarely contained PGCs and was completely separated from the medulla by a layer of connective tissue.

The medulla of the right rudiment from treated embryos was significantly different from that of control embryos. Its size was larger due to a greater number of distended tubules. These tubules were scattered throughout the rudiment and were frequently applied against the germinal epithelium. The solid cords usually occupied the center of the right rudiments. Their outlines were not clearly defined and few PGCs were found among them (figs. 13, 14).

In short, the effect of FSH on the ovaries was greatest in the medulla, particularly in its superficial portion. The enlargement of this region in turn, resulted, in an overall increase in the size of the ovaries of treated embryos.

B. Effects of FSH on the Testes

The effects of FSH on the testes were not as pronounced as on the ovaries. Macroscopically, the right and left testes of treated embryos, were observed to be larger than those of controls. The appearance of the testes from both treated and control embryos was smooth (figs. 3,4).

Microscopically, the germinal epithelium had a similar appearance in the testes from treated and control embryos and did not seem to be affected by the FSH treatment.

This treatment did, however, seem to affect the seminiferous tubules and interstitial components to some extent. On cross section the treated testes were made up of an anastomosis of tubules whose diameters measured 30u. The tubules had fine basement membranes against which were found round nuclei of peritoneal cells and spermatogonia. The tubules of the controls were not well outlined by a basement membrane and their diameters measured about 25u. The nuclei of the tubular cells were not applied against the basement membrane.

The testicular tubules from control embryos had a multi-directional orientation and were cut longitudinally, obliquely, and transversely. They were surrounded by intertubular cells and connective tissue. The intertubular space was better developed in the testes from treated animals. This space was occupied by loose connective tissue, interstitial cells, and blood vessels. In testes from the control animals, the intertubular tissue was

more solid and the tubules approached each other more closely.

The primordial germ cells of this stage were recognized only by the size and shape of their nuclei. All glycogen granules had disappeared.

No significant differance in mitotic activity was found at this stage.

Embryos of 18 Days Incubation

It has been generally accepted that the pituitary gonadotropins are indispensable for the continuation of the growth of the gonads in the later half of the chick incubation period and that prior to this the gonads of the immature chick respond actively and specifically to FSH. The purpose of our study at this age was to confirm the results observed in the gonads of 11-day old embryos (table 10).

Effects of FSH on the Ovaries

The effect of FSH on the ovaries of 18-day embryos was observed to be similar to that found in the 11-day old embryos. The difference between the two age groups lies in the extent of the changes.

Macroscopically, the ovaries of the treated embryos were larger than those of the controls. Under the dissecting microscope, the treated ovaries were lobulated and honeycombed in appearance (figs. 5,6).

Microscopically, the effects of FSH were found in both the cortex and in the medulla (figs. 15,16). The ovarian cortices from treated embryos ranged from zero to 200u in thickness. The thickest portions of the cortices were situated at the cranial and caudal ends and/or on the medial and lateral sides of the ovaries. In the middle portion, the cortex was broken up by the invasion of distended tubules and it was lobulated by dense connective tissue. The primary and secondary tunicae albugineae were well developed (fig.

18).

The ovarian cortices from the control embryos were quite uniform in thickness and at no place did the medulla reach the germinal epithelium (fig. 17).

The ovarian medullae from treated embryos were occupied mainly by distended tubules. The diameters of these tubules measured over 100u. The solid cords were fewer in number and contained only four or five cells in cross section. Fat laden cells were also found among these tubules. The reticular portion occupied the major part of ovaries and consisted of strands of squamous cells.

The right rudiment of treated embryos had increased in size as a result of the treatment and was occupied mostly by distended tubules (figs. 19,20).

PGCs were rarely encountered and there was little mitotic activity.

Effect of FSH on the Testes

In gross examination, the treated testes of this stage of incubation had increased in width as a result of FSH treatment. The increase in size was, however, not very significant (figs. 7,8).

Histological study revealed a greater amount of intertubular material in the treated testes (figs. 21,22). Inasmuch as the size of the tubules from the treated embryos was not uniform, any comparison with tubule size from the control embryos was necessarily difficult (figs. 23,24).

DISCUSSION

The effects of pituitary gonadotropins on the gonads of chick embryos were investigated by Domm and Van Dyke ('32). A similar study on immature fowl has been made by Taber ('48). The latter investigator used FSH, LH, and PMS separately in her experiment. In both cases, the effect of individual or of combined pituitary gonadotropins was reported to be pronounced in the medulla.

The purpose of the present investigation was to study the effects of exogenous FSH on the gonads of chick embryos during the first half of incubation. Some problems were encountered during the course of this study. The most serious difficulty was encountered in the evaluation of gonadal changes in young embryos. The macroscopic comparison of embryonic gonads was seriously impeded by the continuous advance in age. There was a lack of uniformity in the development of embryos from a batch of eggs, incubated at the same time and for the same duration. This normal, differential growth rate is reflected in the development of the gonads as well as in the embryo as a whole. Thus comparisons between treated and control embryos of the same incubation period have less validity than comparisons between treated and control embryos of the same growth stage (as determined by Hamburger and Hamilton '51). In addition, short term treatment has certain liabilities. The effects of exogenous hormone administration require a certain minimal period of time to become demonstrable (detectable). The earliest time an adequate (effective) injection could be made was at the end of the second day of incubation.

Embryos which were sacrificed on the 4th day of incubation had been, therefore, exposed to the treatment for only two days and maximal effects could not be expected during this short treatment period. In order to prolong the exposure period, an effort was made to treat the eggs prior to incubation but this approach had the disadvantage of reducing the amount of hormone capable of being absorbed by the blastodisc. In an attempt to avoid these disadvantages, it was decided to prolong the period of treatment to allow the embryos to continue their development until an older stage.

The comparison of gonadal structures between embryos met with the same difficulty.

The number of PGCs varies greatly from one embryo to another as indicated by recent investigations (Meyer '64, Clawson and Domm '63). No definite standard has been evolved whereby a change in the population of PGCs due to hormonal treatment can be evaluated.

Since the mitotic activity of the somatic cells also undergoes great variation, these details have been omitted from the present study. However, the purpose of the present investigation could not be fulfilled without some description of these factors.

Variations in the morphology of gonadal components (cortex and medulla) from stage to stage and from embryo to embryo tend to obscure the effects of any hormonal treatment and make the comparison between treated and control structures very difficult and hazardous. The observation and description of embryonic gonads from the fourth, fifth, sixth, seventh, eighth, and ninth day of incubation, however, greatly contribute to an evaluation of changes due to hormonal treatment in later stages.

Microscopic observations of gonadal structures before and after sexual differentiation have enabled us to conclude that the effect of FSH on the gonads is gradual and specific.

A s result of our investigation, it was clearly indicated that pituitary FSH definitely affects the gonads of the chick during the first half of the incubation period. These effects were not manifested on the formation of the gonads or on the differentiation of sex but rather on the development of the gonads. The ovine pituitary FSH exerted its influence on the medulla of ovaries and the stroma of testes.

An explanation for the impaired development of the cortex and medulla in the female, and of the tubules and stroma in the male at 11 days of incubation might be found by correlating the results of previous investigations. As a result of investigations by Domm and Gustavson '29, Domm and Van dyke '32, Domm '33a,b, Domm '37, Corner '38 and others, it was well established that the testicular and ovarian medulla secretes androgenic hormone and that the ovarian cortex secretes estrogenic hormone. On the one hand, pituitary FSH has been found to be an antagonist of estrogenic hormones and, on the other hand, synergistic with androgenic hormones (Breneman '36). The poor development of the cortex of treated ovaries has been due to the antagonistic effect of exogenous FSH observed in our experiments. In contrast, the synergistic action of pituitary FSH with androgenic hormone may have stimulated the precocious growth of both the testicular and ovarian medulla. These suggestions depend, of course, on whether or not the gonads produce their own hormones during the first half of the incubation period.

The extent of the effects of FSH on the gonads has been found to depend on a number of factors such as the time of initial injection, the dose of in-

jected hormone, and the duration of treatment.

The greatest effect on the gonads of 11-day chick embryos occurred when the initial injection was made prior to the third day of incubation. This also indicates that the formation of the chick gonads, which begins on the third day of incubation, has not been obstructed by the ovine pituitary FSH. In the presence of exogenous FSH, the gonads form and differentiate into either male or female in accordance with their genetic determination. The illustration of the genotype however, in any given case is beyond the scope of this investigation.

The various doses of hormone have been found to play an important role on the life or death of the embryos. A large dose injected prior to the formation of the embryonic gonads and their sexual differentiation could either kill the developing embryo or suppress its growth. A greater mortality was observed among the male chick embryos than among the female embryos injected with these large doses. Larger amounts of FSH were required to provoke detectable changes in ovarian tissue than in testicular tissue. Also gradual increase in the amount of hormone injected during the succeeding embryonic stages had a more pronounced effect on the gonads than a constant dose.

It is reasonable to expect that any hormonal effects due to treatment require a threshold concentration of the hormone and a constant reservoir of that hormone before such effects can be measured. In our experiment, the longer the treatment, the more profound were the changes observed in the gonads.

The selection of a single FSH for our present investigation was based in part, on our interest in the effect of pituitary FSH on the embryonic gonads

of the chick and in part on the direction of this effect. A single hormone, exogenously administered, could bring about an imbalance in hormonal titer which, in turn, might result in abnormal growth such as tumors or cancer. Our experiments however, terminated on the 11th day of incubation. The apparent impaired growth of the ovarian cortex and the hyperplasia of the testicular and ovarian medulla, due to the treatment of pituitary FSH, needs further investigation. The early effects of FSH on embryonic chick gonads have been studied but the effects on later development and reproduction remain to be determined.

SUMMARY

Ovine pituitary FSH was found to affect the ovarian cortex and the testicular and ovarian medulla of chick embryos injected during the first half of the incubation period.

The extent of this effect was found to be dependent on the time of the initial injection, the dose injected and the duration of treatment.

The ideal time for the first injection during this period was the second day of incubation, that is, about one day before the gonads begin to form.

An effective dose was achieved by gradually increasing the amount of hormone injected with the increase in incubation age.

The effects were specific and the longer the treatments lasted, the more pronounced they became.

The hormone did not affect the formation of the gonads or the differentiation of sex. It did however, result in an increase in the size of the gonads in females and, to a lesser degree, those of males.

Histological study revealed a growth stimulation of the medulla of the left ovary and of the right rudiment, an inhibition of the cortex of left ovaries, and an increase in the intertubular tissue and the size of the tubules of testes.

The mitotic activity of the somatic cells and of the primordial germ cells did not appear to be influenced by the administration of this hormone.

The genetic expression appears to be compatible with the administration of FSH beginning with the onset of the formation of the gonads.

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TABLE 1

MICROSCOPIC STUDY OF EFFECTS OF PITUITARY FSH ON GONADS

Age of embryos in days	Number of treated embryos		Number of control embryos	
	Female	Male	Female	Male
4		3		2
5		3		3
6		9		7
7		6		6
8	1	1	1	1
9	2	6	2	6
10	1	2	2	2
11	23	20	25	23
18	4	9	5	7

TABLE 2

TREATMENT OF 4 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment	Dose in mg	Stage
1-I-1	4	½	3½	0.4	23
1-I-2	4	½	3½	0.4	22
1-I-3	4	½	3½	0.4	21
1-C-1	4	0	0	0	23
1-C-2	4	0	0	0	22

TABLE 3

TREATMENT OF 5 DAY OLD EMBRYOS

Animal	Sacrifice age' in days	Injection age in days	Duration of treatment in days	Dose in mg	Stage
2-I-6	5	½	5½	0.4	25
2-I-7	5	½	5½	0.4	25
2-I-8	5	½	5½	0.4	26
2-C-9	5	0	0	0	26
2-C-10	5	0	0	0	25
2-C-11	5	0	0	0	25

TABLE 4

TREATMENT OF 6 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	Length of gonads in mm Left	Right
3-I-12	6	½	5½	0.6	2.5	2.5
3-I-13	6	½	5½	0.6	2.8	2.2
5-I-1	6	0	6	0.5	2.0	1.7
5-I-2	6	0	6	0.5	1.7	1.7
5-I-3	6	0	6	0.5	1.1	1.0
7-I-1	6	0	6	0.6	2.0	1.8
7-I-2	6	0	6	0.6	1.6	2.0
7-I-3	6	0	6	0.6	2.0	2.0
7-I-4	6	0	6	0.6	2.2	2.0
3-C-14	6	0	0	0	2.0	2.0
3-C-15	6	0	0	0	2.0	2.0
5-C-1	6	0	0	0	1.8	1.7
7-C-1	6	0	0	0	2.0	2.0
7-C-2	6	0	0	0	2.0	2.0
7-C-3	6	0	0	0	2.1	2.1
7-C-4	6	0	0	0	2.0	2.0

TABLE 5

TREATMENT OF 7 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	Length of gonads in mm	
					Left	Right
4-I-16	7	½	6½	0.6	2.2	2.0
4-I-17	7	½	6½	0.6	3.0	3.0
8-I-1	7	0	7	0.5	2.1	2.0
8-I-2	7	0	7	0.5	1.6	1.6
8-I-3	7	0	7	0.5	1.8	1.8
8-I-4	7	0	7	0.5	1.7	1.8
4-C-18	7	0	0	0	2.0	2.0
4-C-19	7	-	-	-	2.2	2.2
8-C-1	7	-	-	-	1.8	1.6
8-C-2	7	-	-	-	2.0	1.5
8-C-3	7	-	-	-	2.0	1.8
8-C-4	7	-	-	-	1.8	2.0

TABLE 6

TREATMENT OF 8 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	Length of gonads in mm	
					Left	Right
4-I-20	8	½	7½	0.8	2.8	2.2
4-I-21	8	½	7½	0.8	2.8	2.5
4-C-22	8	0	0	0	2.5	2.5
4-C-23	8	0	0	0	2.2	1.7

TABLE 7

TREATMENT OF 9 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	Length of Gonads in mm Left	Right	Sex
4-I-24	9	½	8½	0.8	3.0	3.0	♂
4-I-25	9	½	8½	0.8	2.5	2.4	♂
6-I-1	9	2	7	0.8	3.0	2.8	♀
6-I-2	9	2	7	0.8	3.0	3.0	♂
9-I-1	9	6	3	1.4	3.2	3.2	♂
9-I-2	9	6	3	1.4	3.0	3.0	♂
9-I-3	9	6	3	1.4	3.0	2.0	♀
9-I-4	9	6	3	1.4	2.5	2.4	♂
4-C-26	9	0	0	0	2.8	3.0	♂
4-C-27	9	0	0	0	3.0	3.0	♂
6-C-1	9	0	0	0	2.5	2.0	♀
6-C-2	9	0	0	0	3.0	3.0	♂
9-C-1	9	0	0	0	2.8	2.6	♂
9-C-2	9	0	0	0	2.5	2.5	♂
9-C-3	9	0	0	0	2.2	2.0	♂
9-C-4	9	0	0	0	3.0	2.5	♀

TABLE 8

TREATMENT OF 10 DAY OLD EMBRYOS

Animal	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	Length of Gonads in mm Left	Right	Sex
12-I-1	10	0	10	1.5	2.58	3.12	♂
12-I-2	10	0	10	1.5	2.58	2.47	♂
12-I-3	10	0	10	1.5	3.52	2.70	♀
12-C-1	10	-	0	0	2.58	2.35	♀
12-C-2	10	-	0	0	3.17	2.58	♀
12-C-3	10	-	0	0	2.64	2.82	♂
12-C-4	10	-	0	0	2.35	2.47	♂

TABLE 9
TREATMENT OF 11 DAY OLD EMBRYOS

Animal No.	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	No. sex F.	M.	Result
6	11	0	11	1.5	-	2	-
6	11	2	9	1.7	3	-	X
				0.6	12	13	X
74	11	5	6	0.4	1	1	X
				0.2	-	1	X
12	11	6	5	1.5	7	1	X
17	11	7	4	1.5	-	2	-

X - degree of effect
- - no significant effect

TABLE 10

TREATMENT OF 18 DAY OLD EMBRYOS

Animal No.	Sacrifice age in days	Injection age in days	Duration of treatment in days	Dose in mg	No. sex F.	M.	Result
16	18	5	13	1.2	3	6	X
24	18	5	13	0.6	1	3	X
38*	18	5	13	0.6	1	1	X
18*	18	6	12	1.2	13	5	X

X - significant effect
- - no or little effect
* - additional experiments

TABLE 11

WEIGHT LOSS IN TREATED AND CONTROL EGGS DURING INCUBATION

Age in days	Treated (T) Control (C)	No. of embryo	Total weight lost in grams	Mean loss of wt. in grams
7	T	4	8.090	2.025
	C	4	6.080	1.520
10	T	2	5.840	2.920
	C	6	14.150	2.408
11	T	13	28.980	2.230
	C	18	44.000	2.440

TABLE 12

RELATIONSHIP BETWEEN EGG AND EMBRYO WEIGHTS IN TREATED AND CONTROLS OF ELEVEN DAYS INCUBATION

Total No.	Treated (T) Control (C)	Total weight in grams	Mean value in grams	Grams of egg / grams of embryo
Egg 19	T	983.55	51.76	17.02
Egg 22	C	1170.74	53.21	16.42
Embryo 19	T	57.80	3.04	1
Embryo 22	C	71.29	3.24	1

PLATE 1

EXPLANATION OF FIGURES

The embryo of figure 2 was sacrificed twenty-four hours and that of 4, Forty-eight hours following final injection.

1. A control female embryo of 11 days. Development was at stage 37 and body weight 3.05 gm on sacrifice. Note smooth appearance of both ovaries and pointed ends of the left one. X 11.

2 An experimental female embryo of 11 days, stage 37, which had a body weight of 3.03 gm on sacrifice. The embryo received the initial injection of FSH on the second day of incubation and every other day thereafter until the tenth day. The dosages per injection increased from 0.2 to 0.6 mg. The total amount administered was 1.7 mg and the duration of treatment nine days. Note the larger size, rough surface, and rounded ends of the left ovary and right rudiment when compared with control gonads in figure 1. X 11.

3 A control male embryo of 11 days, stage 38, which weighed of 3.50 gm at time of sacrifice. Note the size of both testes. X 11.

4 An experimental male embryo of 11 days, stage 37, which weighed 3.49 mg at time of sacrifice. The embryo received initial injection on the fifth day and every other day thereafter until the ninth day. The dose was 0.2 mg / injection and the total amount administered 0.6 mg. The duration of treatment was six days. Note the increase in the width of both testes when compared with those of control in figure 3. X 11.

FIGURE 1

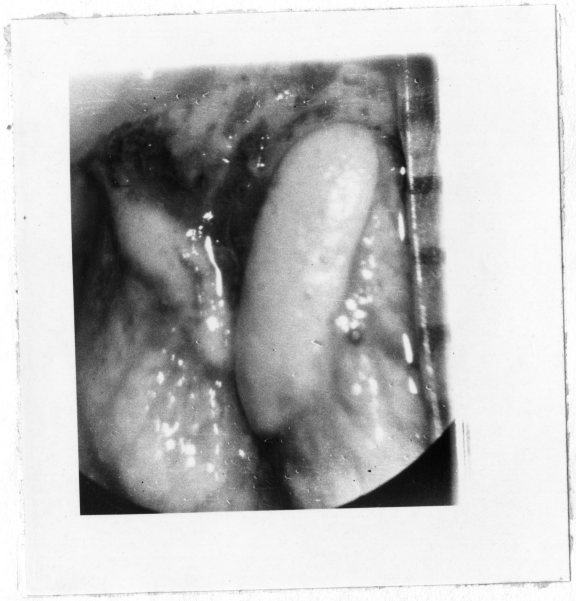

FIGURE 2

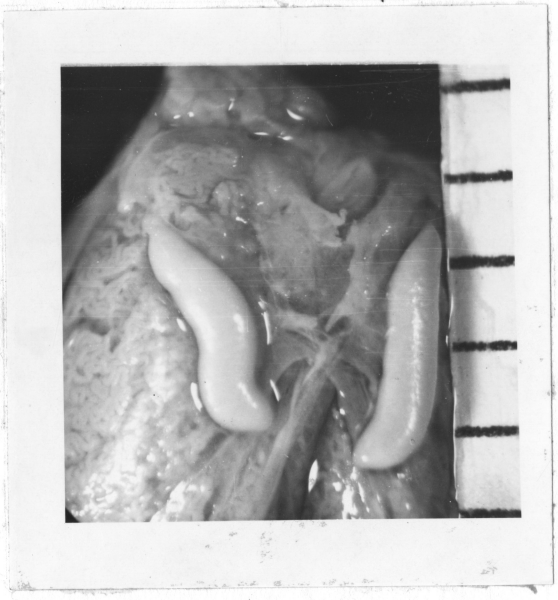

FIGURE 3

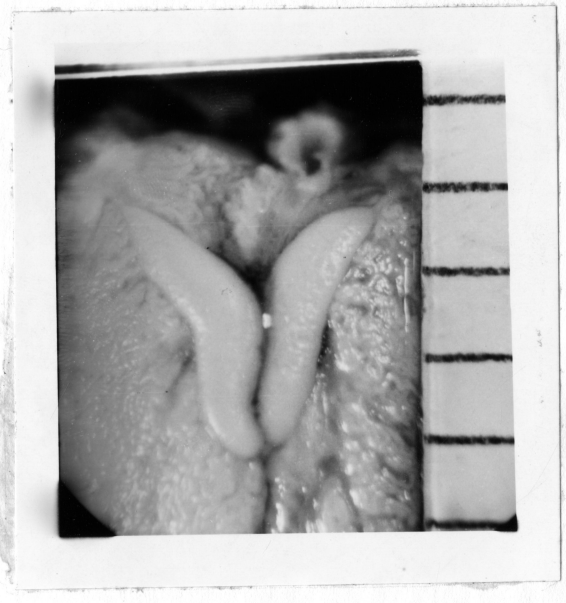

FIGURE 4

PLATE 2

EXPLANATION OF FIGURES

These embryos were sacrificed three days following final injection.

5 A control female embryo of 18 days. Development was at stage 43 and body weight 23.20 gm at time of sacrifice. Note oval shape of the left ovary. X 9.

6 An experimental female embryo of 18 days, stage 42, which had a body weight of 20.00 gm on sacrifice. The embryo received the initial injection of FSH on the fifth day of incubation and every other day thereafter until the fifteenth day. The dose was 0.2 mg / injection and the total amount administered 1.2 mg. The duration of treatment was ten days. Note size of left ovary and of right rudiment and lobulation of the left ovary. X 9.

7 A control male embryo of 18 days, stage 43, which weighed 22.35 mg at time of sacrifice. X 9.

8 An experimental male embryo of 18 days, stage 43, which weighed 22.20 mg on sacrifice. The embryo received initial injection on the fifth day and every other day thereafter until the fifteenth day. The dose was 0.2 mg / injection and the total amount administered 1.2 mg. The duration of treatment was ten days. Note increase in size of testes, particularly of left, when compared with those of control in figure 7, X 9.

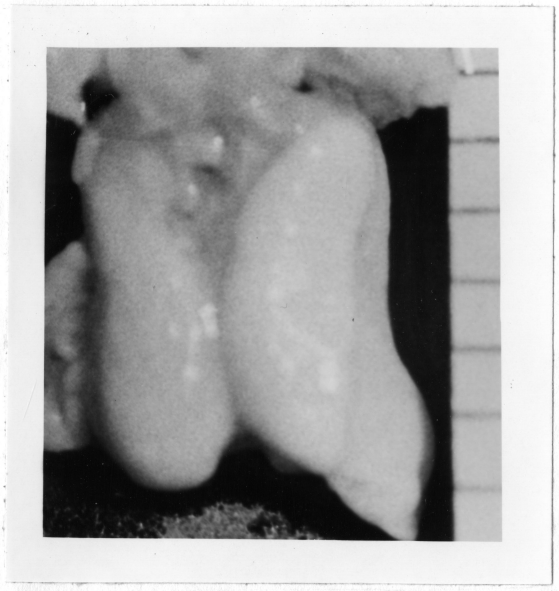

FIGURE 5

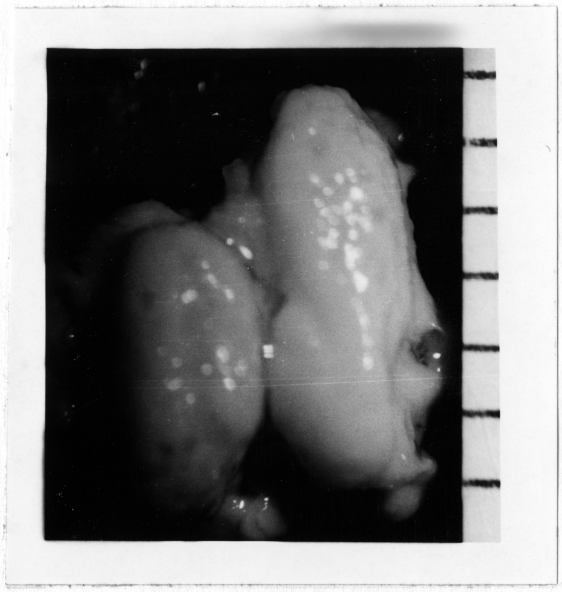

FIGURE 6

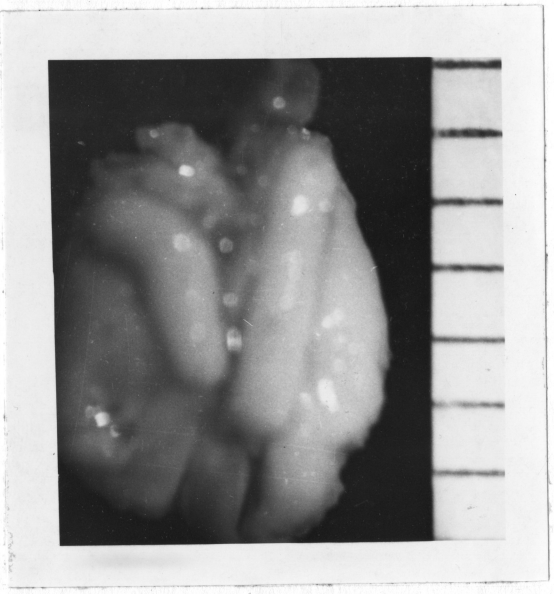

FIGURE 7

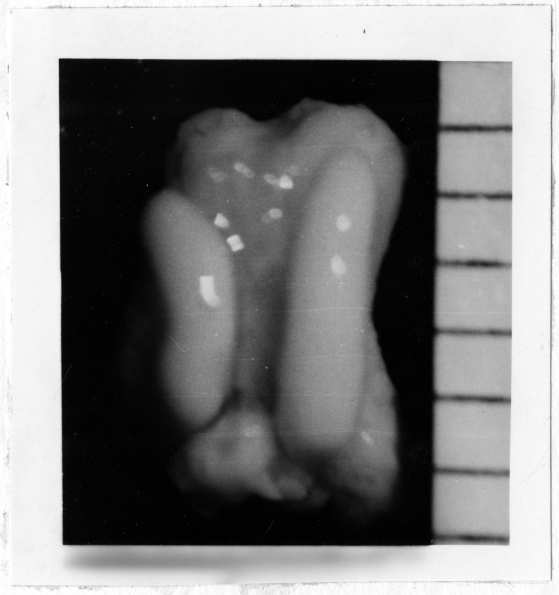

FIGURE 8

PLATE 3

EXPLANATION OF FIGURES

9 A cross section through the caudal third of the left ovary shown in figure 1. Note shape and size of the left ovary (LO) and of the right rudiment (RR). The gonads are quite compact. X 67.

10 A cross section through the caudal third of the left ovary shown in figure 2. The mushroom shape and particularly the greater size of the left ovary is in sharp contrast to that of the control shown in figure 9. Note increase in vacuolation of the medullary portion of the left ovary (LO) and of the right rudiment (RR) when compared with control. X 67.

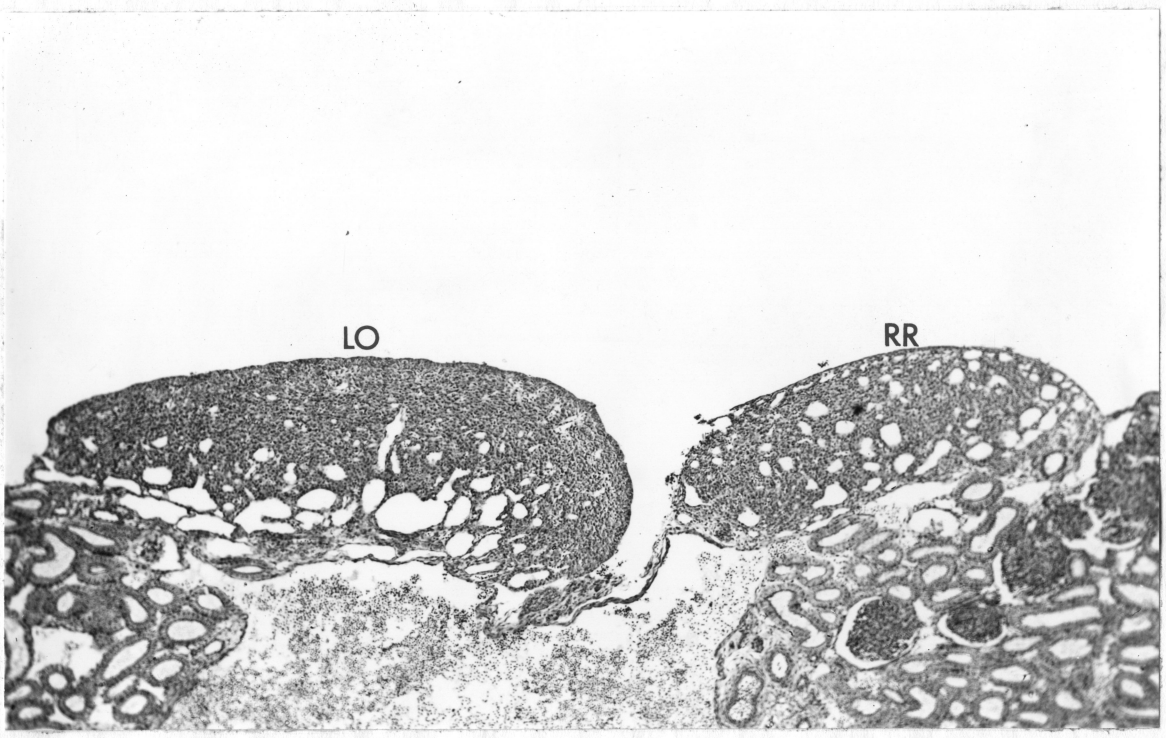

FIGURE 9

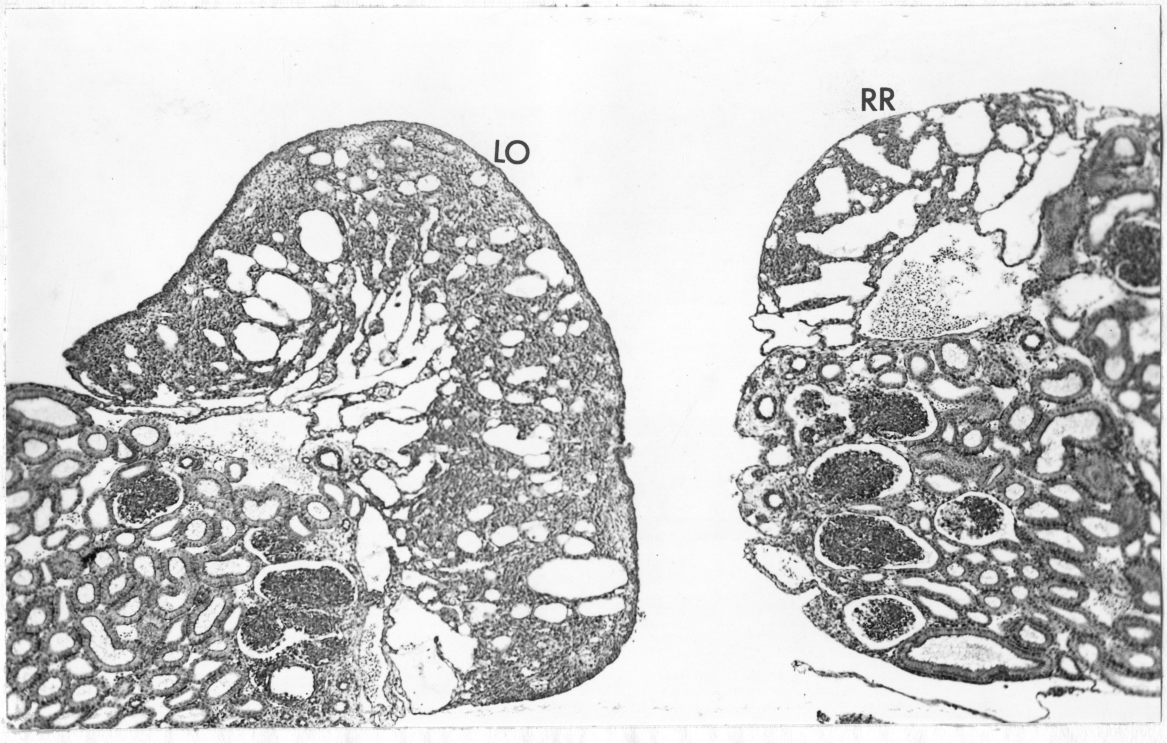

FIGURE 10

PLATE 4

EXPLANATION OF FIGURES

11 A portion of the control left ovary shown in figure 9. Note thick germinal epithelium (GE), well developed cortex (CO), and compact medulla (MED) with small distended tubules (DT). Primordial germ cells (PGCs) may be seen. X 560.

12 A portion of the experimental left ovary shown in figure 10. Note relatively thin germinal epithelium (GE), and a hypertrophied medulla with solid cords (MED) and numerous distended tubules (DT). The distended medullary tubules invade the domain of cortex, in places. Also note the position and relation of the cells lining the tubles. X 560.

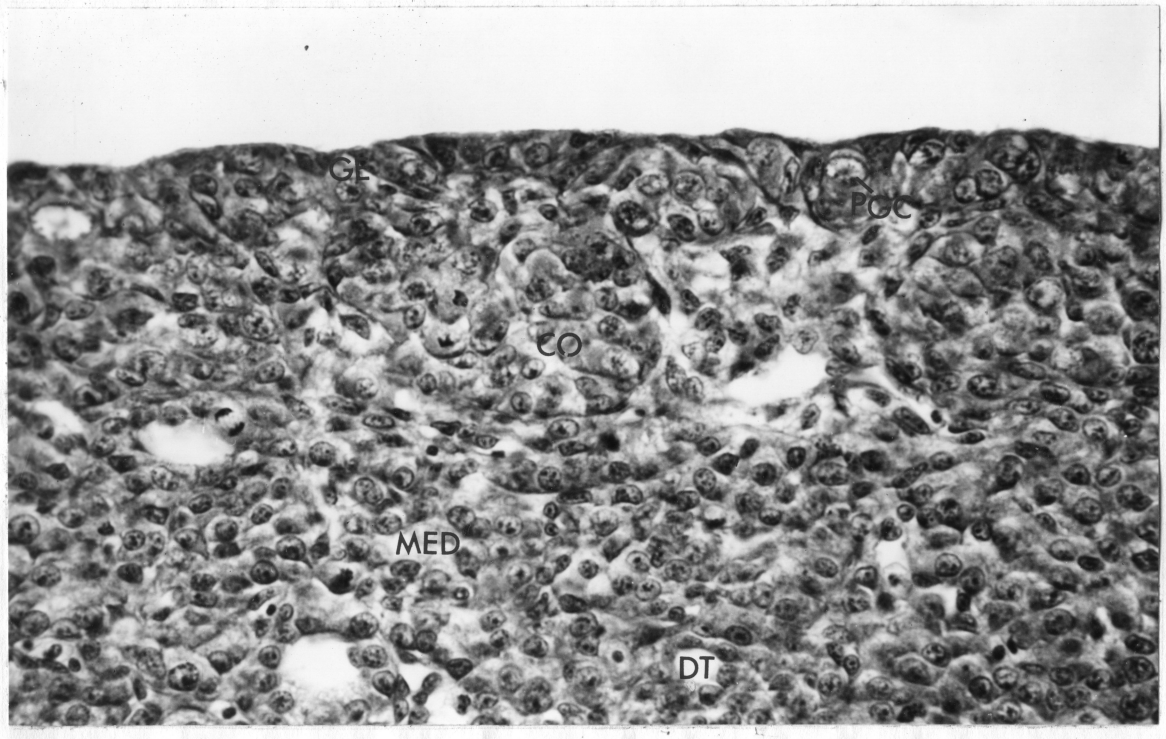

FIGURE 11

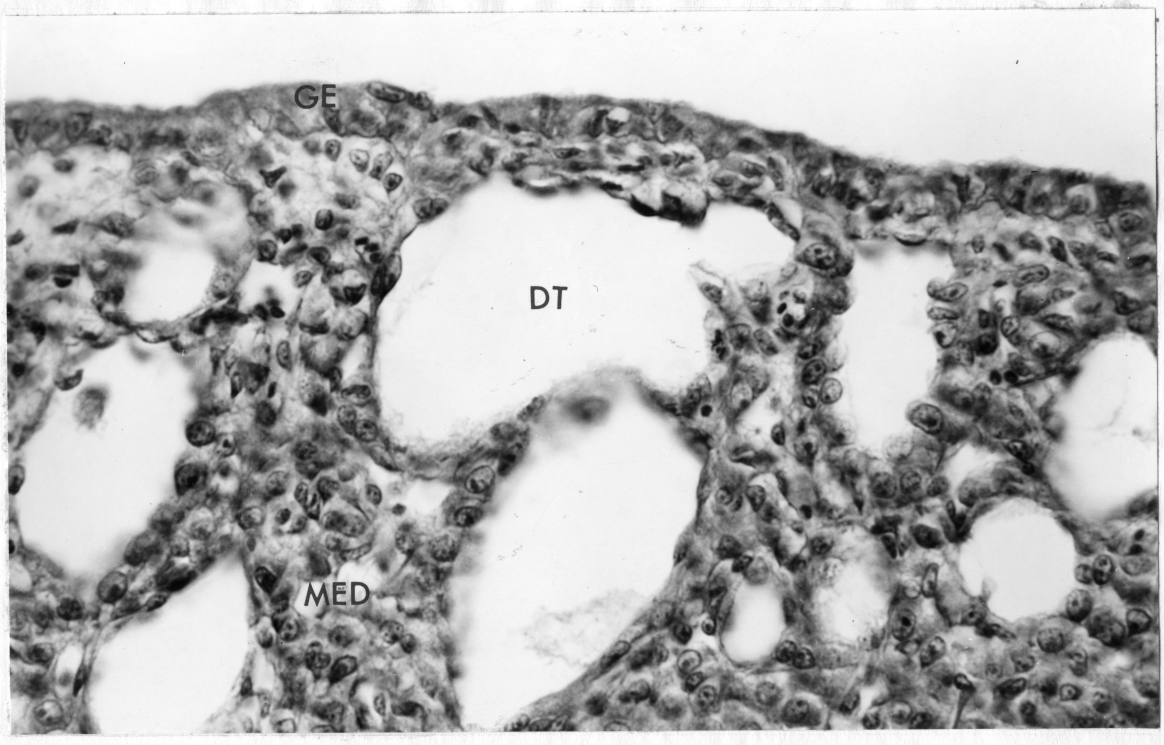

FIGURE 12

PLATE 5

EXPLANATION OF FIGURES

13 A portion of the right rudimentary gonad of control shown in figure 9. Note the relation of the germinal epithelium (GE) and medulla (MED). The medulla consists of solid cords and small distended tubules (DT). X 560.

14 A portion of the experimental right rudimentary gonad seen in figure 10. The germinal epithelium (GE) is not uniform in thickness. Large distended tubules (DT) are the prominent feature. X 560.

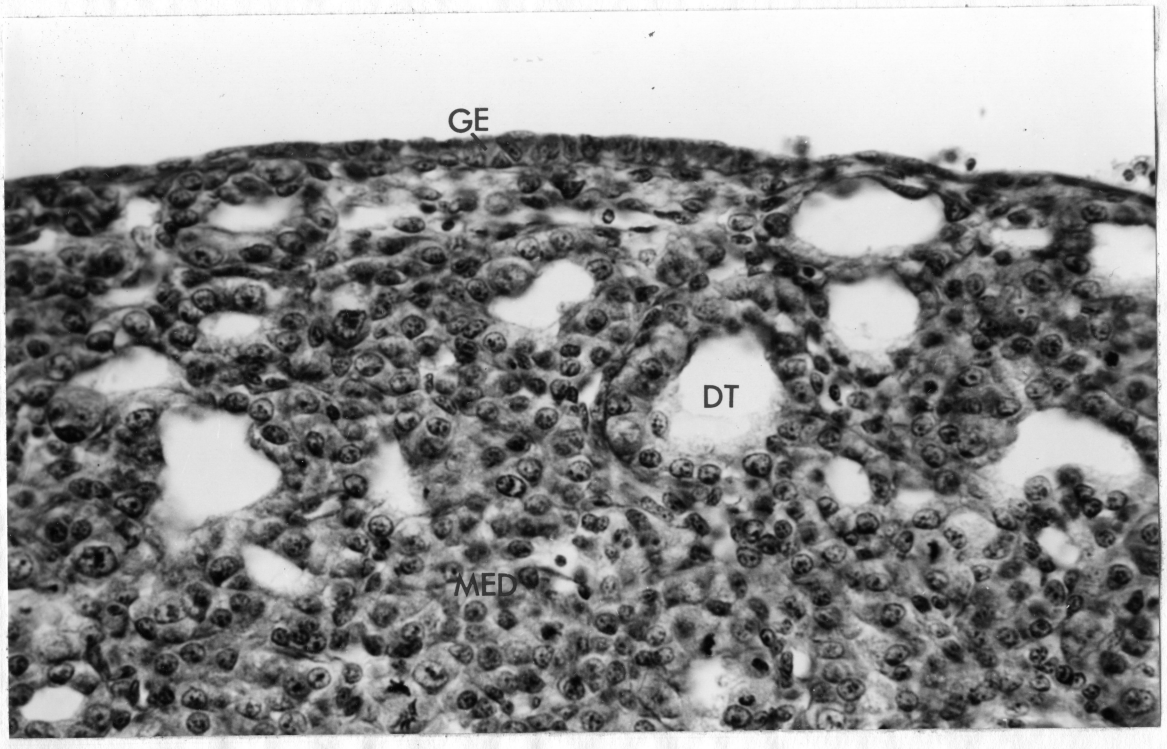

FIGURE 13

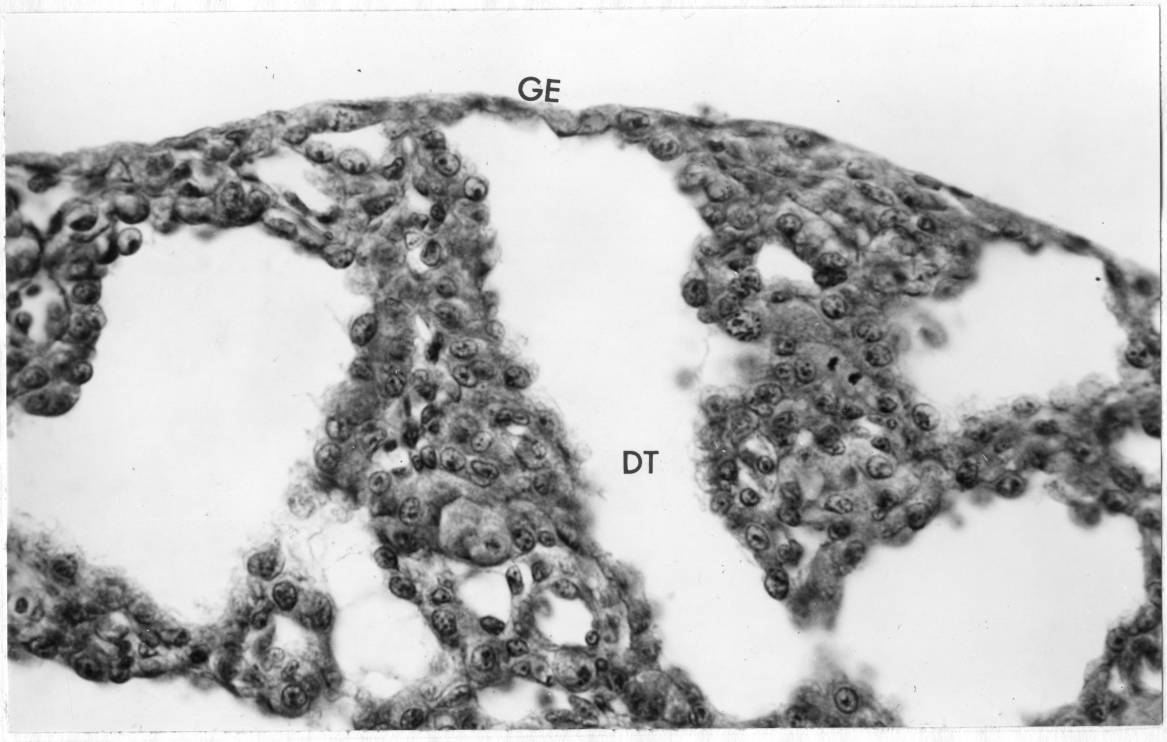

FIGURE 14

PLATE 6

EXPLANATION OF FIGURES

15 A cross section through the caudal third of the gonads of control female embryo shown in figure 5. Note the thick, regular, continuous cortex of the left ovary (LO) and solid medulla of the right rudimentary gonad (RR). X 53.

16 A cross section through the caudal third of the gonads of experimental female embryo shown in figure 6. Note the irregular and discontinuous cortex (LO) and the hypertrophy of the medulla of the left ovary and that of the right rudimentary gonad (RR) which are prominent features of FSH treatment. X 53.

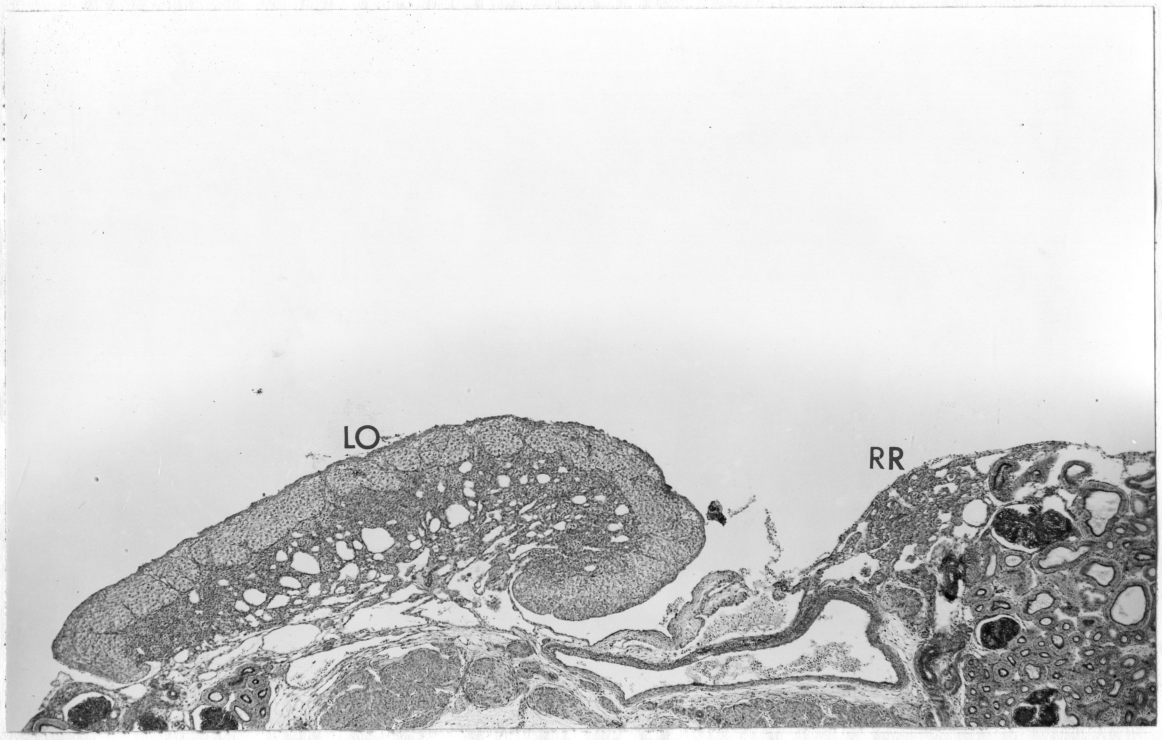

FIGURE 15

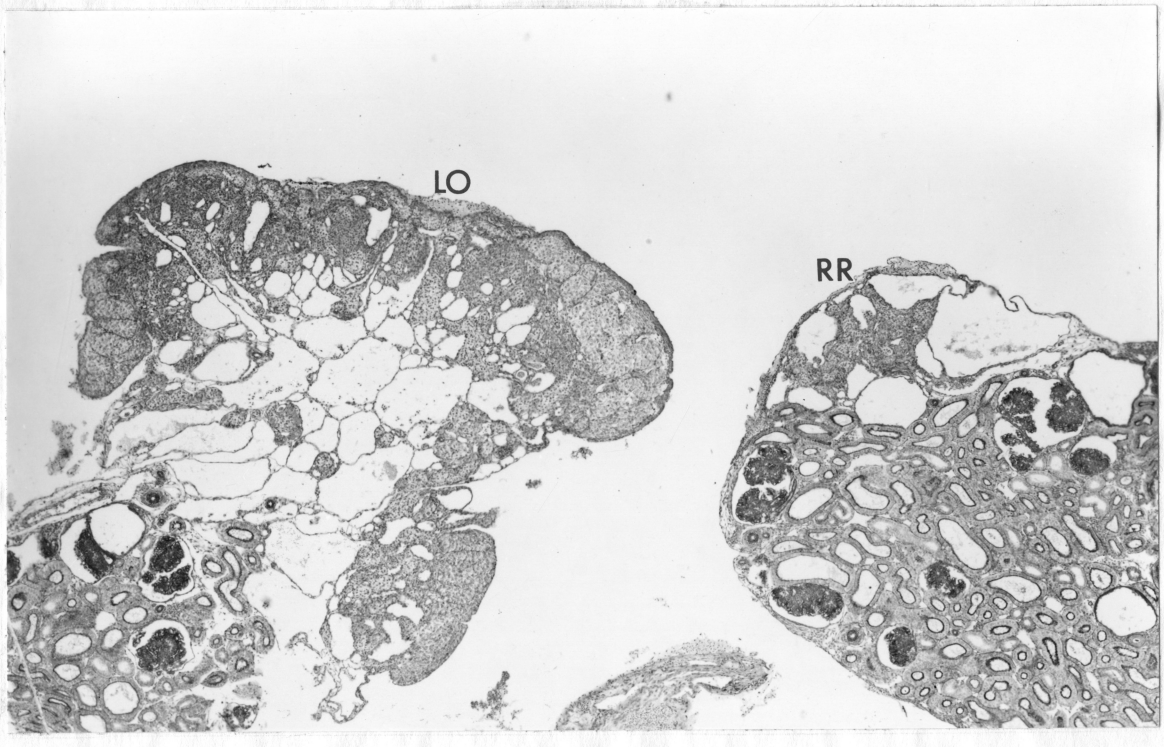

FIGURE 16

PLATE 7

EXPLANATION OF FIGURES

17 A portion of the control left ovary seen in figure 15. Note the compact germinal epithelium (GE) separated from the cortex (CO) by the secondary tunica albuginea (STA). The cortex is well developed and separated from underlying medulla (SM) by the primary tunica albuginea (PTA). X 560.

18 A portion of the experimental left ovary shown in figure 16. Note the relatively less compact germinal epithelium (GE) separated from underlying medulla (SM) by the primary tunica albuginea (PTA). The distended tubules (DT) in this area have pushed toward the surface, reaching the germinal epithelium and occupy the domain of the cortex. X 560.

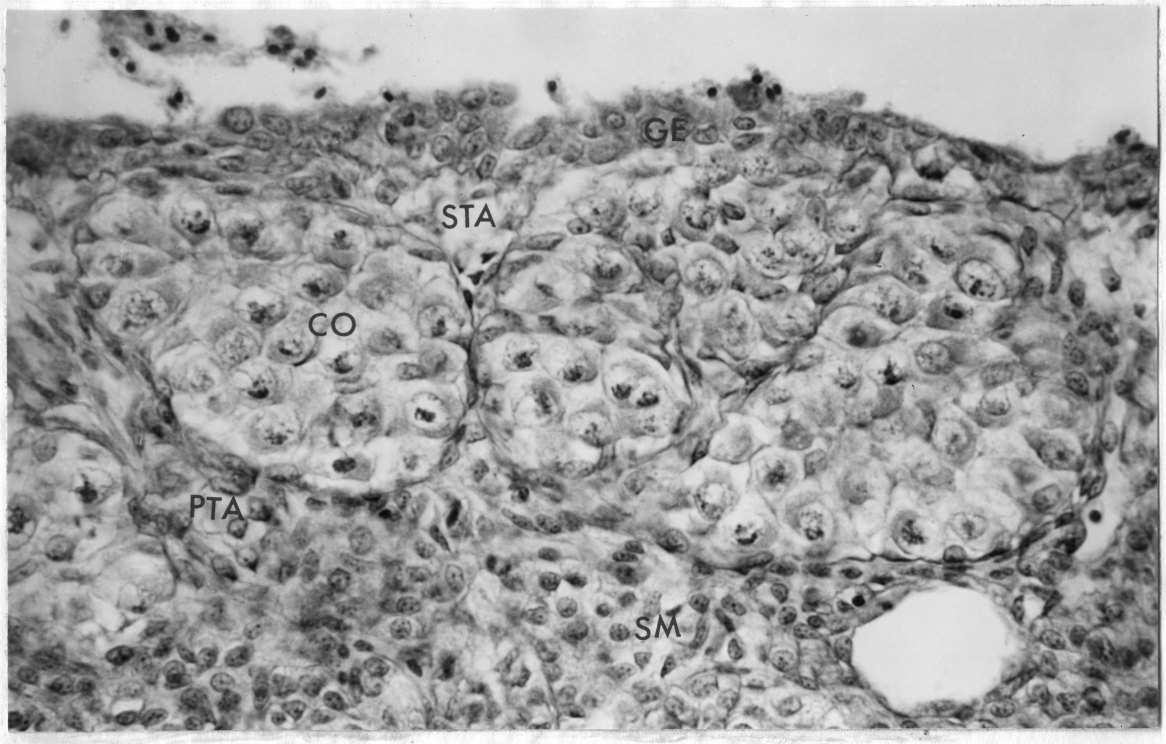

FIGURE 17

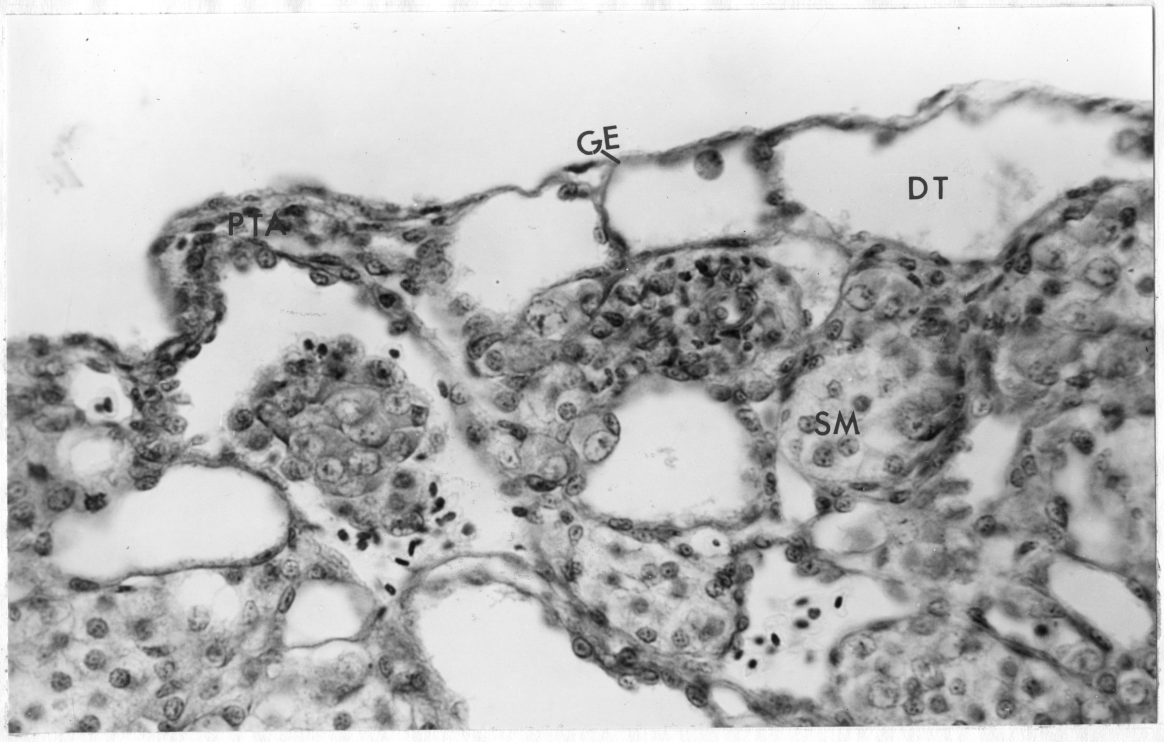

FIGURE 18

PLATE 8

EXPLANATION OF FIGURES

19 A portion of the right rudimentary gonad of control shown in figure 15. The germinal epithelium (GE) is composed of a single layer of squamous cells and is in close relation with the medulla. The right rudimentary gonad is composed primarily of medullary cords (MC) and small distended tubules. Primordial germ cells (PGCs) are seen among the solid cords. X 560.

20 A portion of the experimental right rudimentary gonad shown in figure 16. The germinal epithelium (GE) consists mostly of connective tissue and lies against distended tubules (DT). Note the empty lumina and the cells lining the tubules. The solid portion of the medulla (MED) is small in comparison with that of the control. X 560.

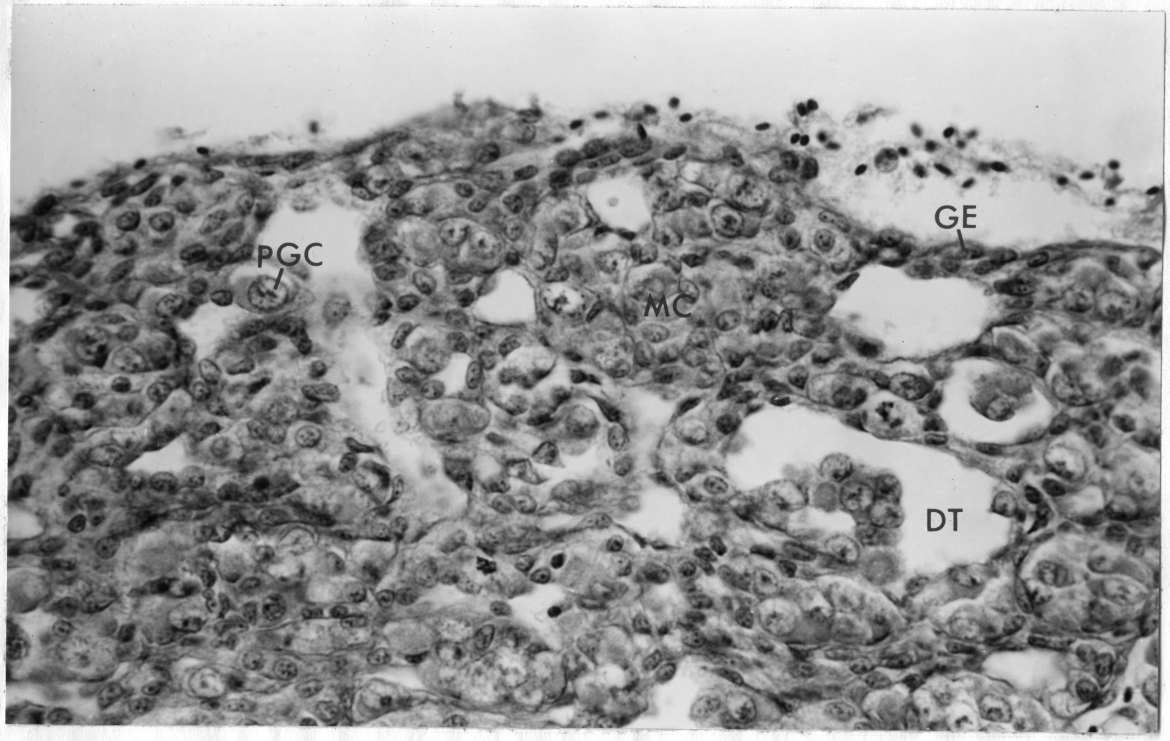

FIGURE 19

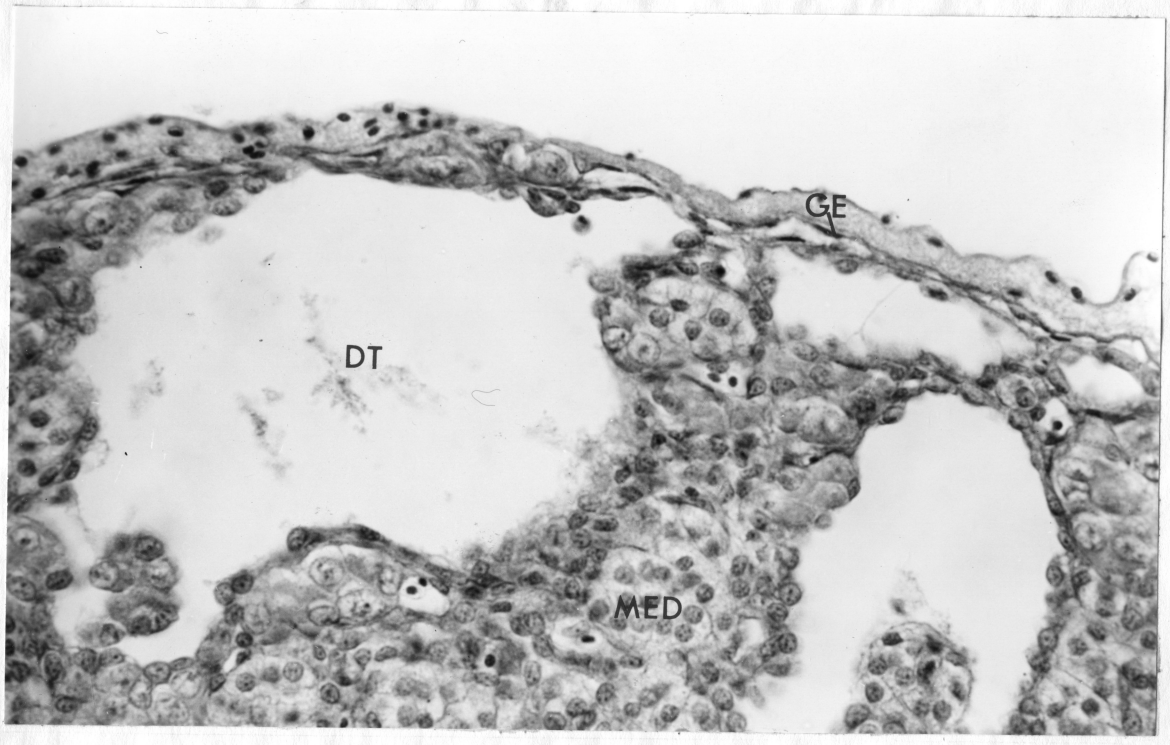

FIGURE 20

PLATE 9

EXPLANATION OF FIGURES

21 A cross section through the caudal half of control testes shown in figure 7. Note the flattened shape of both the left (LT) and the right (RT) testis. Note presence of small, mostly circular, tubules and a relatively abundant intertubular tissue. X 53.

22 A cross section through the caudal half of experimental testes shown in figure 8. The cross section of both the left (LT) and the right (RT) testis has become more or less rounded. Note the presence of larger tubules showing considerable branching and a relative reduction in the intertubular tissues when compared with the control shown in figure 21. X 53.

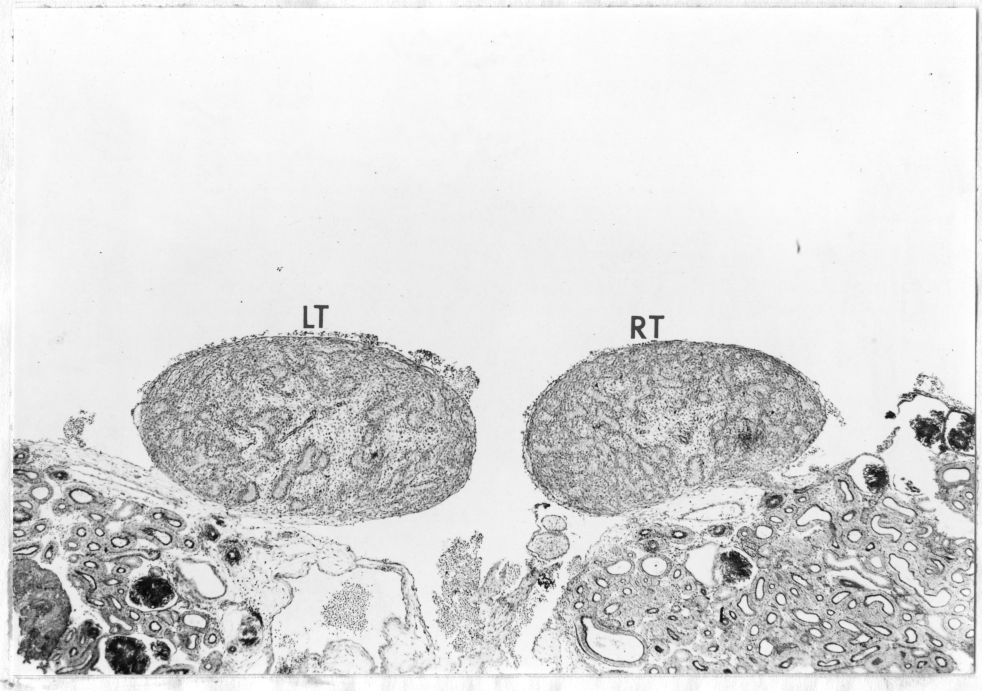

FIGURE 21

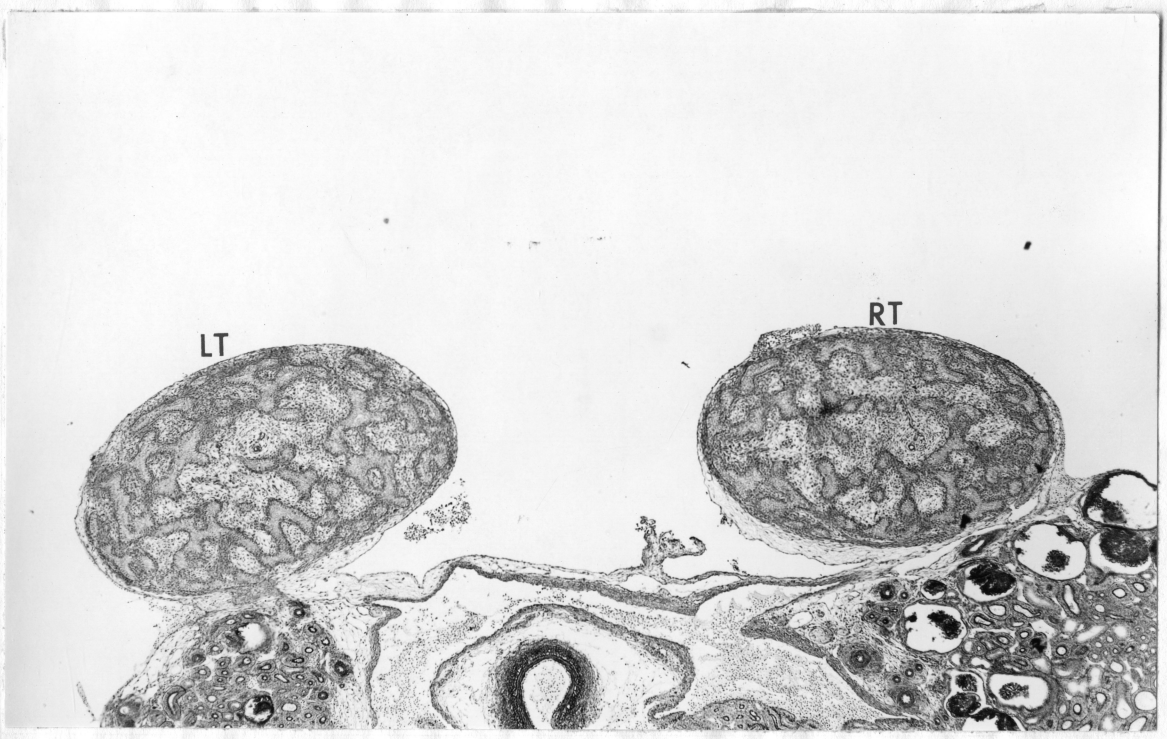

FIGURE 22

PLATE 10

EXPLANATION OF FIGURES

23 A central portion of the control cross section shown in figure 21. The seminiferous tubules (ST) are small, show no lumina, and are relatively numerous. The intertubular tissue (IT) is quite dense and abundant. X 560.

24 A central portion of the experimental cross section shown in figure 22. Note that seminiferous tubules (ST) are noticeably larger but less numerous than in the control. The intertubular tissue (IT) is loosely arranged and less abundant than in the control figure 23. X 560.

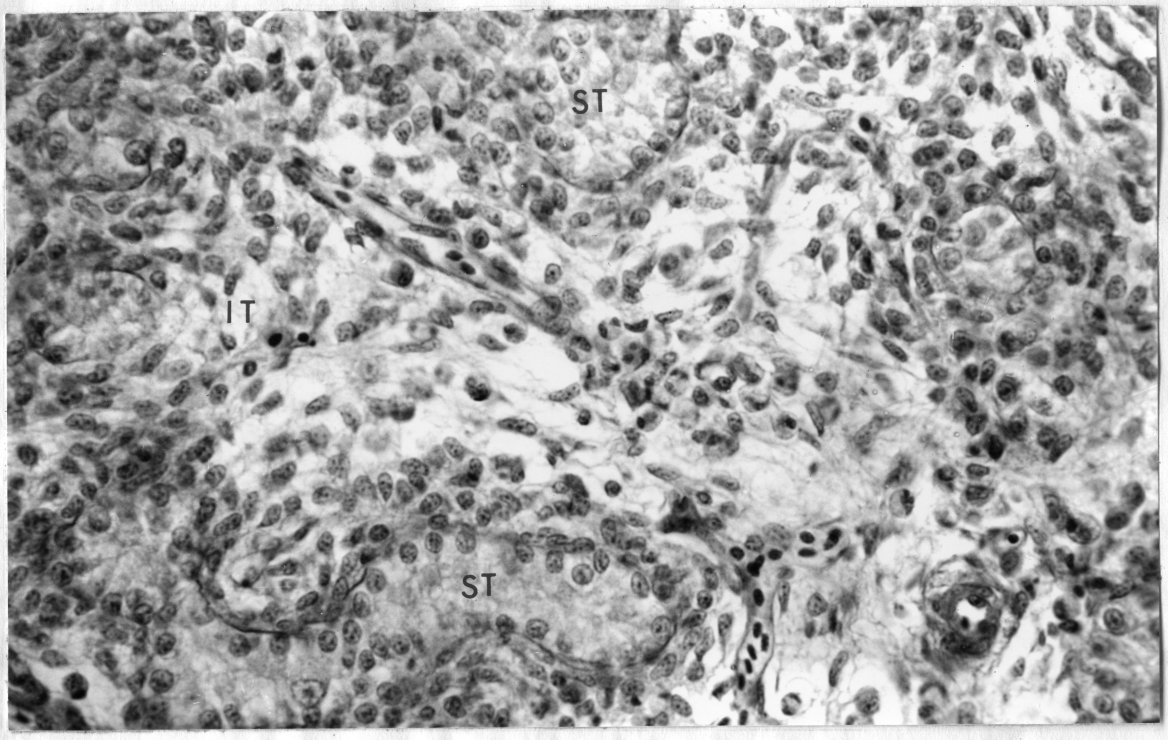

FIGURE 23

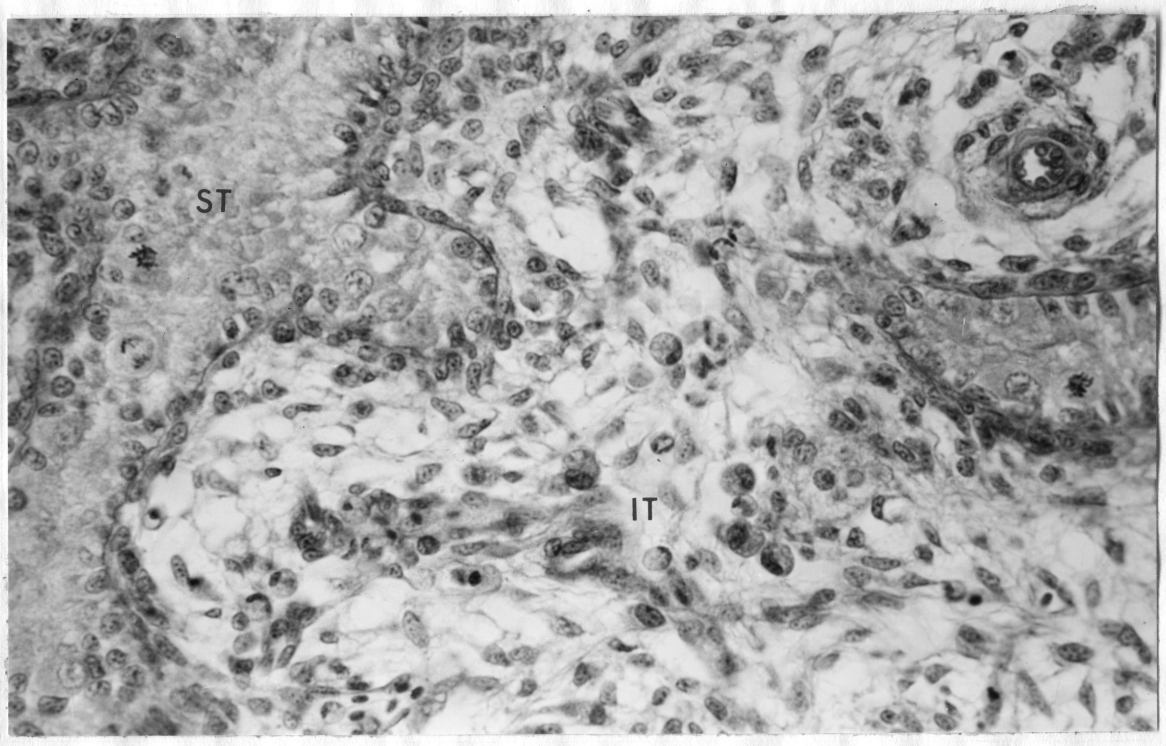

FIGURE 24

APPROVAL SHEET

The dissertation submitted by Pham Duc Tuan has been read and approved by three members of the Board of Examiners.

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

The dissertation is therefore accepted in partial fulfillment for the degree of Master of Science.

May 29, 1967
Date

Signature of Adviser