12-1968

Quantitative and qualitative determinations of gametogenesis and radiation effects in the prenatal bovine testis

George Herman Matschke

To the Graduate Council:

I am submitting herewith a dissertation written by George Herman Matschke entitled "Quantitative and qualitative determinations of gametogenesis and radiation effects in the prenatal bovine testis." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

R. L. Murphree, Major Professor

We have read this dissertation and recommend its acceptance:

R. G. Cragle, J. C. Howell, H. V. Shirley, B. H. Erickson

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 12, 1968

To the Graduate Council:

I am submitting herewith a dissertation written by George Herman Matschke, entitled "Quantitative and Qualitative Determinations of Gametogenesis and Radiation Effects in the Prenatal Bovine Testis." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

R. L. Murphree
Major Professor

We have read this dissertation and recommend its acceptance:

Raymond G Cragle

J. C. Howard

H. V. Shirley

B H Erickson

Accepted for the Council:

Hilton A. Smith
Vice Chancellor for
Graduate Studies and Research

QUANTITATIVE AND QUALITATIVE DETERMINATIONS OF GAMETOGENESIS AND RADIATION EFFECTS IN THE PRENATAL BOVINE TESTIS

A Dissertation

Presented to

the Graduate Council of

The University of Tennessee

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

George Herman Matschke

December 1968

ACKNOWLEDGMENT

The author wishes to express gratitude to Dr. N. S. Hall, Laboratory Director, for providing the financial support and laboratory facilities for conducting this research problem.

Special recognition is given to Dr. R. L. Murphree, Major Professor, for his assistance, guidance, and encouragement through the author's graduate program.

Sincere appreciation is given to Dr. B. H. Erickson for his assistance in suggesting and planning this research study.

Recognition is given to Drs. R. G. Cragle, J. C. Howell, and H. V. Shirley for serving on the graduate committee.

The author is indebted to R. A. Reynolds, J. R. Eblen, B. R. Kyle, H. R. Tinch, and R. B. Graves for their assistance in the semen collection studies and to H. I. Cross for her laboratory assistance throughout the study. Also, recognition is given to E. L. Russell and C. D. Shell for their aid in processing the tissue sections.

Special recognition is given to the secretaries for typing the rough drafts of this dissertation: I. T. Freshour, C. H. Hawkins, E. H. Rampley, M. C. Rice, and to M. R. Kerr for typing the final draft.

My affectionate thanks go to my wife and daughters for their patience and profound understanding during this period of graduate study.

ABSTRACT

The objectives of this study were to (1) trace the development of the prenatal bovine testis, (2) evaluate the quantitative and qualitative changes in the germ cell population, (3) evaluate the effects of gamma radiation on the developing testis, and (4) correlate sperm production with testicular morphology.

The population of germ cells, number of mitotic figures for germinal and supporting cells, and testicular volume were determined in testes from known-age fetuses.

Fifty-six males were irradiated with 400 R (air dose to dam) ^{60}Co gamma radiation at ages ranging from 15-280 days postcoitum. Seven control and 11 irradiated males were unilaterally castrated at birth. Five control and 38 irradiated bulls were castrated at approximately 10 months of age. The testes were prepared for histological analyses. Semen was collected from control, intact, and unilateral-castrated irradiated animals when they were approximately 2 years old.

The germinal ridge was first observed at 32 days postcoitum and sex differentiation occurred as early as day 36. The sex cords were unorganized until about day 70; at this age the supporting cells were located adjacent to the basement membrane and the gonocytes or primitive germ cells were positioned in the lumen of the sex cord or primitive seminiferous tubule.

The gonocyte, prior to day 120, had three small acidophilic nucleoli; at later ages a gonocyte with one large acidophilic nucleolus

appeared and this cellular type was prevalent at birth. Following tubular organization the cytoplasmic to nuclear ratio increased with age.

The overall germ cell population increased from 44 at days 32-35 to 12,000,000 at days 151-180. Only a slight increase in germ cell numbers was noted from 211-240 days until birth (283 days). The mitotic figure index (number of mitotic figures per 50,000 germ cells) was high while the gonad was in the germinal ridge stage and reached a peak at 36-38 days. The index remained high until day 90 and then dropped abruptly during the 91-120 day period. Some germinal mitoses were noted throughout the remainder of the prenatal period with a minor increase occurring in the last days preceeding parturition. Necrotic gonocytes were noted at days 120-150 and their numbers peaked at 241-280 days; necrosis, however, never affected more than 1 percent of the population at any given stage in gestation. The germ cell population prior to day 70 was apparently unaffected by irradiation (400 R to dam) but following irradiation at days 80-130 germ cell numbers were reduced to about 7 percent of the control value in testes excised at 3-19 days postpartum.

In testes removed 10 months postnatally, no damage was observed in testes irradiated prior to day 70, but in testes irradiated during the 70-90 day period 69 percent of the 1200 cross-sections observed were sterile. Sterility among tubular cross-sections increased to 88 percent at 91-120 days postcoitum, remained essentially unchanged to day 210 and decreased to 20 percent at 241-280 days of gestation.

Bulls irradiated at 130 days of gestation produced 70 and 78 percent fewer sperm-per-ejaculate than their counterpart intact and unilateral-castrate controls, respectively. Sperm motility in the irradiated intact and unilateral castrates was 37 and 20 percent less than the intact and unilateral-castrate controls.

Since 400 R is a near-lethal dose for the dam and since bulls irradiated at a stage of maximum sensitivity produced seven-tenths billion sperm-per-ejaculate, a number well above the 6 million motile sperm required for optimum fertility in artificial insemination, the probability of sterilizing the bull in the prenatal state with a single-acute whole-body dose of gamma-irradiation is low.

TABLE OF CONTENTS

LIST OF TABLES

TABLE PAGE

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Gametogenesis and prenatal testicular morphology follow the same general pattern in all mammalian species. The germ cells migrate to the germinal ridge and later become incorporated into the sex cords along with the supporting cells. As the organism ages, germ cells increase in numbers and size.

Prior to birth a large number of the germ cells undergo degeneration. Despite this interspecific uniformity in growth and development of the germ cells, radiosensitivity is not uniform but varies according to the species.

The objectives of this study were to (1) trace the histology of the prenatal bovine testis (2) measure qualitatively and quantitatively the germ cells in the testis and (3) measure sperm production of the prenatally-irradiated bulls and to correlate this production with testicular morphology.

CHAPTER II

REVIEW OF LITERATURE

EMBRYOLOGY OF THE TESTIS

Gross Development

The earliest appearance of the gonads is associated with the formation of the nephric system. The gonads arise as ridgelike thickenings (gonadial ridge, germinal ridge) on the ventral border of the mesonephric kidney. The germinal ridge is comprised of closely-packed cells covered by mesothelium. The mesothelium is continuous with the mesothelial covering of the mesonephric kidney (Patten, 1946). The germinal ridge is an indifferent gonad. The mesothelium differentiates from the adjacent kidney mesothelium into the germinal epithelium. Before the gonad develops into a testis or an ovary, cells of the germinal epithelium first grow into the underlying mesenchyme and form cord-like masses. In males these cords become sharply delimited and differentiate into the seminiferous tubules. At the same time developing directly beneath the germinal epithelium is a conspicuous zone of young connective tissue called the tunic albuginea. In the female the cords become less distinct and the germ cells are scattered in the mesenchymal stroma, and a less conspicuous zone of connective tissue beneath the germinal epithelium is formed (Patten, 1946).

The formation of the tunica albuginea and, consequently, sex differentiation of males occur at different ages of gestation. This

structure is visible on approximately day 14 in the rat, day 27 in swine, and day 36 in cattle (Hargitt, 1926, Erickson, Murphree, and Andrews, 1963, and Krehgbiel, 1963).

Primordial germ cells or gonocytes are incorporated inside the sex cord, but intracord cellular organization is lacking until day 17 in the rat, day 50 in the pig and day 80 in cattle (Erickson, 1965). At this time the supporting cells become aligned with the periphery of the cord, leaving the gonocytes at the center.

Chiquoine (1954) selectively stained mouse germ cells using the Gomori histochemical technique for alkaline phosphatase. The germ cells were stained black by a precipitate of cobalt sulphide. The primordial germ cells according to Chiquoine (1954) are first recognizable in 8-day embryos and are seen scattered among cells of the caudal end of the primitive streak, the root of the allantoic mesoderm, and the underlying yolk sac splanchnopleure. Within the splanchnopleure of the yolk sac the germ cells are mixed with entodermal or mesodermal cells. Initially, the germ cells migrate caudally and ventrally to the yolk sac and thence caudally into the allantoic mesoderm. At 9 days these cells have migrated to the embryo proper and were seen by Chiquoine (1954) in the areas of the lateral and caudal face of the open mid gut, in the stalk of the allantois, and in the splanchnopleure of the hind gut. In the 11 and 12 day embryos the cells were found in the earlier position of the germinal ridges. During the course of their migration individual germ cells sometimes exhibit pseudopod-like cell processes. During the course of their migration, only a few germ cells deviate to so great an extent from the described pathway that they can be considered as lost to the gonad (Chiquoine, 1954).

Ultrastructure of Germ Cells

At 15.5 days postcoitum (p.c.) male rat germ cells lose their rounded shape and become somewhat angular. At 16.5 days p.c. the most prominent change within the germ cells consists of the accumulation of mitochondria at one pole of the cell (mitochondrial polarization), when mitotic activity begins to decline. The germ cells remain closely packed and cytoplasmic bridges between adjacent gonocytes are common. By 19 days p.c. mitochondria again become distributed more evenly throughout the cytoplasm, and specific organelles (B and A bodies) appear in the cytoplasm. Three varieties of B bodies are found: B_1, B_2, and B_3. The B bodies disappear after birth from germ cells which become attached to the basement membrane. The persistence of B bodies in unattached germ cells and their abundance in cells with swollen endoplasmic reticulum and abnormal mitochondria suggest that they may somehow be involved in spontaneous degeneration. The establishment of cytoplasmic contact between germ cells and the basement membrane appears to be a prerequisite for subsequent survival and mitotic activity. The nature and function of granular cytoplasmic inclusions and A bodies, are obscure (Franchi and Mandl, 1966).

Spermatogenesis

The germ cells or gonocytes of rats are larger than the somatic cells and possess spherical or near spherical nuclei. As the gonocytes increase in size with age their nuclei become less chromophilic, the nucleoli become round and prominent, and the cytoplasmic:nuclear ratio increases (Beaumont and Mandl, 1963). From the 20th day of gestation

large numbers of germ cells show changes indicative of degeneration, i.e., enlargement of the nucleus, crinkling or fragmentation of the nuclear membrane or both, and contraction of the cytoplasm. Rat testes aged 4-6.5 days postpartum (p.p.) contain several morphologically distinguishable cell-types; they are normal or degenerating gonocytes and a third type known as transitional cells. These transitional cells migrate toward the basement membrane of the cord. They lose their round outline and become elongated, and their nuclei are frequently lobed. The nucleoli become associated with the nuclear membrane and are rodshaped, while the chromatin becomes increasingly condensed and chromophilic. These cells differentiate into type-A spermatogonia. Nuclei of spermatogonia are smaller than those of gonocytes or transitional cells; they are ovoid in shape and possess a densely staining chromatin with several prominent nucleoli. These cells are characteristically aligned on the basement membrane of the seminiferous cord (Beaumont and Mandl, 1963).

Type-A spermatogonia constitute 96 percent of all germ cells at 9 days p.p. (Mandl, 1966). The division of spermatogonia leads to the formation of spermatocytes. Leblond and Clermont (1952) claim that this division is unequal and produces two cells, one which remains as a type-A spermatogonium and the other which differentiates into a new spermatogonium referred to as intermediate type. The intermediate type divides by mitosis to give type-B spermatogonia, cells that are characterized by nuclei containing large clumps of chromatin and a thick nuclear membrane. Mitotic division of type-B spermatogonia gives rise to primary spermatocytes. Meiotic division gives rise to secondary spermatocytes which persist only a few hours before another meiotic division

which gives rise to spermatids (Cole and Cupps, 1959). Leblond and Clermont (1952) have divided spermiogenesis into four main phases which are subdivided into 19 different stages. The first phase is the Golgi phase consisting of three stages leading to the formation of the acrosomic granule. The second phase consists of four stages during which the head cap grows out of the acrosomic granule. The acrosome phase consists of seven stages during which the head caps and acrosomic granules become oriented toward the basement membrane of the tubule and the acrosomic granule transforms into the rod-like acrosome. The maturation phase consists of five stages. At the completion of this phase, the spermatids are referred to as spermatozoa.

Cellular associations recognized during a cycle of the seminiferous epithelium permit the distinction of various phases or stages. These associations of cells do not occur at random but develop in close relationship to one another, with the result that at any given area in the seminiferous epithelium there is a constant succession of cellular organizations that takes place with a cyclic regularity.

Two principle methods of classification have been developed, one proposed by Leblond and Clermont (1952) which is based on the development of the acrosomic system during spermiogenesis, and the other by Curtis (1918) which is based on the meiotic divisions, variation in shape of the spermatid nucleus and the release of spermatozoa into the lumen of the seminiferous tubule.

Lacy (1967) proposed a hypothesis explaining the cyclic production of sperm. Each generation of sperm that attains maturity sheds "residual bodies," which are engulfed by Sertoli cells. The residual

bodies act as a highly localized chemical messenger and as a source of raw materials to be utilized by Sertoli cells in the formation of some specific steroid or steroids, referred to as the Sertoli cell hormone (SCH). The elaboration of SCH is dependent upon follicle stimulating hormone (FSH). As the titre of SCH increases newly formed primary spermatocytes are able to complete their maturation division. This division occurs shortly after the release of sperm.

Germ Cell Population Estimates

Beaumont and Mandl (1963) described the dynamics of the germ cell population in rats aged 14.5 days p.c. to 4.5 days p.p. The total population of primitive germ cells rises from 20,000 at 14.5 days p.c. to a plateau of 120,000-140,000 between 20 days p.c. and 2 days p.p. The number of gonocytes then declines to 50,000 at 6-6.5 days after birth. This decrease is due to the elimination of degenerating germ cells and the transformation of gonocytes into definitive germ cells or spermatogonia.

RADIATION EFFECTS ON THE TESTIS

Prenatal

Radiation response of the testis is not uniform throughout gestation but is known to depend on the developmental stage at the time of exposure (Beaumont, 1960a). The undifferentiated gonad of the rat (prior to day 14 p.c.), boar (prior to day 27 p.c.), and bull (prior to day 40 p.c.) is not materially affected by ionizing radiation (Ershoff, 1959; Erickson, Murphree, and Andrews, 1963; Erickson, 1965).

Beaumont (1960a) reported that the radiosensitivity of the rat testis increased during late fetal life in parallel with gonadal differentiation. Earlier work reported by Lengerova and Vojtiskova (1957), Rugh and Jackson (1958), and Ershoff (1959) demonstrate this same trend. An adverse effect of radiation on the germ cells is noted only when irradiation occurs after differentiation of the gonad. Complete sterility occurred in 36 percent of the 15.5 day fetal males and 37 percent of the 16.5 day fetal male mice exposed to 200 R X rays (Rugh and Jackson, 1958). Radiosensitivity of the rat testis is at a maximum on the 15th day p.c. according to Lengerova and Vojtiskova (1957). Results of other workers, however, do not support this observation. Radiation sensitivity was noted at all ages in the rat by Erickson (1965), but sensitivity increased markedly at day 17. From day 18 to parturition complete sterility was induced. Correspondingly, Beaumont (1960a) found sterility at day 19 in the prenatally irradiated male rat.

The prenatally irradiated pig showed a similar response to the rat. Minor damage was observed at 35 and 45 days p.c., but at days 50 through 90 the radiation effect was markedly intensified. In contrast to the rat, however, complete sterilization did not occur (Erickson, 1965).

Mechanisms underlying gonocyte radiosusceptibility are still unknown. Beaumont (1960a) reported that radiosusceptibility may be related to gonocyte mitotic activity, since mitotic activity is reportedly high among rat gonocytes prior to day 17 but practically nonexistent thereafter or when radiosensitivity is highest. However, in the pig and bull the gonocyte remains mitotically active throughout gestation (Erickson, 1965). Erickson (1965) reported that the initiation of pronounced gonocyte sensitivity

could be related to changes in the sex cord or primitive seminiferous tubules. Increased susceptibility begins with the formation of the sex cord.

At 17 days p.c. for the rat, 80 days p.c. for the bull, and 50 days p.c. for the pig, the supporting cells become aligned with the periphery of the cord, and the gonocytes remain mainly in the center. At these three ages the sensitivity of the gonocyte is markedly increased. Morphological changes of the gonocyte at this time were not detected.

Beaumont (1960b) reported that secretory and germinal components of the fetal testis respond independently to irradiation. She exposed pregnant rats to 100 R at 19.5 days of gestation. This dose was sufficient to cause almost complete elimination of the germinal elements; there was, however, apparently no diminution in the output of androgen by the testis as judged by the weight of the accessory sex organs at autopsy.

Erickson (1964) showed that 200 R of gamma irradiation retarded the morphogenesis of the Leydig cell of the neonatal boar, but no lasting damage was observed. An acute dose of 300 R to adult male mice did not reduce the number of Sertoli cells per tubule at intervals of 2 to 42 days postexposure. These cells did not show shrinkage, separation from the membrane or other evidence of pathology (Fogg and Cowing, 1951).

Doses exceeding 100 R may affect the interstitial tissue as reported by Ershoff (1959). A reduction occurs in the weight of the seminal vesicles of sexually mature rats exposed to 150 and 300 R acute X irradiation on the 18th day of pregnancy.

Neonatal

One hundred roentgen of X rays given to rat testes at birth inhibits the division of gonocytes but does not prevent them from differentiating into transitional cells. These cells fail to divide to form type-A spermatogonia. Instead they increase in size so that by 8 days p.p. the testes of irradiated animals contain numerous giant transitional cells which are destined to degenerate (Mandl, 1966). Labeled radioactive precursors of DNA, RNA, and protein demonstrate that synthetic processes continue in transitional cells whose division had been blocked (Lett, 1968). Synthesis of RNA is largely unaffected by irradiation. DNA synthesis may be prolonged after exposure, but the amount of H-thymidine incorporated by the nucleus declines from 5 days p.p. and does not keep pace with nuclear growth. Nuclear protein synthesis increases from 5 days after irradiation and the quantity of phenylalanine taken up by giant cells seems to be proportional to nuclear size. Thus the accumulation of nuclear protein appears to be the main factor in nuclear enlargement (Lett, 1968). Mandl (1966) confirmed Klimek and Vlasinova's (1963) conclusion that the dose of radiation influences the incidence rather than the ultimate size of giant cells. Franchi and Mandl (1966) reported that a dose of 100 R does not inhibit the formation of centrioles in transitional cells, which normally precedes the onset of mitotic prophase, but prevents the cells from proceeding further into mitotic prophase itself.

Postpuberal

Two factors are thought to contribute to the reduction of numbers

of spermatogonia in the postpuberally irradiated testis; one is cell death and the other is mitotic inhibition.

Low doses of either X- or gamma-radiation drastically depleted the population of late type-A, intermediate, and type-B spermatogonia (Oakberg, 1955a). Oakberg and Clark (1961) detected a decrease in the number of spermatogonia after as little as 3 R gamma rays. Type-A spermatogonia showed heterogeneous resistance with a significantly higher proportion surviving exposure to 30-100 R. According to Oakberg and Clark (1961) damaged cells that are not quickly eliminated die at the meiotic metaphase or anaphase.

Twelve, one-week-old mice were exposed to either a partial or whole-body dose of 600 R of X rays and were sacrificed at intervals from one hour to 28 days postirradiation. The number of type-A spermatogonia dropped 60 percent in 12 hours. A minimum of 1.9 and 1.8 percent of normal was reached at 5 and 7 days, respectively, after irradiation (Oakberg, 1955a). Cell death occurred at late interphase or early prophase in type-A and intermediate spermatogonia, and at anaphase or telophase in type-B cells. Type-B spermatogonia were relatively resistant to cell killing for about 5 to 7 hours after completion of DNA synthesis (Monesi, 1962). He concluded that the much longer duration of the sensitive period (interphase) and the much shorter duration of the resistant period (prophase) in intermediate and type-B spermatogonia may account for the greater sensitivity of these cells in comparison to type-A spermatogonia. Radiation induced inhibition of mitosis in spermatogonia was reported by Eschenbrenner and Miller (1950). They exposed mice to various levels of X rays and then sacrificed them at weekly intervals.

Loss and recovery of testis weights and alterations of testicular histology during the recovery period were explained on the basis of a selective effect of radiation on spermatogonia. Bryan and Gowen (1956) concluded that depletion was due to inhibition of spermatogonial mitosis rather than radiation induced spermatogonial necrosis after irradiation of mice with 320 R of X rays. The frequency of spermatogonia reached its lowest point in 3 days following irradiation and regeneration was not seen until 10 days after irradiation.

Casarett and Casarett (1957) concluded that spermatogonial depletion in rats given 324 or 500 R whole-body irradiation was largely due to a marked inhibition of mitosis of type-A and of normal differentiation of type-B cells.

Very high doses of X rays are required before any loss of spermatocytes at stages prior to maturation division can be detected (Oakberg, 1955a). Subsequently, Oakberg and di Minno (1960) estimated the pre-leptotene to be the most sensitive and diakinesis-metaphase the least sensitive stage. Sensitivity appears to decline more or less progressively during the intervening stages, though differences between individual phases were not clear cut. They suggested that there may be two periods of high sensitivity, one at pre-leptotene and the other at early to mid-pachytene.

Oakberg (1955b) was unable to detect any structural change in cells irradiated as spermatids with exposures up to 1500 R, but irradiation of spermatids in both the rat and mouse is known to lead to a high incidence of mutations in the F_1 generation (Bateman, 1956). Chang, Hunt, and Romanoff (1957) used radiocobalt-irradiated spermatozoa to inseminate

female rabbits and drew the following conclusions: Motility of spermatozoa was impaired only at very high doses of radiation (32,000 to 65,000 R). Though inability of sperm to reach the site of fertilization may occur at these doses, the fertilizing capacity per se appears to be undamaged. Cleavage of fertilized ova was very much retarded. The disturbance was related to dose and the higher the dose, the earlier the failure of cleavage. When sperm was irradiated with 90 R of the X rays, the proportion of apparently normal fetuses was not significantly lower than after normal mating; irradiation at 800 R resulted in impaired cleavage and only 10 percent of the zygotes developed into blastocysts. Once a normal blastocyst was formed, implantation and normal development occurred. Cleavage was severely inhibited when sperm were exposed to 6500 R. Since the fertilizing capacity of sperm was not damaged following extremely high doses of irradiation (32,000 to 65,000 R) it is obvious that the physiological mechanisms involved in the process of fertilization are not damaged by irradiation of sperm.

SEMEN STUDIES

Prenatal

In a study conducted by Parish, Murphree, and Hupp (1962) two groups of pregnant cows were irradiated with 400 R of gamma radiation at a dose rate of 0.41 R per minute. One group was irradiated at 136 days p.c. (range 116-180 days) and the other at 230 days p.c. (range 218-243 days). Semen was collected from their male offspring by electroejaculation at 19 and 31 months of age. The number of sperm per ejaculate for those irradiated at 5 and 8 months was 44 and 52 percent less, respectively,

than for controls for both collection periods. In an additional study conducted by Pace, Murphree, and Hupp (1961) pregnant sows were irradiated with 400 R of gamma radiation between 54 and 64 days of gestation. Semen was collected from five control and four irradiated boars by use of an artificial vagina. Sperm production by the irradiated group was 35 percent less than by the control boars.

Postnatal

Erickson (1963) irradiated three bulls at 8, 22, and 29 days of age and at 2 years of age semen analyses and testicular evaluations were made. Irradiated animals produced 31 percent fewer sperm than the controls. No difference was found in either sperm morphology or motility. Microscopic examination of the irradiated testes showed that of the 600 seminiferous tubule cross-sections, 21 percent were sterile or subfunctional.

Brooks (1968) irradiated bull calves at approximately 60 days of age with 300 R gamma radiation. At 100 days the right testis was excised; at 2 years of age the irradiated bulls produced 66 percent fewer sperm per ejaculate; also, the irradiated group produced 22 percent fewer live sperm and 57 percent more morphologically abnormal sperm than the control group.

Erickson (1963) exposed prepuberal male calves aged 1 to 150 days to 200 R of gamma-rays (whole-body) and castrated them at 30, 60, and 210 days after irradiation. He concluded that the bovine gonocyte is most sensitive to a 200 R exposure of gamma-rays during the postnatal period extending from birth to approximately 70 days of age. A lesser

sensitivity was apparent from 80 days to 105 days and from 120 to 150 days only minor damage was produced. Radiosensitivity of the germ cells can be correlated with changes in their developmental status. Loss of relatively high sensitivity at day 70 is paralleled by marked increase in testis-weight, germinal mitoses, and germ cell numbers. Lower sensitivity seen at days 120-150 was probably due to the rapid increase in number of type-A spermatogonia.

Postpuberal

Fifteen Hereford bulls, approximately 2 years of age, were exposed to radiation levels of 100, 200, 400, or 800 R by Gillette, et al..(1964). Semen was collected once weekly for approximately one year. Sperm numbers decreased by about 50 percent at 7 weeks after exposure to 800 R, at 8 weeks after exposure to 400 R, at 9 weeks after exposure to 200 R, and at 11 weeks after exposure to 100 R. Regardless of dose, sperm numbers reached a low 14 weeks after exposure. At 40 weeks the sperm concentration of bulls exposed to 800 R returned to only 65 percent of the pre-irradiation value and was maintained at this level until termination of the study at one year postirradiation. The semen values from bulls exposed to 200 R or 400 R were equal to control values 32 weeks after irradiation; 100 R bulls recovered after 22 weeks. Histologic examination of the testes one year after exposure to 800 R revealed that approximately 20 percent were showing erratic spermatogenesis. Ten percent of the tubules of the 400 R bulls were abnormal.

Parish (1958) exposed bulls to doses of 100, 200, 300, and 400 R gamma radiation. No effect was noticed on sperm production for the first

6 weeks. By the ninth week there was a reduction in number as well as percent live, motile, and normal sperm. At 12 weeks sperm production was at its lowest level. At 24 weeks after irradiation sperm production had not reached the preirradiation level.

Welch (1965) irradiated 15 mature bulls at 50 R per week or 100 R biweekly at the rate of 0.57 R per minute to an accumulated dose of 600 R. Sperm production began to decline 9 weeks following initial exposure and was significantly different from the controls by the 13th week after initial exposure. At the 23rd week the irradiated animals were approaching the control level. Histological study revealed that only one-half of the tubule cross-sections were normal.

CHAPTER III

MATERIALS AND METHODS

Prenatal Morphology and Germ Cell Population Study

Pregnant cows of known breeding dates were slaughtered and the fetuses recovered. The gonads were removed and fixed in FAA, except in younger embryos where the entire embryo or the caudal half was fixed and serially sectioned. Germ cells in male embryos up to 39 days of age were enumerated by direct counts in every other tissue section.

The population of germ cells in the testes of fetuses 39 to 90 days of age was calculated according to the method outlined by Beaumont and Mandl (1962). The relative volume of testicular tissue occupied by germ cells was calculated by Chalkley's (1943) method. Random sections throughout the testis were scanned, under oil immersion, and an average of 750 nuclear hits was recorded for each animal and classified as follows: resting or dividing gonocytes, or nongerminal tissue. The population was estimated by the following formula:

$$\text{Population estimate} = \frac{\text{Total testicular volume x percent germ cells}}{\text{Mean nuclear volume of germ cell}}$$

The germ cell population of fetuses aged 91 to 280 days postcoitum (p.c.) was estimated by the method outlined by Beaumont and Mandl (1963). Percentage of tubular tissue was estimated by Chalkley's (1943) method for each animal. The diameter of 20 seminiferous tubules, cut at right angle to their long axis was measured from the basement membranes. Mean area was calculated per cross-section and the volume determined for 250 cross-sections cut at 5 microns (μ). Gonocytes in 250 cross-sections

were counted, and a correction factor of 1.4 determined. This factor was derived by counting the number of serial sections possessing the same nucleus (40 separate determinations were made).

The germ cell population was estimated in the following manner:

Total volume of testis: $4.0\ mm^3$

Percent tubular tissue: 75

Calculated volume of tubular tissue: $3.0\ mm^3$

Mean diameter of seminiferous tubules: 80μ

Mean area of cross-section: $5072\mu^2$

Volume occupied by 250 cross-sections cut at 5μ: 6.284×10^6

$$250 \times 5\mu \times 5072\ \mu^2 = 6.284 \times 10^6$$

Multiplication factor:

$$\frac{\text{Volume tubular tissue } (3.0 \times 10^9)}{\text{Volume occupied by 250 cross-sections } (6.284 \times 10^6)} = 477.4$$

Correction factor: 1.4

Number of resting gonocytes in 250 cross-sections: 300

Total population of germ cells: $\frac{300 \times 477.4}{1.4} = 1{,}023{,}000$

Determination of Mitotic Index of Germinal and Supporting Cells

Mitotic figures for germinal and supporting cells were counted, under oil immersion, in three center testicular sections in animals aged 39 to 280 days p.c. The two cell types were distinguished by cell size, germinal metaphase plates measuring 8μ or larger and metaphase plates of supporting cells 7μ or less. The number of sections per testis was divided into the number of germ cells yielding the relative population of mitotic figures per cross-section. Knowing the number of mitotic

figures per cross-section and the germ cell population per section, a mitotic figure index was then calculated on a 50,000 germ cells basis.

Counts of germinal and interstitial mitotic figures were made on every second section of gonadal tissue aged 32-38 days p.c. Again the index was based on 50,000 germ cells.

Measurements of the Size of Germ Cells at Different Stages of Development

Measurements of nuclear and cellular diameter of germ cells at different developmental stages were made under oil immersion using an ocular micrometer. Two maximum diameters at right angles were measured and the volumes were calculated from the mean diameters by the following formula: $4/3\ \Pi\ R^3$. The use of this formula is based on the assumption that the nuclei and cells are spherical (Beaumont and Mandl, 1962).

Estimation of Testicular Volume

Testicular volume was estimated by the method outlined by Dornfield, Slater and Scheffe (1942). Testes were serially sectioned at 5μ and every 10th section mounted. Sections were projected by a camera lucida at a magnification of 2.5 to 18 power and the outline of the testis was measured by the usual planimetric method. The cross-section measurements were plotted on graph paper and the area beneath the curve measured by a planimeter. Volume was calculated by the formula: $V = (X\ Y\ T/M^2)\ P$

Where V = volume of testis in cubic centimeters; P = planimeter reading in square centimeters; M = linear magnification of the camera lucida projection; T = thickness of section in microns; X = number of sections per horizontal centimeter on the graph; and Y = square centimeters per vertical centimeter on the graph.

Irradiation and Semen Studies

Fifty-six males were irradiated with 400 R ^{60}Co gamma radiation (air dose to dam) in utero at ages ranging from 15-280 days p.c. The dose rate was 0.44 R per minute in the radiation facility described by Wilding, Simmons, and Rust (1952). Twenty males served as controls. Seven control and 11 irradiated males were unilaterally castrated at 3-19 days postpartum (p.p.). The testes were prepared and enumerated in the same manner as previously described. Thirty-eight males were castrated at approximately 10 months of age along with 5 control males. The remaining intact and unilateral castrate animals remained on pasture until they were approximately 2 years old, at which time semen collection began. The bulls were divided into two groups. Collections were taken from one group on Mondays and Thursdays, and from the second group on Tuesdays and Fridays. The finger electrode method of electroejaculation as described by Austin, Hupp, and Murphree (1961) was used. Depletion of sperm reserves in each bull was accomplished by 10 collections of 10 ml. per collection during the first collection day; after that, 4 collections of 10 ml. per collection were taken each collection day for 4 weeks to keep epididymal sperm storage at a minimum (Hupp et al., 1962).

Semen was collected in a prewarmed glass centrifuge tube and the volume measured to the nearest 0.1 ml. for each ejaculate. A drop of undiluted semen from each ejaculate was immediately placed on a warm glass slide (37° C.) mounted on a microscope stage. Motility and percent motile sperm were recorded under 35 power according to the method described by Herman and Madden (1963). Sperm morphology and live-dead ratio was evaluated by placing a drop of undiluted semen on a prewarmed glass slide.

Staining was effected with nigrosin-eosin. Sperm concentration was determined with a Klett-Summerson colorimeter using the technique described by Hupp et al. (1962). The semen was diluted 1:40 or 1:10 in 2.8 percent sodium citrate, and the optical density was converted to sperm concentration using previously established curves (Hupp et al., 1962).

Following completion of the semen collection, all bulls were castrated. The testes were stripped free of their adnexa, weighed, and measured. Sections at the middle and both poles were taken for histological analysis and fixed in Bouins fluid for 24 hours. Sections were cut at 5μ and stained with hematoxyline and counter-stained with orange-G.

Twelve hundred seminiferous tubule cross-sections were viewed at random from the three different sites. Tubules were evaluated as follows: (1) number of sterile tubule cross-sections, (2) number of cross-sections that contained spermatogonia only, (3) number of tubule cross-sections that contained only spermatogonia and spermatocytes, (4) number of tubule cross-sections with spermatogonia, spermatocytes, and spermatids in reduced numbers, and (5) number of tubule cross-sections.

The frequency of the eight stages of seminiferous epithelium as outlined by Curtis (1918), Roosen-Runge and Giesel (1950) and adapted by Ortavant (1954) to the ram, bull, and boar was determined.

The stages are defined as follows:

Stage I. Extends from absence of spermatozoa in the lumen to the onset of elongation of the spermatids.

Stage II. Extends from the start of elongation of the spermatids to the onset of grouping of elongated spermatids into distinct bundles.

Stage III. Extends from the formation of spermatid bundles to the first maturation division of primary spermatocytes.

Stage IV. Extends from the beginning of the first maturation division to the end of the second maturation division of spermatocytes.

Stage V. Extends from the end of the second maturation division to the time spermatid nuclei show a dusty appearance.

Stage VI. Extends from the time the spermatid nuclei show a dusty appearance to the time all spermatozoa have left the Sertoli cells and moved toward the lumen.

Stage VII. Extends from the beginning to the end of the movement of spermatozoa toward the lumen.

Stage VIII. Extends from the time the spermatozoa line the lumen to their complete disappearance from the lumen.

Type-A spermatogonia were counted in 100 Stage I tubule cross-sections. Three hundred seminiferous tubule cross-sections per testis removed at 3-19 days postnatal were scored for the number of gonocytes per tubule. Only round or nearly round tubular cross-sections were classified.

Statistical analyses of the data were conducted by use of procedures presented in Steel and Torrie (1960) and Snedecor (1956). Duncan's multiple-range test as modified by Kramer (1957) was used for separation of means when an analysis of variance indicated that differences among treatment means was a significant source of variation.

CHAPTER IV

RESULTS

QUALITATIVE OBSERVATIONS

Testicular Histology

The germinal ridge was first observed on the ventro-mesial face of the mesonephric kidney in the 32 day postcoitum (p.c.) embryo. The tunica albuginea of the testis was present as early as day 36, thus making sex microscopically distinguishable. Gonocytes were observed in the germinal ridge at 32 days. These cells were distinguished from somatic cells by their large spherical or nearly spherical nuclei and their three acidophilic nucleoli located near the periphery of the nuclear membrane (Figure 1). By day 39 most gonocytes were restricted to sex cords or primitive seminiferous tubules. At this stage the germinative and somatic elements of the cords were intermixed (Figure 2), but at about day 70 the supporting cells were situated adjacent to the basement membrane and the gonocytes were positioned in the lumen, thus forming the definitive sex cord (Figure 3).

Gonocyte morphology undergoes one change prenatally and this change follows tubular organization. Prior to this time the cytoplasmic to nuclear ratio was low, averaging 1.9 to 1. The nucleus contained one to three small acidophilic nucleoli and the chromatin material was clumped along the nuclear membrane, leaving the nuclear center essentially free of chromatin. Following tubular organization the cytoplasmic

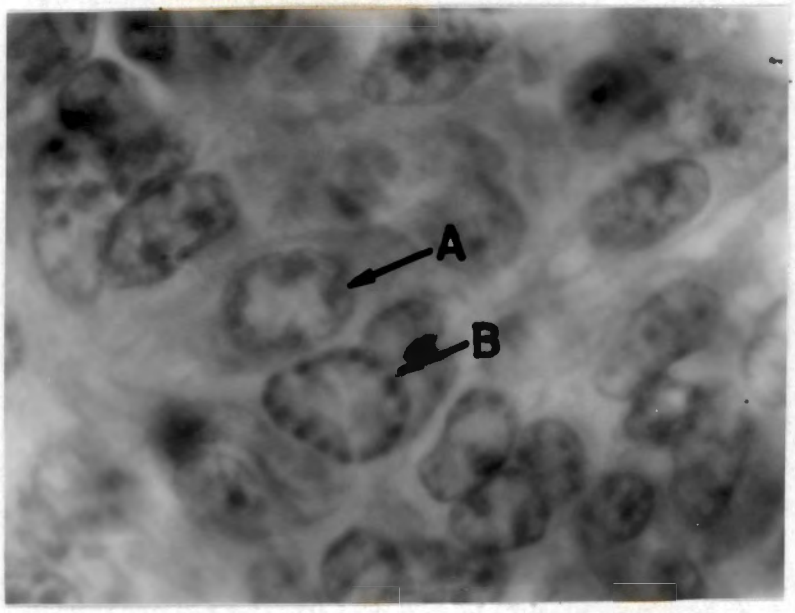

Figure 1. Two primordial germ cells at 32 days postcoitum. Note germ cell at interphase with three prominent acidophilic nucleoli (A) and germ cell in early prophase (B). X 1000.

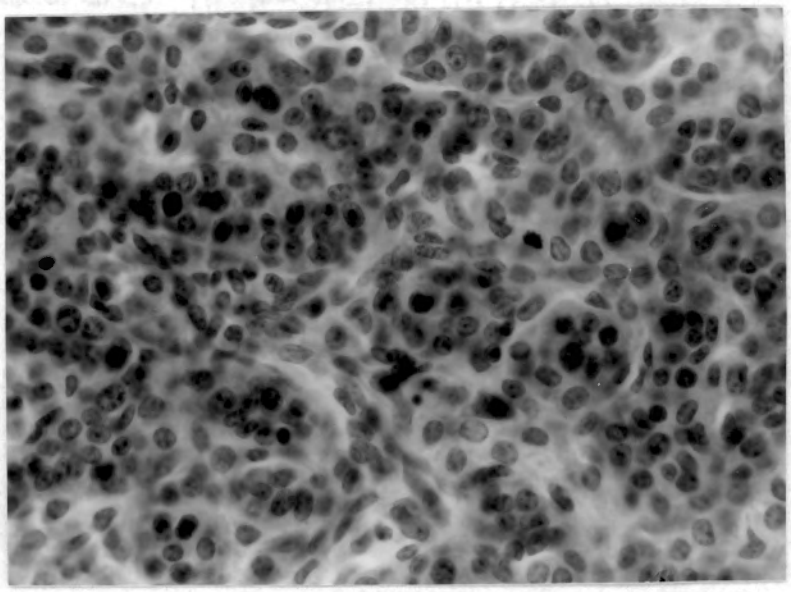

Figure 2. Testis cross-section at 60 days postcoitum. Note lack of tubular organization. X 400.

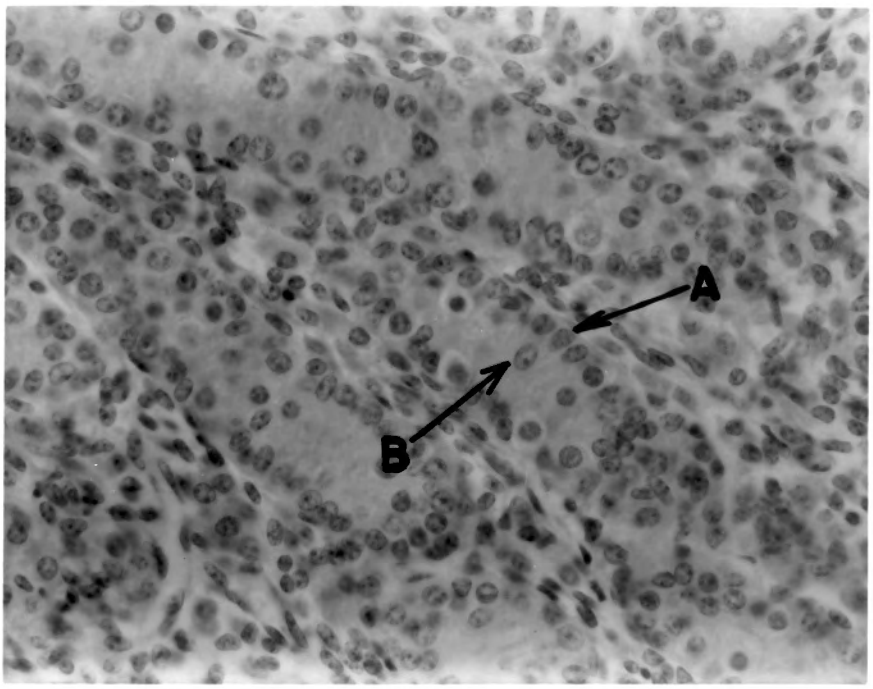

Figure 3. Testis cross-section at 90 days postcoitum. Note alignment of supporting cells (A) along basement membrane and gonocytes (B) positioned in lumen of tubule. X 400.

to nuclear ratio increased with age and at 71-80 days it was 2.4 to 1 and reached 6.9 to 1 at 241-280 days. The nuclear chromatin was precipitated in small granules that were distributed throughout the nucleoplasm.

Changes in nuclear morphology were first observed at 120 days when either the 1 to 3 acidophilic nucleoli fused to form one large nucleolus or two nucleoli disappeared and the remaining nucleolus enlarged. This change did not occur in all gonocytes but was present in about 90 percent of the gonocytes at 241-280 days (Figure 4).

Leydig cells became distinguished from somatic cells around day 40. These cells possessed either a round or ovular nucleus, but the round nucleus predominated from 60-105 days p.c. when the nucleus became and remained crenated until birth. The distinguishing feature of the Leydig cell up to 170 days was the acidophilic nucleolus capped with basophilic chromatin material, but the cap was absent past day 170.

Size of Germ Cells at Different Stages of Development

The cellular to nuclear ratio was lowest in the 30-40 day group and remained low until tubular organization occurred. Detectable gonocyte growth commenced between 71-80 days p.c. and plateaued by 121-150 days (Figure 5). This growth was principally cytoplasmic, since nuclear size remained constant throughout gestation (Table 1).

Testicular Volume

Testicular volume increased throughout gestation; however, changes were not uniform (Table 2). At 51-60 days, testicular volume exceeded the preceding period by 3.8 times. During the 71-80 day period testicular

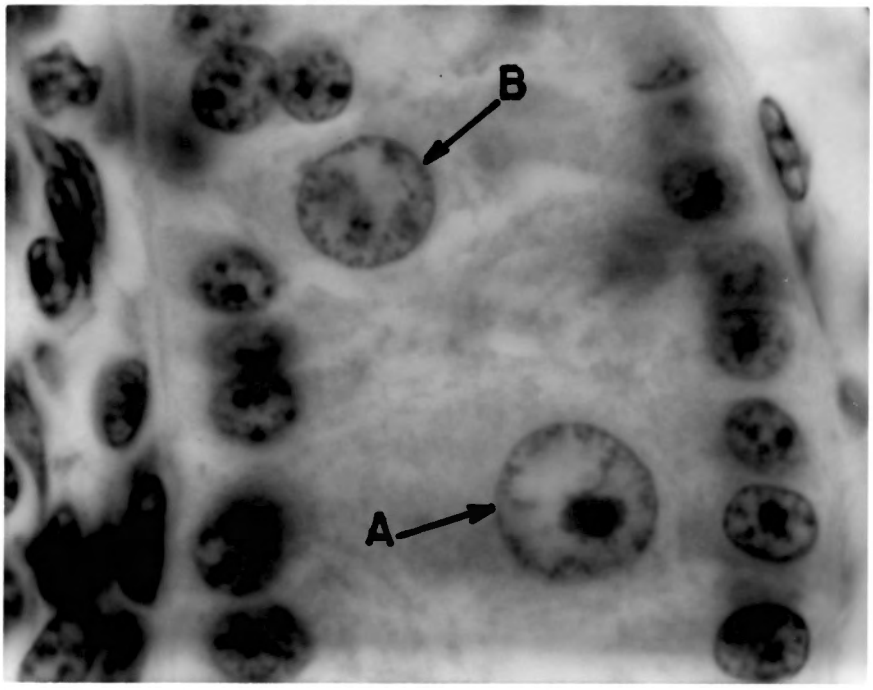

Figure 4. Tubule cross-section at 280 days showing a gonocyte with one large nucleolus (A) and another gonocyte with three nucleoli (B). X 1000.

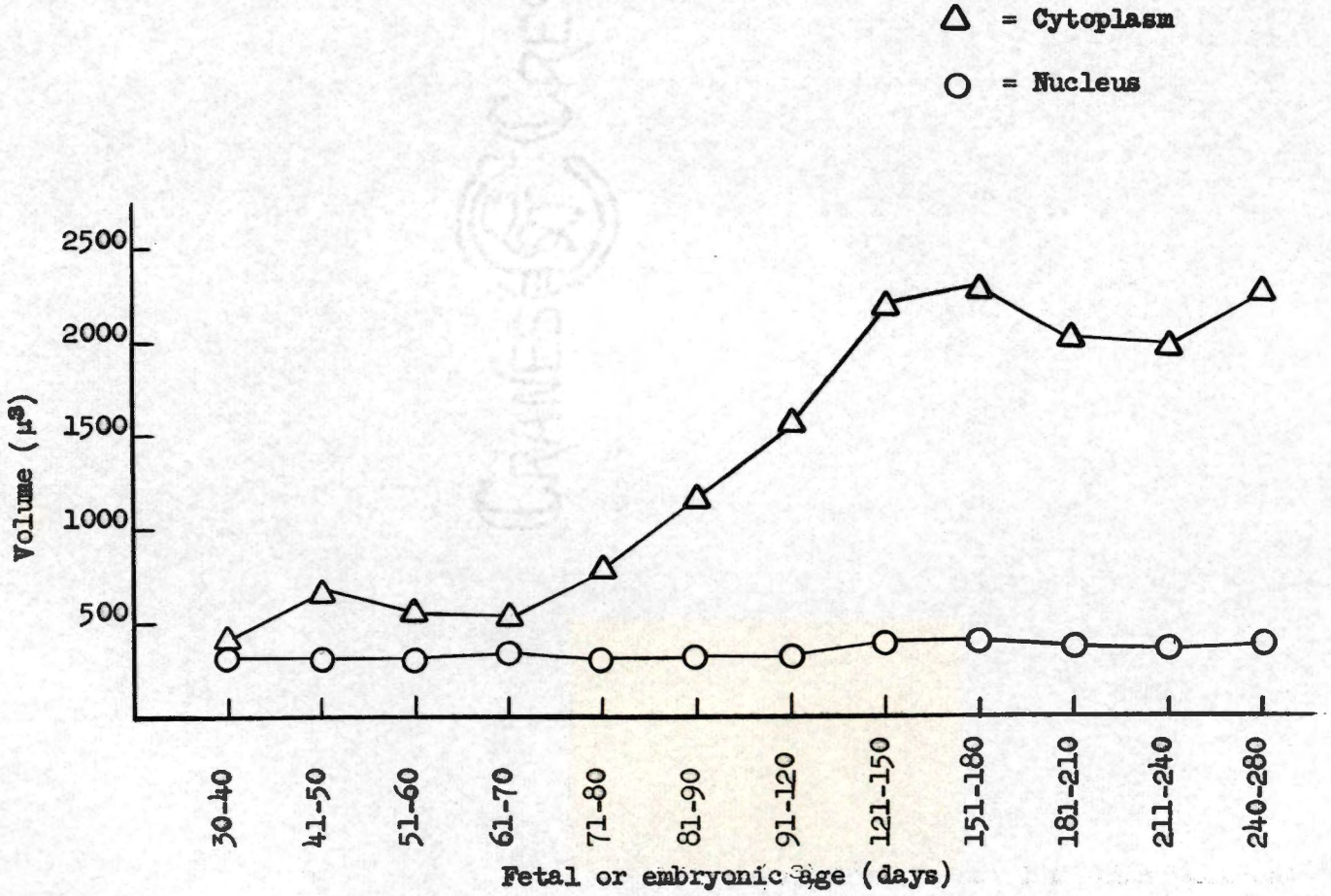

Figure 5. Calculated volume of germ cells and their nuclei at various stages of gestation.

TABLE 1

MEAN SIZE OF INTERPHASE GERM CELLS AND THEIR NUCLEI AT DIFFERENT STAGES OF DEVELOPMENT

Age in Days	Number Cells Measured	Volume of Interphase Germ Cell (μ^3)[a]	Volume of Interphase Germ Cell Nuclei (μ^3)
30-40	30	390 ± 29	298 ± 25
41-50	45	667 ± 42	307 ± 13
51-60	15	584 ± 25	300 ± 26
61-70	60	534 ± 25	341 ± 11
71-80	15	773 ± 45	318 ± 24
81-90	60	1143 ± 50	323 ± 13
91-120	60	1558 ± 104	327 ± 11
121-150	60	2180 ± 77	401 ± 11
151-180	60	2260 ± 181	376 ± 12
181-210	60	2027 ± 193	382 ± 17
211-240	15	1961 ± 150	309 ± 22
241-280	15	2250 ± 132	328 ± 15

[a]Mean ± standard error.

TABLE 2

TESTICULAR VOLUME IN FETAL BULLS

Age in Days	Number Animals Examined	Volume of Single Testis (mm^3)
30-40	4	0.25 ± 0.05[a]
41-50	5	0.34 ± 0.09
51-60	6	1.30 ± 0.20
61-70	6	1.60 ± 0.40
71-80	4	8.7 ± 3
81-90	6	13.7 ± 4
91-120	9	48.3 ± 8
121-150	3	91.5 ± 22
151-180	5	360.0 ± 38
181-210	12	680.0 ± 92
211-240	2	980.0 ± 110
241-280	8	1230.0 ± 74

[a]Mean ± standard error.

weight increased 5.4 times. The 91-120 and 151-180 periods were characterized by increases in volume that were 3.5 times the preceding period. Subsequently, growth continued at a lesser rate.

QUANTIFICATION OF GERM CELLS

Number of Germ Cells

The overall population of germ cells increased up to the 150-180 day stage (Table 3). The apparent discrepancy at the 211-240 day stage is presumably due to the small number of animals. The youngest embryos examined (32-35 days) had a mean population of only 44 gonocytes. The period of most rapid growth was between the 36-38 and 39-50 day groups when the population increased approximately 130 times. Germ cell numbers increased in each of the next four age groups by approximately 7, 1.5, 4, and 2 times over the previous age group. The population then remained fairly static between 91 and 150 days, and then increased about four-fold (Table 3).

Number of Germinal and Somatic Mitoses

The mitotic-figure index per 50,000 germ cells was high while the gonad was in the germinal ridge stage (32-35 day group), but the highest index recorded was in the 36-38 day group where an index of over 9,800 was recorded (Table 4). This represents a three-fold increase over the previous age group. The index then dropped to 6,400 over the next two age groups (39-50 and 51-60) and decreased sharply to 2949 figures during the 61-70 day period. Number of mitotic figures remained static during the 71-80 and 81-90 day periods and decreased by 87 percent in

TABLE 3

CALCULATED POPULATION OF GERM CELLS PER TESTIS

Age in Days	Number Testes Examined	Population of Germ Cells[a]
32-35	2	44 ± 5
36-38	4	145 ± 23
39-50	11	19,632 ± 3,000
51-60	5	156,866 ± 37,000
61-70	4	258,064 ± 75,000
71-80	2	1,956,131 ± 315,000
81-90	4	4,517,182 ± 559,000
91-120	5	3,541,050 ± 276,900
121-150	7	7,273,190 ± 612,600
151-180	8	12,130,300 ± 138,830
181-210	12	13,770,000 ± 132,150
211-240	2	19,460,089 ± 198,750
241-280	8	13,519,100 ± 210,750

[a]Mean ± standard error.

TABLE 4

MITOTIC FIGURE INDEX FOR GERMINAL AND SUPPORTING CELLS[a]

Age in Days	Number Testes Examined	Number Mitoses per 50,000 Germ Cells[b]	Number Mitotic Supporting Cells per Cross-Section
32-35	4	3,478 ± 1037	2
36-38	3	9,821 ± 1275	3
39-50	3	7,191 ± 1209	12
51-60	3	6,436 ± 1115	6
61-70	3	2,949 ± 440	7
71-80	3	1,513 ± 368	7
81-90	3	1,272 ± 372	8
91-120	3	159 ± 33	10
121-150	3	342 ± 24	14
151-180	3	129 ± 24	16
181-210	3	268 ± 34	13
211-240	3	379 ± 79	13
241-280	3	682 ± 116	21

[a] Three center cross-sections per testis were exmained per animal.

[b] Mean ± standard error.

the 91-120 age group. The index then remained static throughout gestation except for a slight increase in the 241-280 day group (Table 4, page 33).

Mitotic figures for supporting cells were seen infrequently during early gestation but they became more common during the last two-thirds of gestation.

Necrotic Gonocytes

Necrotic gonocytes were not consistently observed until the 151-180 day stage (Table 5). In the 121-150 day group only the 150 day animal had degenerating germ cells, but in the 151-180 day group all testes examined contained necrotic gonocytes. In the 181-210 day group 0.04 percent of the population were necrotic and the frequency of necrotic gonocytes increased to almost 1 percent of the population at birth. Morphological changes included an increase in both cell and nuclear size with the chromatin becoming clumped along the nuclear membrane. The nuclear membrane was crinkled or broken, thus releasing the nucleoplasm into the cytoplasm. The majority of necrotic cells were in interphase but an occasional abnormal mitosis was observed (Figure 6). In some cells only the division of the nucleus occurred resulting in binucleated cells or karyokinesis without cytokinesis. Also, some cells were observed with four to eight nuclei (Figure 7).

Irradiation Effects on Testicular Morphology

Data on effect of prenatal irradiation on testicular morphology at 10 months postpartum (p.p.) are summarized in Table 6. The gonocyte population prior to day 60 is relatively resistant to irradiation damage as only 4 percent of the 1200 tubule cross-sections examined were

TABLE 5

MEAN NUMBER OF NECROTIC GONOCYTES AT DIFFERENT STAGES OF DEVELOPMENT[a]

Age in Days	Number Animals	Number Necrotic Gonocytes[b]
39	1	0 ± 0
41-50	3	0 ± 0
51-60	3	0 ± 0
61-70	3	0 ± 0
71-80	3	0 ± 0
81-90	3	0 ± 0
91-120	3	0.1 ± 0
121-150	3	2 ± 1
151-180	3	4 ± 1
181-210	3	17 ± 7
211-240	3	19 ± 4
241-280	3	30 ± 3

[a]Three center cross-sections per testis were examined.

[b]Mean ± standard error.

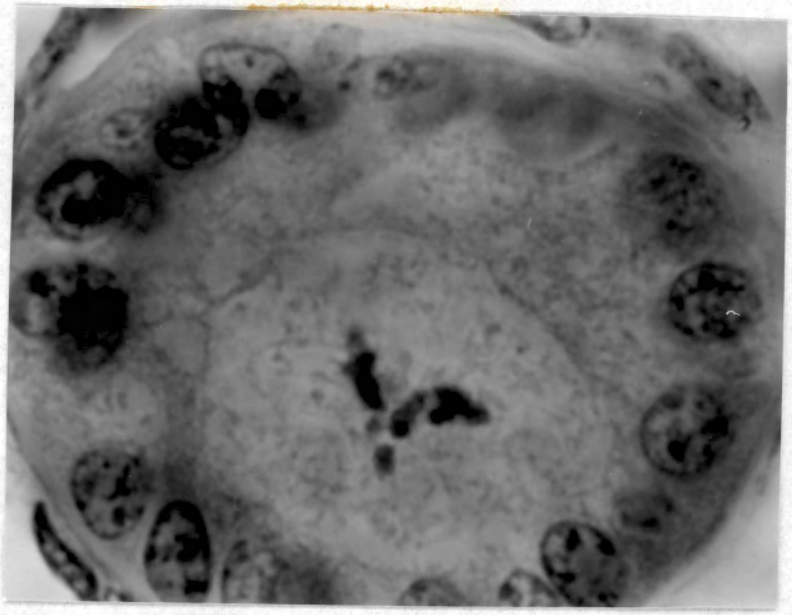

Figure 6. Degenerating primordial germ cell at metaphase. X 1000.

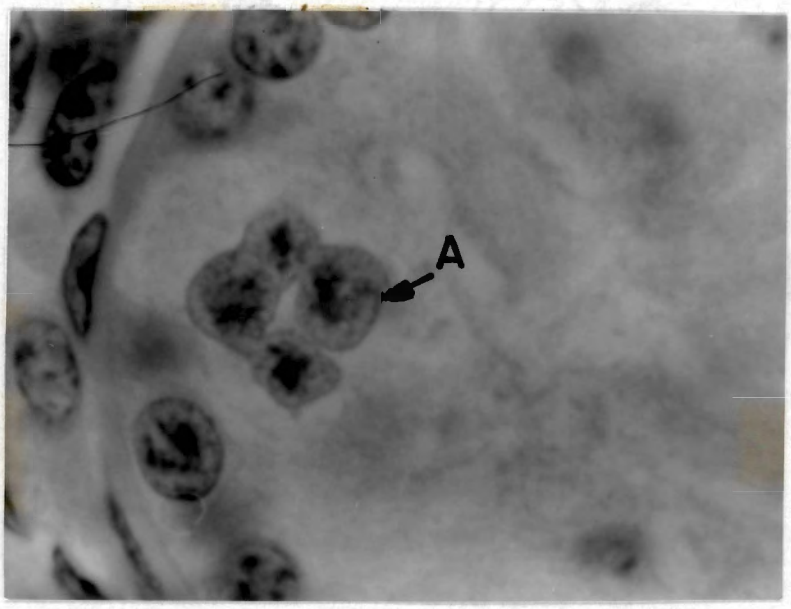

Figure 7. Multinucleated degenerating germ cell (A). Note the apparent budding of the nucleus. X 1000.

TABLE 6

NUMBER AND PERCENT OF 1200 SEMINIFEROUS TUBULE CROSS-SECTIONS CONTAINING NO GERM CELLS; SPERMATOGONIA ONLY; SPERMATOGONIA PLUS SPERMATOCYTES; SPERMATOGONIA, SPERMATOCYTES, AND SPERMATIDS IN REDUCED NUMBERS, IN TESTES EXCISED AT 10 MONTHS OF AGE

Age at Irradiation (days)	Number Animals	No Germ Cells (sterile)	Spermatogonia Only	Spermatogonia Plus Spermatocytes	Spermatogonia, Spermatocytes, and Spermatids in Reduced Numbers
Control	5	0.8 ± 0.8[a]	6 ± 2	58 ± 20	8 ± 8
		0[b]	0	0	1
40-60	3	1 ± 1	0.3 ± 0.3	33 ± 8	14 ± 2.5
		0	0	3	1
70-90	4	836 ± 149	35 ± 16	201 ± 96	136 ± 43
		69	3	17	11
91-120	1	1058	6	69	71
		88	0	6	6
121-150	2	894 ± 49	38 ± 2	117 ± 14	85 ± 16
		74	3	10	7
151-180	5	828 ± 55	25.8 ± 14	92 ± 19	81 ± 16
		68	2	8	7
181-210	2	978 ± 45	3 ± 0	80 ± 35	29 ± 2
		81	0	7	3
211-240	3	466 ± 203	91 ± 27	149 ± 27	117 ± 64
		57	6	10	14
241-280	3	257 ± 129	80 ± 55	233 ± 61	136 ± 54
		20	6	18	10

[a] Mean ± standard error.

[b] Percentage of total number of tubules examined (1200).

subfunctional (Table 6). One-hundred percent damage occurred in the 70-120 day group. The percentage of subfunctional tubules decreased to between 85 and 94 percent for the next four age groups. Fifty-four percent of the tubule cross-sections in the 241-280 day group were subfunctional. (Subfunctional tubules include the following four categories: (1) no germ cells; (2) spermatogonia only; (3) spermatogonia plus spermatocytes; and (4) spermatogonia, spermatocytes and spermatids occurring in reduced numbers).

An analysis of variance indicated that the radiation effect was a significant source of variation in the number of subfunctional tubules ($P < 0.05$). Each treatment mean was then compared with all other treatment means by Duncan's multiple-range test as modified by Kramer (1957). There was no significant difference between the control and 40-60 day irradiated group, or among the 70-280 day age groups ($P > 0.05$). There was, however, a significant difference between the control and 40-60 day group when compared to the 70-80 day age group ($P < 0.05$).

Frequency of subfunctional tubules was also evaluated in prenatally irradiated testes excised at 25 months p.p. (Table 7). The percentage of subfunctional tubules was only 2 percent for the control and 0 percent for the day 25 irradiated bull. Damage in the 91-120 and 121-150 day groups was 66 and 69 percent, respectively. The percent of subfunctional tubules decreased the last 2 months of pregnancy to 28 percent for the seventh month and 19 percent for the ninth month. An analysis of variance indicated a significant F test, and the means were compared as before (Table 8).

TABLE 7

NUMBER AND PERCENT OF 1200 SEMINIFEROUS TUBULE CROSS-SECTIONS CONTAINING NO GERM-CELLS; SPERMATOGONIA ONLY; SPERMATOGONIA PLUS SPERMATOCYTES; SPERMATOGONIA, SPERMATOCYTES, AND SPERMATIDS IN REDUCED NUMBERS, IN TESTES EXCISED AT 25 MONTHS OF AGE

Age at Irradiation (days)	Number Animals	No Germ-Cells (sterile)	Spermatogonia Only	Spermatogonia Plus Spermatocytes	Spermatogonia, Spermatocytes, and Spermatids in Reduced Numbers
Control	7	1 ± 0.6[a]	3 ± 1.6	11 ± 6	8 ± 4
		0[b]	0	1	1
25	1	5	0	3	3
		0	0	0	0
91-120	2	674 ± 16	8 ± 5	81 ± 15	34 ± 7
		55	1	7	3
121-150	5	610 ± 114	7 ± 4	150 ± 42	76 ± 32
		50	1	12	6
217	1	153	40	104	30
		13	3	9	3
274	1	78	18	112	35
		6	1	9	3

[a]Mean ± standard error.

[b]Percentage of total number of tubules examined (1200).

TABLE 8

COMPARISON OF MEANS BY DUNCAN'S MULTIPLE-RANGE TEST OF SUBFUNCTIONAL TUBULES IN PRENATALLY IRRADIATED TESTES EXCISED AT 25 MONTHS OF AGE[a]

Age at Irradiation					
0-25	Control	241-280	211-240	91-120	121-150
Means[b]					
11	23	243	327	796	843

[a] Subfunctional tubules include the following four categories: (1) no germ cells (2) spermatogonia only (3) spermatogonia plus spermatocytes, and (4) spermatogonia, spermatocytes, and spermatids occurring in reduced numbers.

[b] Means not underscored by the same line are significantly different at the $P < 0.05$ level.

Three control and 10 irradiated males were castrated at 48-75 months p.p. and the frequency of sterile tubules was compared with the bulls castrated at 10 and 25 months p.p. The data are summarized in Table 9. The percentage of sterile cross-sections was at a minimum in animals irradiated under 60 days of age. In the 10 month castrates 69 percent of the tubules were sterile in the 70-90 day group and the percentage increased to 88 in the 91-120 age group. Damage to the 10 month castrates slowly decreased through the next four age groups to 57 percent in the 211-240 day age group, and then dropped to 20 percent in the 241-280 day age group. A similar trend was found in the 25 month group and in those testes excised at 4 or more years after irradiation; but a considerable degree of recovery from the effects of prenatal irradiation is evidenced by the decline in number of sterile tubule cross-sections with age.

The assumption of homogeneity of variances among the irradiated groups was tested by the method of Bartlett (Steel and Torrie, 1960). The assumption of homogeneity was accepted. Further calculations indicated that irradiation at the different ages was a significant source of variation in the number of sterile tubules ($P < 0.05$). Each treatment mean was then compared with all other treatment means by Duncan's multiple-range test as modified by Kramer (1957). The data in Table 10 show the comparison of mean number of sterile tubules. No difference in sterile tubules was noted between the ages of 91 and 210 days but these groups did differ significantly from control, also no significant difference occurred between the control and those testes irradiated at 15-60 days and 241-280 days p.c.

TABLE 9

PERCENT OF 1200 SEMINIFEROUS TUBULE CROSS-SECTIONS CONTAINING NO GERM CELLS (STERILE) IN TESTES EXCISED AT 10-75 MONTHS OF AGE

Age at Irradiation (days)	Age at Castration (months)	Number of Animals	Percentage of Sterile Tubules
Control	10	5	0
	25	7	0
	48	3	0
12-25	25	1	0
	52	7	0.3
40-60	11	5	0.8
70-90	10	4	69
91-120	10	1	88
	25	2	55
	74	1	11
121-150	10	2	74
	25	5	55
	75	1	24
151-180	10	5	68
181-210	10	3	62
211-240	10	3	57
	24	1	13
	63	1	1
241-280	10	3	20
	25	1	6
	75	1	12

TABLE 10

COMPARISON OF MEAN NUMBER OF STERILE TUBULES BY DUNCAN'S MULTIPLE-RANGE TEST

Age at Irradiation[a]									
Control	40-60	12-25	241-280	211-240	91-120	70-90	121-150	181-210	151-180
Means									
3	3	4	230	358	641	673	693	750	828

[a]Means not underscored by the same line are significantly different ($P < 0.05$).

Normal and degenerating gonocytes were counted in testes of irradiated and control animals that were unilaterally castrated at 3-19 days after birth (Table 11). The normal gonocyte number in the 80-133 days p.c. irradiated group was 11 and 9 percent of the control for the normal and degenerating gonocytes, respectively. An analysis of variance on the number of normal gonocytes revealed a significant difference between groups ($P < 0.05$). Duncan's multiple-range test was used to compare the differences among the means. No significant differences existed between the control and 30-70 day p.c. irradiated group ($P > 0.05$), but there was a significant difference between the 80-130 day p.c. irradiated group and the other two groups ($P < 0.05$). The necrotic-gonocyte means were tested in the same manner. Again no difference occurred between the control and 30-70 day group ($P > 0.05$), but a significant difference existed between the 80-130 day group p.c. irradiated group and the other two groups ($P < 0.05$).

Semen Study

The average number of sperm-per-ejaculate and testes weights are shown in Table 12.

Variations in number of sperm-per-ejaculate were large among the intact control bulls but the lowest sperm-per-ejaculate for the intact control bull was higher than the highest intact irradiated bull. The intact and unilateral castrate control bulls produced 78 and 70 percent more sperm-per-ejaculate than the intact and unilaterally-castrate irradiated bulls, respectively.

However, comparison of the means by Duncan's multiple-range test as modified by Kramer (1957) showed that there was no difference in the

TABLE 11

NUMBER OF NORMAL AND NECROTIC GONOCYTES PER 600 TUBULE CROSS-SECTIONS[a]

Age at Irradiation (days)	Number Animals	Number of Normal Gonocytes	Number of Necrotic Gonocytes
Control	7	421 ± 65	70 ± 14
30-70	4	562 ± 43	55 ± 11
80-133	7	39 ± 12	7 ± 2

[a] Animals were castrated at 5 to 19 days postpartum.

[b] Mean ± standard error.

TABLE 12

SPERM PER EJACULATE AND TESTES WEIGHTS OF 25-MONTH-OLD BULLS

Treatment	Animal Number	Sperm per Ejaculation (in billions)	Testis Weight (grams)[a]
Intact Controls	133	5.5	496
	228	2.7	491
	162	1.6	454
	33	3.3	605
	92	2.5	504
	125	2.1	586
	62	1.4	378
	27	1.4	471
		2.6 ± 0.5[b]	516 ± 29
Unilateral-Castrate Controls	148	1.0	245
	164	1.2	389
	120	1.5	351
	69	1.8	348
	108	2.7	380
		1.6 ± 0.3	343 ± 26
Intact Irradiated	46	1.1	352[a]
	78	0.8	261
	121	0.7	505
	189	0.9	332
	222	0.4	264
	203	0.4	308
	100	0.3	222
		0.7 ± 0.1	320 ± 35
Unilateral-Castrate Irradiated	36	0.7	311
	103	0.5	175
	77	0.8	251
		0.7 ± 0.1	246 ± 39

[a]Combined weight of both testes for intact males and weight of left testes for unilateral-castrated males.

[b]Mean ± standard error.

sperm-per-ejaculate between the intact control and unilateral-castrate control, or between the unilateral-castrate control and the two irradiated groups ($P > 0.05$).

Following characterization of sperm production, the number of type-A spermatogonia per 100 tubule cross-sections at Stage I of the seminiferous epithelium was determined (Table 13). Unilateral-castrate controls had the highest number of A's per tubule cross-section. Comparison of means by Duncan's multiple-range test showed a significant difference ($P < 0.05$) between the unilateral-castrate controls and the other combination of means ($P > 0.05$).

Sperm Motility

Sperm motility in the irradiated group (intact and unilateral castrates) was 37 and 20 percent less, respectively, than the intact and unilateral-castrate controls (Table 14). This difference was significant ($P < 0.01$). The percentage of motile sperm was 34 and 9 percent less, respectively, in the ejaculates of intact and unilateral-castrate irradiated bulls than the intact and unilateral-castrate controls. This difference was significant ($P < 0.05$) for the intact controls when compared with intact irradiated, but the difference between the unilateral-castrate groups was not significant ($P > 0.05$).

The difference in percent of abnormal sperm between the intact controls and irradiated groups was highly significant; also the difference between the unilaterally-castrated control and irradiated groups was significant ($P < 0.01$).

The eight stages of the seminiferous epithelium were enumerated in both the irradiated and control animals (Table 15). The data

TABLE 13

FREQUENCY OF TYPE-A SPERMATOGONIA IN 100 STAGE I SEMINIFEROUS TUBULE CROSS-SECTIONS AT 25 MONTHS OF AGE

Treatment Group	Number	Number Type-A Spermatogonia
Intact Controls	92	171
	133	204
	228	116
	27	231
	125	148
	33	167
		181 ± 12[a]
Unilateral-Castrate Controls	148	228
	164	336
	120	394
	69	348
	108	317
		324 ± 27
Intact Irradiated	48	178
	78	220
	121	166
	189	156
	222	195
	203	155
	100	164
		176 ± 9
Unilateral-Castrate Irradiated	36	240
	103	175
	77	206
		207 ± 19

[a]Mean ± standard error.

TABLE 14

SPERM MOTILITY AND MORPHOLOGY

Treatment Group	Animal Number	Degree (0 - 5)	Percent Motile	Percent Live	Percent Abnormal
Intact Controls	133	3.6	73	84	28
	228	3.3	67	74	27
	162	3.1	62	80	23
	33	2.7	56	76	8
	92	2.4	46	78	8
	125	2.4	48	84	8
	62	1.6	26	79	15
	27	2.1	35	65	9
		2.7 ± 0.2[a]	51 ± 6	76 ± 2	16 ± 33
Unilateral-Castrate Control	148	1.4	19	52	12
	164	1.9	31	66	12
	120	1.9	38	83	7
	69	2.2	40	83	8
	108	2.0	33	71	11
		1.9 ± 0.1	32 ± 4	71 ± 6	10 ± 2
Intact Irradiated	46	2.2	45	74	29
	78	1.3	27	66	29
	121	1.7	34	77	27
	189	1.8	38	76	29
	222	1.1	23	85	32
	203	1.9	38	72	17
	100	1.6	33	60	27
		1.7 ± 0.1[b]	34 ± 3[d]	73 ± 3	27 ± 2[b]
Unilateral-Castrate Irradiated	36	1.9	39	83	25
	107	1.2	26	74	20
	77	1.1	23	44	36
		1.5 ± 0.2[c]	29 ± 5	67 ± 12	27 ± 5[c]

[a]Mean ± standard error.

[b]Significantly different from the intact control group ($P < 0.01$).

[c]Significantly different from the unilaterally castrated control group ($P < 0.01$).

[d]Significantly different from the intact control group ($P < 0.05$).

TABLE 15

RELATIVE FREQUENCY OF THE EIGHT STAGES OF THE SEMINIFEROUS EPITHELIUM AS DETERMINED FROM TESTES AGED 9-25 MONTHS

Age at Irradiation (days)	Number of Animals	Stage of Seminiferous Epithelial Cycle							
		I	II	III	IV	V	VI	VII	VIII
Control	15	44 ± 3[a]	6 ± 0	23 ± 0	1 ± 0	7 ± 1	4 ± 0	3 ± 0	14 ± 1
15-25	5	47 ± 1	6 ± 0	19 ± 2	1 ± 0	8 ± 1	4 ± 1	4 ± 1	11 ± 1
40-60	3	50 ± 8	6 ± 0	18 ± 1	1 ± 0	4 ± 2	3 ± 1	2 ± 1	14 ± 4
61-90	6	Spermatogenic cycle was not complete.							
91-120	3	43 ± 3	6 ± 1	20 ± 2	1 ± 0	7 ± 2	4 ± 1	3 ± 1	16 ± 2
121-150	6	41 ± 3	6 ± 1	25 ± 1	1 ± 0	8 ± 2	4 ± 1	1 ± 0	15 ± 1
151-180	3	38 ± 2	7 ± 1	26 ± 1	2 ± 0	9 ± 3	1 ± 0	1 ± 1	15 ± 2
181-210	2	39 ± 3	4 ± 2	25 ± 0	1 ± 0	6 ± 0	2 ± 1	2 ± 0	21 ± 4
211-240	3	40 ± 4	7 ± 1	28 ± 0	1 ± 0	7 ± 2	3 ± 1	2 ± 0	12 ± 2
241-280	3	33 ± 2	8 ± 2	26 ± 4	1 ± 1	8 ± 2	4 ± 1	3 ± 1	16 ± 2

[a]Mean percentage of 1200 tubule cross-sections examined ± standard error to nearest whole number.

indicate that the spermatogenic sequence in the postpuberal bull was not affected by prenatal irradiation. The hypothesis that the relative frequency of the eight stages of the seminiferous epithelium was not different between the control and irradiated groups was tested by a Chi square test using a R x C contingency table (Steel and Torrie, 1960). A nonsignificant X^2 value was obtained; thus the hypothesis was accepted.

CHAPTER V

DISCUSSION

This study substantiates the observations of Jost and Prepin (1966) regarding the age of genital ridge formation (32 days) and Krehgbiel's (1963) observation on the age of testicular formation (36 days) in the prenatal bull. However, the literature is believed to contain no within species parallel to either the qualitative or quantitative information reported in this dissertation.

Like the rat (Beaumont, 1960) gonocyte morphology changes in the prenatal bull during gestation. The changes include a less chromophilic nucleus, more round and prominent nucleoli, and an increase in volume of both cytoplasm and nucleus. Changes that occur in the bull but not in the rat are: (1) the nucleus remains constant in size but the cytoplasmic volume increases and (2) the multiple nucleoli either fuse or one increases in size as the other two disappear.

Cells considered degenerating were observed in the rat during late gestation from day 20 postcoitum (p.c.) onward; in the bull necrotic gonocytes were first observed at day 116 and their numbers slowly increased until day 181-210 and then increased sharply until birth. Morphological changes such as enlargement of the nucleus, crinkling or complete breakdown of the nuclear membrane, and contraction of the cytoplasm were similar in the two species. However, in the bull binucleated cells were common in late gestation, a condition not reported in the rat.

Testicular growth in the prenatal bull was not uniform as was the case in the rat. Rat testes grew steadily between 14.5 days p.c. and birth; this growth represented a 30-fold increase in size. Four periods of rapid growth characterized the testis of the bull and the bull testis, being of larger volume than the rat, increased about 4900 times in volume from sex differentiation to birth.

Differences in the germ cell population occurred between the rat and the bull prior to sex differentiation. Evidently germinal mitosis in the rat, prior to this morphological change, occurs at a higher rate than in the bull, as the germ cell population of the rat numbered 20,000 at this time compared to about 150 for the bull. At the time of tubule organization the rat gonocyte population increased four times compared to about 13,000 times in the bull. Germ cell numbers in the rat reached a peak 2 days prior to birth and decreased 27 percent at bith. The gonocyte population in the bull peaked between days 211-240 and then decreased by an apparent 31 percent at day 241-280. However, the peak population may not have been as great if the sample size at day 211-240 had been larger.

Beaumont (1960) reported that in the rat the population of gonocytes is radiosensitive from sex differentiation until birth, but indicated that sensitivity increased with fetal age. Following 150 R of X rays to the dam gonocyte sensitivity increased markedly at day 17 (Erickson, 1966). This event coincided with two morphological changes in the testis, tubular organization, and a decrease in the number of germinal mitotic figures. At day 18-22 p.c. complete sterility occurred (Erickson, 1965).

The pattern of radiosensitivity of the bull gonocyte (400 R to dam) was as follows: low sensitivity prior to day 70, a marked increase after day 70, high radiosensitivity through day 210, and a trend to lesser sensitivity through the remainder of gestation. Although the population of gonocytes appeared to be radioresistant during the last 70 days of gestation, the individual gonocyte after day 210 may be just as radiosensitive as the gonocyte prior to day 210. The apparent radioresistance shown during this period may be explained by the large population at this time (19,000,000 at day 211-240 and 13,000,000 at day 241-280). Thus the overall effect of a single damaged gonocyte may be masked by surviving gonocytes that will repopulate the tubules in the postnatal period. The volume of the testes also increased during this period (Table 2, page 30). That the sensitivity of the late prenatal gonocyte remains high is also evidenced by the response of the early postnatal gonocyte (Erickson, 1963). The population of gonocytes in testes aged 1 to 5 days postnatal at irradiation (200 R ^{60}Co) was reduced to less than 23 percent of the controls, when examined 60 days post-irradiation (Erickson, 1963). This can be compared with the 7 percent surviving prenatal irradiation (day 80-133) as seen in testes excised at 3-19 days after birth (Table 11, page 45).

The germ-cell of the bull follows essentially the same pattern as the germ-cell of the rat in its response to irradiation, but qualitative and quantitative differences exist. Irradiation (400 R to dam) did not cause complete sterility in the bull at any time of development (Table 9, page 42). whereas 100 R of X rays to male rat fetuses produced sterility from day 18 to parturition (day 22).

Rate of germinal mitosis was reported high among rat gonocytes prior to day 17, coinciding with low radiosensitivity, but practically nonexistent thereafter, coinciding with high radiosensitivity, Beaumont (1960); whereas, in the bull the mitotic rate was high during the first 90 days of gestation and continued at a lower rate through gestation (Table 4, page 33). Germ cell sensitivity in the bull coincided with the alignment and segregation of the cellular components of the primitive seminiferous tubules and an increase in the cytoplasmic to nuclear ratio. Also, Erickson, Murphree, and Andrews (1963) reported that alignment and segregation were noted to parallel the beginning of pronounced germ cell radiosensitivity in the boar at day 50. No abrupt change in the cytoplasmic to nuclear ratio was noted, however, in the rat gonocyte at the time of peak radiosensitivity (Beaumont and Mandl, 1963).

Seven percent of the gonocytes irradiated 80-130 days p.c. (400 R ^{60}Co to dam) survived to 30 days postnatal (Table 11, page 45). From this low number subsequent repopulation of the tubules occurred. From the data in Table 9 (page 42) it is evident that repopulation of sterile tubules with functional type-A spermatogonia is a continuous process. Testes irradiated at 91-120 days p.c. and examined at 10 months postnatal were 88 percent sterile compared to 55 and 11 percent sterile at 25 and 75 months, respectively. Despite the fact that 55 percent of the tubules were sterile at 25 months, sperm was produced at 26 percent of control levels (Table 12, page 46). Recovery, however, is probably never complete as 11 percent of the tubular apparatus of the 75-month-old animals was sterile.

Irradiation does not alter the relative frequency of the eight stages of the seminiferous epithelium as no difference existed between the irradiated and control groups (Table 15, page 50). Irradiation also did not affect the number of type-A spermatogonia per tubule in the intact irradiated compared to control groups (Table 13, page 48). Evidently unilateral castration in the prepuberal bull caused hyperplasia among the germ cells as reflected by the 66 percent increase in type-A spermatogonia per tubule; this response was also reflected by an increase in testes weights (Table 13, page 48). Irradiation evidently suppressed the hyperplasia in the irradiated unilateral castrate as the number of type-A spermatogonia per tubule was significantly different from unilateral-castrate controls but irradiation did not suppress testis weights (Table 12, page 46).

Irradiation reduced semen production in intact irradiated bulls by about 72 percent as compared with the controls (0.7 billion vs. 2.6 billion) when sampled at 25 months of age. Motility, both degree and percent, and percent abnormal sperm were significantly lower ($P < 0.05$) than in the intact controls (Table 14, page 49), but the percentage of live sperm was unaffected. This reduction in sperm per ejaculate is greater than that reported by Parish, Murphree, and Hupp (1962) who irradiated bulls 5 and 8 months p.c. These animals subsequently produced 42 and 52 percent less sperm per ejaculate, respectively, than controls.

Because of the small number of bulls collected per age group and the wide range of germ cell numbers observed between different individuals per age group, the population estimate per age group can be regarded only

as approximate estimates of the overall population means.

Complete sterility did not occur after a dose of 400 R whole-body gamma irradiation, although the gonocyte population was low in the group exposed at 80-133 days (Table 11, page 45). No animal was sterile when examined at 10 months of age (Table 6, page 37). Recovery apparently continued postnatally until natural degeneration began.

The seven-tenths billion sperm per ejaculate in the 25-month-old bulls is well above the minimum number of 6 million required in artificial insemination for optimum fertility (Branton, Kellgren, and Patrick, 1953). Therefore this study agrees with Erickson's statement (1963) that the chance of sterilizing the bull in the prenatal state with a single-acute whole-body dose of ionizing radiation is low.

BIBLIOGRAPHY

BIBLIOGRAPHY

Austin, J. W., E. W. Hupp, and R. L. Murphree. 1961. Comparison of bull semen collected in the artificial vagina and by electro-ejaculation. J. Dairy Sci. 44:2292.

Bateman, A. J. 1956. Sensitivity of immature mouse sperm to the mutagenic effects of X rays. Nature, Lond. 178:1278.

Beaumont, H. M. 1960a. Changes in the radiosensitivity of the testis during foetal development. Int. J. Rad. Biol. 2:247.

Beaumont, H. M. 1960b. Effect of irradiation during foetal life on the subsequent structure and secretory activity of the gonads. J. Endocr. 24:325.

Beaumont, H. M. and A. M. Mandl. 1962. A quantitative and cytological study of oogonia and oocytes in the foetal and neonatal rat. Proc. Roy. Soc. B. 115:557.

Beaumont, H. M. and A. M. Mandl. 1963. A quantitative study of primordial germ cells in the male rat. J. Embryol. Exp. Morphol. 11:715.

Branton, C., H. D. Kellgren, and T. E. Patrick. 1953. The importance of numbers of spermatozoa in relation to semen quality and fertility of dairy bulls. J. Dairy Sci. 36:301.

Brooks, F. T. 1968. Effect of gamma radiation and unilateral castration on the testis of the prepuberal bull as determined through histological and semenological techniques. M. S. Thesis. University of Tennessee, Knoxville.

Bryan, J. H. D. and J. W. Gowen. 1956. A histological and cytophotometric study of the effects of X rays on the mouse testis. Biol. Bull. 110:229.

Chalkley, H. W. 1943. Method for the quantitative morphologic analysis of tissue. J. Nat. Cancer Inst. 4:47.

Casarett, A. P. and G. W. Casarett. 1957. Histological investigations of mechanisms of X rays effect on spermatogenesis in the rat. Univ. of Rochester Atom. Eng. Proj. UR 496:7.

Chang, M. C., D. M. Hunt, and E. B. Romanoff. 1957. Effects of radiocobalt irradiation of rabbit spermatozoa in vitro on fertilization and early development. Anat. Record 129:211

Chiquoine, A. D. 1954. The identification, origin and migration of the primordial germ cells in the mouse embryo. Anat. Record 118:135.

Cole, H. H. and P. T. Cupps. 1959. Reproduction in Domestic Animals. Vol. II. Academic Press, New York.

Curtis, G. M. 1918. The morphology of the mammalian seminiferous tubule. Am. J. Anat. 24:239.

Dornfield, E. J., D. W. Slater, and H. Scheffe. 1942. A method for accurate determinations of volume and cell numbers in small organs. Anat. Record 82:255.

Erickson, B. H., R. L. Murphree, and J. F. Andrews. 1963. Effects of prenatal gamma irradiation on the germ cells of the male pig. Radiation Res. 20:640.

Erickson, B. H. 1963. Effects of gamma irradiation on the primitive germ cells of the prepubertal bovine testis. Int. J. Rad. Biol. 7:361.

Erickson, B. H. 1964. Effects of neonatal gamma irradiation on hormone production and spermatogenesis in the testis of the adult pig. J. Reprod. Fert. 8:91.

Erickson, B. H. 1965. Symposium on atomic energy in animal science: Radiation effects on gonadal development in farm animals. J. Animal Sci. 24:568.

Erschenbrenner, A. B. and E. Miller. 1950. Effect of roentgen rays on the testis. Quantitative histological analysis following whole-body exposure of mice. Arch. Path. 50:736.

Ershoff, B. M. 1959. Effects of prenatal X irradiation on testicular function and morphology in the rat. Am. J. Physiol. 196:896.

Fogg, L. C. and R. C. Cowing. 1951. The changes in cell morphology and histochemistry of the testis following irradiation and their relation to other induced testicular changes. Can. J. Res. 11:23.

Franchi, L. L. and A. M. Mandl. 1964. The ultrastructure of germ cells in foetal and neonatal male rats. J. Embryol. Exp. Morphol. 12:289.

Franchi, L. L. and A. M. Mandl. 1966. The ultrastructure of male germ cells in rats X irradiated at birth. Proc. Roy. Soc. B. 165:136.

Gillette, E. L., M. L. Hopwood, W. D. Carlson, and F. X. Gassner. 1964. The effects of X irradiation of bovine testicles on semen. Radiation Res. 22:264.

Hargitt, G. F. 1926. The formation of the sex glands and germ cells of mammals, II. The history of the male germ cells in the albino rat. J. Morphol. 42:253.

Herman, H. A. and F. W. Madden. 1963. The Artificial Insemination of Dairy and Beef Cattle. A Handbook and Laboratory Manual. Lucas Brothers, Columbia, Missouri.

Hupp, E. W., J. W. Austin, N. R. Parish, and R. L. Murphree. 1962. Sperm production of Hereford bulls at different intensities of collection. J. Animal Sci. 21:272.

Jost, P. A. and J. Prepin. 1966. Donnees sur la migration des cellules germinales primordiales du foetus de veau. Arch. d'Anatomie Microscopique 2:161.

Klimek, M. and M. Vlasinova. 1963. Independence of the increase in volume of x irradiated Hela cells on radiation doses. Folia Biol. 9:314.

Kramer, C. Y. 1957. Extension of multiple range tests to group correlated adjusted means. Biometrics 13:13.

Krehgbiel, E. B. 1963. Differentiation of gonads in the bovine embryo. Dissertation Abstr. (No. 6798) 24:01.

Lacy, D. 1967. The seminiferous tubule in mammals. Endeavour 98:101.

Leblond, C. P. and Y. Clermont. 1952. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann. N. Y. Acad. Sci. 55:548.

Lengerova, A. and M. Vojtiskova. 1957. Spermatogenesis in rats following irradiation during embryogenesis. Folia Biol. 3:282.

Lett, I. C. 1968. The synthetic activity of primordial germ cells in normal and irradiated neonatal male rats. J. Embryol. Exp. Morphol. 19:239.

Mandl, A. M. 1966. The population of germ cells in immature male rats following irradiation at birth. Proc. Roy. Soc. B. 165:103.

Monesi, V. 1962. Relation between X ray sensitivity and stages of the cell cycle in spermatogonia of the mouse. Radiation Res. 17:809.

Oakberg, E. F. 1955a. Sensitivity and time of generation of spermatogenic cells irradiated in various stages of maturation in the mouse. Radiation Res. 2:369.

Oakberg, E. F. 1955b. Degeneration of spermatogonia of the mouse following exposure to X rays and stages in the mitotic cycle at which cell death occurs. J. Morphol. 97:39.

Oakberg, E. F. and R. L. di Minno. 1960. X ray sensitivity of primary spermatocytes of the mouse. Int. J. Rad. Biol. 2:196.

Oakberg, E. F. and E. Clark. 1961. Effect of dose and dose rate on radiation damage to mouse spermatogonia and oocytes as measured by cell survival. J. Cellular Compl. Physiol. 58:173.

Ortavant, R. 1959. Reproduction in Domestic Animals (H. H. Cole and P. T. Cupps, eds.) Vol. III. Academic Press, New York.

Pace, H. B., R. L. Murphree, and E. W. Hupp. 1961. Effects of prenatal gamma radiation on testicular morphology and function in swine. Am. J. Vet. Res. 22:406.

Parish, N. R. 1958. The effect of whole-body irradiation on the semen of bulls. M. S. Thesis. Univ. of Tennessee, Knoxville.

Parish, N. R., R. L. Murphree, and E. W. Hupp. 1962. Growth and sexual development of prenatally irradiated cattle. J. Animal Sci. 21:473.

Patten, B. M. 1946. Reproductive organs and gametogenesis. Foundations of Embryology. McGraw-Hill Book Co., New York.

Roosen-Runge, E. C. and L. O. Giesel. 1950. Quantitative studies on spermatogenesis in the albino rat. Am. J. Anat. 87:1.

Rugh, R. and S. Jackson. 1958. Effects of fetal X irradiation upon the subsequent fertility of the offspring. J. Exp. Zool. 138:209.

Snedecor, G. W. 1956. Statistical Methods. The Iowa State College Press, Ames, Iowa.

Steel, R. G. D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., New York.

Welch, P. R. 1965. Effects of fractionated doses of total-body irradiation on semen of bulls. M. S. Thesis. Univ. of Tennessee, Knoxville.

Wilding, J. L., C. S. Simmons, and J. H. Rust. 1952. A multicure irradiation site for exposure of large animals to whole-body gamma irradiation. Nucleonics 10:36.

VITA

George Herman Matschke, son of Herman Wilhelm and Martha (Manns) Matschke was born in Morristown, New Jersey, on July 4, 1933. He attended elementary and junior high school in Denville, New Jersey and graduated from Dover High School, Dover, New Jersey in 1951. He attended Auburn University from 1951 to 1956, receiving the degree of Bachelor of Science in Game Management in June, 1956. He was immediately called to active duty with the Army and spent 21 months in the infantry. Released in March 1958, he reentered Auburn University and received a Master of Science degree in Game Management in 1961. He was employed as project leader of the European Wild Hog Project for the Tennessee Game and Fish Commission on the Tellico Wildlife Management Area from 1960 until he entered graduate school at the University of Tennessee in 1965. He was married August, 1956, to Stephanie, daughter of Stephen and Katie (Lucus) Etheridge. They have two children: Martha Marie, age 10 years and Karen Ann, age 3 years.