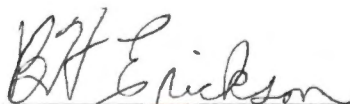


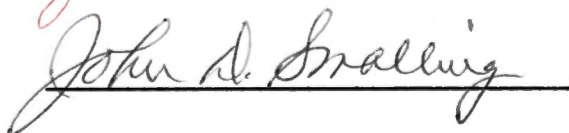
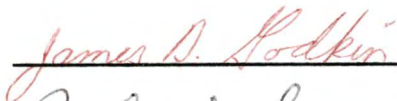
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THE EFFECTS OF CONTINUOUS PRENATAL EXPOSURE TO IONIZING
RADIATION ON GERM CELL SURVIVAL AND FOLLICULAR
DEVELOPMENT IN THE BOVINE

A Thesis

Presented for the

Master of Science

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ABSTRACT

It is known that the germ cell of the prepuberal mammal is sensitive to acute doses of radiation; but only limited information is available on the effects of continuous irradiation. Gravid cows were continuously irradiated with either 8 or 15R of γ -rays for each of 10 days during select intervals of gestation. Fetuses absorbed approximately one-third of the air dose. Ovaries of prenataally irradiated heifers, aged 15 to 18 months, were removed at slaughter, serially sectioned, and prepared for microscopic analysis. Testes excised from newborn bull calves were prepared for microscopic analysis as well. Numbers of primary, growing, and vesicular follicles were determined from ovarian cross-sections, and primitive spermatogonia (gonocytes) were enumerated in seminiferous tubule cross-sections.

Reductions (18 to 21% of control) in number of primary follicles were observed only in heifers irradiated between 60 and 110 days of gestation ($P < 0.01$). During this period the germ-cell population is comprised mainly of mitotically active oogonia, and it is, therefore, likely that this cell type is most susceptible to continuous irradiation. The process of follicular development, as reflected by counts of growing and vesicular follicles, was unimpeded ($P < 0.01$). Number of gonocytes was significantly reduced (10 to 40% of control) in bull calves that were irradiated between 60 and 270 days ($P < 0.01$); a developmental period that corresponds with the shift of the gonocyte from a mitotically active to a mitotically inactive state. The oocyte

of the primordial follicle and the mitotically active gonocyte were relatively resistant to continuous irradiation.

Gonocyte losses in the male were probably insufficient to materially affect sperm production, but the reproductive capacity of females irradiated between 60 and 110 days of gestation would likely be reduced.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION.	1
II. REVIEW OF LITERATURE.	3
Mammalian Oogenesis	3
Radiation Effects on Oogenesis.	4
Irradiation Response of Primordial Germ Cells and Oogonia	4
Irradiation Response of Oocytes	8
Prenatal and Prepuberal Aspects of Mammalian Spermatogenesis	10
Radiation Effects on Spermatogenesis (Prenatal and Prepuberal Stages).	12
Irradiation Response of the Gonocytes	12
Irradiation Response of Undifferentiated Spermatogonia.	15
Irradiation Response of the Differentiating Spermato- gonia, Spermatocytes, Spermatids, and Spermatozoa	17
III. MATERIALS AND METHODS	18
Irradiation	18
Ovarian Histology	19
Testicular Histology.	23
Statistical Analysis.	27
IV. RESULTS	28
Germ-Cell Radioresponse in the Female	28
Primary Follicles (80R)	28

CHAPTER	PAGE
IV. (CONTINUED)	
Primary Follicles (150R).	31
Growing and Vesicular Follicles	31
Combined Effects.	37
Follicle Class Correlation.	37
Germ-Cell Radioresponse in the Male	40
Correlations.	45
V. DISCUSSION.	48
Female.	48
Follicle Class Correlation.	51
Male.	51
VI. CONCLUSIONS	56
BIBLIOGRAPHY.	57
VITA.	63

LIST OF TABLES

TABLE	PAGE
1. Effects of Continuous ^{60}Co γ Radiation (80R, 8R/Day for 10 Days) on the Germ Cells of the Prenatal Bovine Ovary as Reflected by Postnatal Follicular Development. .	29
2. Effects of Continuous ^{60}Co γ Radiation (150R, 15R/Day for 10 Days) on the Germ Cells of the Prenatal Bovine Ovary as Reflected by Postnatal Follicular Development. .	32
3. Combined Effects of 80R and 150R of Continuous ^{60}Co γ Radiation on the Germ Cells of the Prenatal Bovine Ovary as Reflected by Postnatal Follicular Development.	38
4. Correlation Coefficients for the Different Follicle Classes by Period	39
5. Response of the Germ Cells of the Prenatal Bovine Testes to Continuous ^{60}Co γ Radiation as Determined at Birth. .	41
6. Response of Germ Cells of the Prenatal Bovine Testes to Combined Doses of 80R (8R/day for 10 Days) and 150R (15R/Day for 10 Days) of Continuous ^{60}Co γ Radiation as Determined at Birth	46
7. Correlation Coefficients for Testicular Characteristics by Period	47

LIST OF FIGURES

FIGURE	PAGE
1. Cross-Section of a Type 1 Primary Follicle.	20
2. Cross-Section of a Type 2 Primary Follicle.	20
3. Cross-Section of a Type 3 Primary Follicle.	21
4. Cross-Section of a Type 4 Primary Follicle.	21
5. Cross-Section of a Growing Follicle	22
6. Cross-Section of a Normal Vesicular Follicle.	24
7. Cross-Section of an Atretic Vesicular Follicle.	24
8. Seminiferous Tubule Cross-Section (Fertile)	25
9. Seminiferous Tubule Cross-Section (Sterile)	25
10. Seminiferous Tubule Cross-Section (Dead Germ Cell).	26
11. Effect of Continuous Prenatal Irradiation on Number of Primary Follicles	30
12. Effect of Continuous Prenatal Irradiation on Number of Growing Follicles	34
13. Effect of Continuous Prenatal Irradiation on Number of Normal Vesicular Follicles.	35
14. Effect of Continuous Prenatal Irradiation on Number of Atretic Vesicular Follicles	36
15. Sterilizing Effects of Continuous Radiation Administered to the Prenatal Bovine Testis	42
16. Continuous Radiation Effects on the Germ Cells of the Prenatal Bovine Testis.	43

CHAPTER I

INTRODUCTION

Dating back to the early 1900's radiation studies have been conducted on numerous species with varied methodology to determine its effects on reproduction. Early research was characterized by the extensive use of the rodent as a test animal, while radiation was usually administered in acute doses. The most important fact determined in these studies was the presence of particular radiosensitive stages in the morphogenesis of the germ cell; however, many questions remained unanswered. For instance, how sensitive was the germ cell of either sex with respect to dose or dose-rate, and could interspecies extrapolation be made from the response seen in the rodent?

With continued research in both the short-lived species such as the rodent and the long lived species such as the porcine, bovine, and monkey, great variation in radioresponse was found to exist between the sexes, species, and types of exposure. While acute doses of radiation appeared to be quite detrimental to the reproductive processes of the short-lived species, with the male more sensitive than the female, the effects of similar exposures to the long-lived species were minimal. The fact that the germ cell's developmental period in the long-lived species was relatively more protracted raised the question of whether other types of radiation exposure would affect these species. The use of continuous low-level radiation in research ensued and the responses found were quite different. While having

little effect on the germ cells of the short-lived species, an apparent susceptibility to continuous irradiation was found to exist in the germ cells of the long-lived species. This was determined in the pig (Erickson and Martin, 1976) in an effort to estimate the possible effects of continuous radiation in man, of which little was known. Knowledge concerning continuous radiation effects were almost exclusively limited to the rodent and were hardly applicable to the human. Erickson and Martin (ibid, 1976), therefore selected the pig as a comparable subject to the human in terms of developmental events for their radiation study. The intention of this research was to further our understanding of the effects of continuous irradiation in mammals, and the selection of the bovine for this purpose was two-fold. First, the effects of continuous radiation on the bovine had not been researched, and it was our aim to fill this void in radiation research in domestic animals. Secondly, the duration of the developmental processes of the bovine are more comparable to the human than the pig, thus, the bovine's radioresponse could be more reliably extrapolated to man.

CHAPTER II

REVIEW OF LITERATURE

Mammalian Oogenesis

Primordial germ cells appear very early in embryonic life and migrate to the primitive gonads via the bloodstream or by ameboid movement along the dorsal mesentery. Sex differentiation occurs following the arrival of the germ cells in the gonads and, relative to the female, the absence of the Y chromosome in these cells results in the formation of the ovaries (Wells, 1962; Wolff, 1962). In the ovaries, the germ cells, referred to as oogonia, greatly increase their number by repeated mitoses. Following a species dependent period of division, a variable number of oogonia differentiate into oocytes and enter into the first meiotic prophase. During this phase, the oocytes pass through a series of differentiating stages until they reach the stage of rest commonly called the diplotene phase. The oogonia that do not undergo this differentiation, decrease in number up until birth due to cessation of mitotic activity and autolysis. After birth, the remaining oogonia and prediplotene oocytes undergo autolysis resulting in only a finite number of diplotene oocytes (Baker, 1971; Baker and Wai Sum O, 1976; Cran and Moore, 1980). Some of these oocytes continue to grow. Meiosis is suspended, however, until the period just prior to ovulation when this first meiotic division is complete. A second meiotic division follows shortly afterwards and is not complete until fertilization occurs (Baker and Wai Sum O, 1976).

Folliculogenesis is the process of the development of the ovarian follicle and coexists with the process of oogenesis. A close association appears to exist between the germ cells and specific somatic cells from the coelomic epithelium early in prenatal life. It is thought that these somatic cells are the precursors of the granulosa, or follicle, cells (Everett, 1943; Gillman, 1948; Franchi et al., 1962). It is during the diplotene stage of oocyte meiosis that the granulosa cells envelope the growing oocyte. These resulting follicles constitute the pool of primordial follicles from which is drawn those that continue to grow and either undergo atresia or ovulate. The formation of growing follicles (oocyte encompassed by a multi-layer of granulosa cells without a fully formed antrum or vesicle) and vesicular, or graafian follicles, (oocyte encompassed by a multi-layer of granulosa cells with a fully-formed vesicle) in the prenatal bovine results in a few vesicular follicles at birth, however, these follicles do not undergo ovulation but rather become atretic. Only after an animal reaches puberty and gonadotrophin levels are adequate does ovulation occur.

Radiation Effects on Oogenesis

Irradiation Response of Primordial Germ Cells and Oogonia

Primordial germ cells do not appear to be very sensitive to radiation during the period of their migration from the yolk sac stalk to the gonadal ridges. Beaumont (1962), selectively staining rat germ cells by the azo-dye technique for alkaline phosphatase, found

the germ cell population increased while migrating toward the genital ridges prior to Day 15 of gestation, however, negligible radiation effects on these cells were found. The period of increased radiosensitivity appears to be after the germ cells have reached the gonads and mitosis and differentiation of the oogonia is occurring. In the same study, Beaumont (1962) found that 50R or 100R of X-rays, administered at Day 15 of gestation reduced subsequent oocyte numbers to 15% of control values. An increase in oogonia mitosis was noted in ovaries of controls of similar ages and this was believed to be the reason for the elevated sensitivity. Erickson (1966) found similar responses in the bovine germ cell. The exposure of prenatal bovine germ cells to 400R of gamma radiation (whole body to dam) caused no observable germ cell loss prior to Day 60 of gestation; however, the germ cell population decreased at Day 60 under the same treatment. This period is considered to be the beginning of high mitotic activity of the oogonia in the bovine and normally persists until approximately Day 120 of gestation. In another study, Erickson and Reynolds (1978) acutely irradiated cows bearing fetuses varying in age from 40 to 270 days of gestation and determined that oogenesis (reflected by follicular oocyte count) was most severely affected between Days 70 and 90 of gestation. They found no effect on oogonia in earlier stages with respect to numbers, and several possibilities for this finding were discussed. A lower mitotic rate during this earlier period could have been the reason for a refractoriness or resistance to radiation. Another possibility

was that the oogonia early in mitosis were in fact, sensitive to the lethal effects of radiation; however, a sufficient time period remained before the end of mitosis for the surviving oogonia to multiply and compensate for early losses. More evidence corroborating the increased radiosensitivity of oogonia during high mitotic activity was presented by Baker and Beaumont (1967) with studies involving the monkey. Oogonia in the fetal monkey are mitotically active from two months to five months, resulting in a peak number of germ cells at approximately five months. These researchers, prenatally irradiating fetuses at various stages of development with doses ranging from 350R to 1,800R of X-radiation, determined that the largest decrease in germ cell number occurred at five months. They also observed that doses of 1000R or more resulted in the greatest depletion of germ cells, and estimated that a dose of approximately 2000R or more would be required to sterilize the neonatal monkey. Marked changes in germ cell susceptibility to irradiation associated with stage of development were reported by Erickson and Martin (1976, 1984) in the continuously irradiated pig. The exposure of oogonia during the period of peak mitotic activity (40-80 days) to continuous gamma radiation resulted in significant reductions in the germ cell population.

Interspecies differences in radioresponse is prevalent during this period of germ cell development, and the consequences to subsequent reproduction is of pivotal importance. At the completion of this mitotic stage, oogonial numbers are fixed for life in the

female (Beaumont and Mandl, 1962), and any depletion of this oogonial population in any way could result in permanent impairment of the animals reproductive capacity.

Short-lived species, such as the mouse or rat, have a much shorter oogonial phase of mitosis than the long-lived species. The killing of germ cells during this relatively short period would be devastating to the subsequent reproduction of these species because only a limited amount of time exists for possible regeneration of the oogonia population from surviving oogonia.

In the rodent, acute exposures to radiation are far more detrimental to oogonia than are continuous exposures. Only small doses are accumulated with continuous irradiation and are not as destructive as the abrupt large doses of acute irradiation. Beaumont (1966) reported that the population of germ cells of the female rat, when subjected to 100R of acute X-irradiation at 15.5 to 18.5 days, was reduced by two-thirds within five days. Also, no significant restoration of the cellular population, when radiation occurred during this period, was noted by Beaumont (1965, 1966). Nash (1969), exposing mice to 80R, 160R, and 320R at 6, 10, 14, and 17 days of gestation, reported that the single whole-body doses of X-rays at all levels resulted in sterility of the female mice irradiated at 14 and 17 days. In contrast, Erickson and Martin (1976), continuously irradiating rats with 7, 3, 1 and 0.5 rads/day during the first 21 days of a 22 day gestation period, found that while the 7 rad/day treatment was partially injurious, the 1 rad/day treatment had no effect at all on the germ cell population.

Essentially the opposite is true in the long-lived species. Although harmful radiation effects on oogonia in these species have been observed with acute exposures (Erickson, 1966), the effects on subsequent fertility are not as severe as reported in the short-lived species. As alluded to earlier, the period of oogonial mitosis in these animals is much longer and any loss of germ cells due to acute exposures can be replenished by surviving germ cells. Also, the oogonia in the long-lived species appears to be less sensitive to irradiation effects. While the germ cell population was reduced to 14% of control in the rat with an acute dose of radiation (Beaumont, 1961), the same dose reduced germ cell number to only 50% of control in the pig (Erickson, 1977) and 74% of control in the cow (Erickson and Reynolds, 1978). As stated earlier, continuous irradiation appears to have a more profound effect in the long-lived species. Continuous doses of 7, 3, or 1 rad/day administered to the prenatal pig throughout its developmental period resulted in sterility in most cases (Erickson and Martin, 1976).

Irradiation Response of Oocytes

With the exception of the rodent, mammalian oocytes are generally more resistant to the effects of ionizing radiation than oogonia, but exceptions do exist. Oakberg (1979) reported that the dictyate oocyte of the mouse was extremely sensitive to irradiation effects and that doses as low as two or three rads significantly reduced the oocyte population. Beaumont (1962) noted similar radiosensitivities in the neonatal rat. Irradiation of five day old rats, when the

oocytes are in the diplotene phase of meiosis, resulted in a reduction of the total population to approximately 8% of controls. These findings are rather unique in that almost all animals studied have shown that the diplotene stage of oocyte meiosis is fairly resistant to irradiation. Mandl (1959), directly exposing the ovaries of rats to acute doses of X-radiation, reported that the earliest stages of meiotic prophase (pre-leptotene and leptotene) were more susceptible to radiation damage than the latter stages. A dose of 300R was required to destroy most of the oocytes in early development, and the subsequent periods required exaggerated doses before degenerating. Later studies revealed that the period just prior to ovulation--when the oocytes progress through the dictyate stage and reach the first metaphase (maturation division)--is also associated with a rise in radiosensitivity (Beaumont, 1962; Mandl, 1963). The reverse was reported by Oakberg (1978) in the guinea pig. Young oocytes were observed to be resistant while the growing oocytes sensitive.

In the long-lived species, it appears that the oocyte becomes fairly resistant to radiation early in the diplotene stage. The human female (Baker, 1963) as well as the cow (Erickson, 1966) exhibit increased number of oocytes beyond Day 90. Erickson (1965, 1966, 1967), in the bovine, has demonstrated that radiation during this period causes little harm to the oocyte population when administered at doses of 300-600R; however, more elevated doses were damaging

to some degree, and the later stages of meiosis appeared to be more sensitive than the earlier stages. The failure of X-ray doses as high as 600 rads to impair reproductive performance of the human female has been reported by Lindop (1969) and Baker (1971). In the prenatal monkey, Baker and Beaumont (1967) observed that diplo-tene oocytes increased from 1% at 90 days to 20% at 120 days of prenatal development. Exposure of the fetal ovaries during these periods to 1000R or more of X-radiation seemed to have little effect on germ cell survival. Ozzello and Rugh (1965) reported that the ovaries of monkeys irradiated with doses of 200-400R of X-rays during this same period were unaffected. The response of the oocyte to continuous irradiation is somewhat different. Erickson (1978), continuously irradiating the prenatal pig, reported that both oogonium and possibly the preleptotene oocyte were susceptible. As already described, the oocyte of the rodent is sensitive to doses as low as one rad per day, thus vulnerable to continuous irradiation.

Prenatal and Prepuberal Aspects of Mammalian Spermatogenesis

The primordial germ cells, or gonocytes as they are designated, first appear in the primitive gonad at approximately Day 42 of gestation in the bovine (Courot et al., 1970) and Day 12 of gestation in the mouse (Chiquoine, 1954). Mitotic division of the gonocyte occurs in the earlier stages of prenatal development in an effort to establish a large germ cell population. From a peak, mitotic

activity declines as many of the gonocytes are experiencing a state of arrest. Gonocyte mitosis remains low for some time before resuming to previous levels and subsequently differentiating in the post-natal animal to form definitive stem spermatogonia. The actual process of spermatogenesis is initiated when this differentiation occurs. Though the kinetics of spermatogenesis from this period on are out of the realm of this study, a brief summary of these developmental events is in order.

Mitosis of stem spermatogonia results in two different types of cells: 1) those that undergo differentiation (Type A) and eventually become viable spermatozoa, and 2) those that remain undifferentiated and reside as stem spermatogonia for future production of spermatozoa. Several developmental events occur before the formation of viable spermatozoa. Continued differentiation and mitosis of type A spermatogonia results in the formation of more mature spermatogonia, namely intermediate. These in turn differentiate into type B spermatogonia. The final mitotic division of the B spermatogonia yields primary spermatocytes. These are the cells which will undergo the first meiotic division. The primary spermatocytes enter the period of meiotic prophase where they progress through a series of developmental nuclear changes. Eventually, duplication of chromosomes occurs, the cells divide, and two diploid germ cells (secondary spermatocytes) are formed. These cells undergo a second meiotic division, in which no further DNA synthesis occurs, to form haploid cells known as spermatids.

The final period of spermatogenesis is known as spermiogenesis and is characterized by a series of morphological changes, without division, of the spermatid to form mature spermatozoa, (Courot et al., 1970).

Radiation Effects on Spermatogenesis (Prenatal and Prepuberal Stages)

Irradiation Response of the Gonocytes

After the gonocytes experience a variable degree of mitotic activity in the pregonadal and early gonadal stages, these germ cells enter a period of mitotic arrest--extending from Day 18 of gestation to Day 3 of postnatal life in the rat (Beaumont and Mandl, 1963) and Day 70 to Day 230 of gestation in the bull (Matschke and Erickson, 1969). Erickson (1985) and Beaumont (1962) in studies with the bull and rat, respectively, have both reported that the radiosensitivity of the mitotically active gonocyte is minimal when exposed to acute doses of radiation, although, dose-rate effects have been noted in the rat (Erickson and Martin, 1972). During the period of mitotic arrest, however, the gonocyte appears to be extremely sensitive to the ionizing effects of radiation. Mandl (1966) reported that following exposure of one-day old rats to only 19R of X-rays the subsequent germ cell population was reduced to 50% of control. Beaumont (1960) sterilized rats by exposing them to 100R of X-rays during the period of gonocyte mitotic arrest (19-22 days). Sterility was also reported by Hughes (1962) when one-day old rats were irradiated with 150R of X-rays. Selby (1973),

on the other hand, has shown that newborn mice remained fertile after exposure to 300 rads of X-radiation. This contrasting observation is explained, in part, by the fact that gonocyte content of the tubules of mice is three times in excess of that found in the rat during the same developmental stage (Erickson, 1978).

Similar effects of the gonocyte are seen in the long-lived species when irradiation occurs during this period of mitotic arrest. Matschke and Erickson (1969) reported that 150 rads of ^{60}Co gamma rays at 0.5R/minute inflicted little damage in those bull testes irradiated prior to Day 70; however, at Day 70 when much of the gonocyte population is in mitotic arrest, this same treatment reduced the gonocyte population to 31% of control. In another study involving the bovine, Erickson et al. (1972) found that dose of 19 rads killed 50% of the gonocyte number, while a dose of 38 rads practically destroyed the population of gonocytes. This same dose of 38 rads or more was also ascertained to permanently impair sperm-producing ability of the bulls when administered between Day 80 of gestation and Day 70 postnatal. In the pig, an attempt to determine the initial period of radiosensitivity via histological changes in the gonocyte was inconclusive (Erickson, et al., 1963). Comparisons drawn between the rat and pig with respect to testicular development, however, appeared to show that increased gonocyte radiosensitivity of the pig occurs during the same developmental period as the rat.

During the period of mitotic arrest, the gonocyte appears to be more sensitive to total dose rather than dose rate. Thus, while similarities in radioresponse are seen between the short-lived species and the long-lived species exposed to acute doses of radiation, a different response is found when the mitotically arrested gonocyte is exposed to a continuous dose of radiation. In the short-lived species, the period of mitotic arrest is much shorter than that of the long-lived species; therefore, the accumulation of a dose of radiation large enough to cause severe harm to the gonocyte is improbable. This observation was supported by the study of Erickson (1978). In this study, prenatal mice, rats, guinea pigs, and pigs were continuously irradiated with gamma radiation throughout their respective prenatal periods. While dose rates of 0.25 rads/day and 1 rad/day reduced germ cell number in the pig to 38% of 2% of control, respectively, 71% of the germ cells in the mouse survived a dose rate of 1 rad/day, as did 50% in the rat, and 41% in the guinea pig.

In the many studies concerning the radioresponse of the gonocyte, it is evident that the period of gonocyte arrest is the most sensitive of all developmental stages. Both sterility and a decrease in sperm production has been reported upon irradiation of this germ cell type; however, with respect to interspecies differences, dose and dose rate vary in their abilities to cause these detrimental effects.

Irradiation Response of Undifferentiated Spermatogonia

Gonocytes first give rise to definitive stem spermatogonia on Day 4 postpartum in the rat (Beaumont and Mandl, 1963; Hughes, 1962), Day 70 postpartum in the bull (Erickson, 1963), and at approximately 10 years in man (Charney et al., 1952). Mitoses of these stem spermatogonia result in two different types of cells: 1) those which differentiate and give rise to succeeding spermatogonial generations, and 2) those which do not differentiate and remain in the stem spermatogonial pool.

Unlike their progenitor the gonocytes, the stem spermatogonia are very resistant to irradiation; however, radioresponse differs across species with the short-lived mammal being the most resistant. In the mouse, Withers et al. (1974) calculated at D_0 of 180 rads for the stem spermatogonia. Oakberg (1971), however, reported the stem spermatogonia of mice survived acute radiation doses of 100, 500, 1,000, and a fractionated 1000R exposure, although the doses of 500R and 1000R decreased the cell population to 13% and 3% control, respectively. Exposures of 200R of ^{60}Co gamma radiation was found to have very little effect, if any, on undifferentiated stem cell numbers in the mouse (Erickson, 1981) and rat (Erickson, 1976). Shaver (1953) and Jones (1960) have reported that doses of as much as 1000-1100R are required to render the male rat infertile.

Studies involving the long-lived species suggest an increased radiosensitivity of the spermatogonial stem cell over the short-lived species. Irradiation of the boar testes with 100, 200, and

400 rads of gamma radiation reduced the stem spermatogonial population to 51%, 37%, and 34% of control, respectively (Erickson and Martin, 1984). Research by Lyon et al. (1976) on the monkey and Rowley et al. (1974) and Sanderman (1966) on man have revealed that these species are particularly sensitive to irradiation.

Continuous irradiation is less effective in reducing stem cell numbers than is acute exposure. Spermatogenesis in the rat was not permanently impaired with a continuous dose of gamma radiation of as much as 6 rads/day (Erickson, 1978). Although a chronic dose of 2.17 rads/day of gamma radiation sterilized the Rhesus monkey, this occurred only after a period of over three years of exposure (Cheng et al., 1982).

In comparison to the gonocyte during its period of arrest, the stem spermatogonia are highly tolerant to the ionizing effects of radiation. This is very advantageous to the male in view of the fact that heavy depletion of these stem cells would result in permanent damage to sperm production. Extremes in radiation dose will cause some detriment to the stem cells, and dose-rate effects have been noted (Erickson and Blend, 1978); however, significant impairment of subsequent fertility is difficult to obtain. This is probably due to the fact that the stem-cell sensitivity occurs only during the mitotic phase of the cell cycle. Because only a portion of the stem cells are in mitosis at any one given time (Clermont and Harvey, 1965), only a small percentage of the stem cell population would be susceptible to the ionizing effects of radiation (Erickson, 1976).

Irradiation Response of the Differentiating Spermatogonia, Spermatocytes, Spermatids, and Spermatozoa

While many studies have revealed that the differentiating spermatogonia are radiosensitive, it appears that the succeeding generation of germ cells are relatively resistant to irradiation. Oakberg (1957) reported that the doses of 22.5 and 21.4R were lethal to 50% of the type A and type B spermatogonia of the mouse, respectively. The relative sensitivity of the various types of spermatogonia of the rodent have been observed by many other researchers as well (Jones, 1960; Craig et al., 1961; Monesi, 1962; Partington et al., 1962; Erickson, 1978). Rowley et al. (1974), studying the effects of acute irradiation on the human testis, found that an exposure of 100 rads of X-rays resulted in a marked depletion of spermatogonial populations. A period of 9-18 months were required for complete recovery of sperm production and doses of 400 to 600 rads resulted in infertility for five years or more. The responses of the spermatocytes, spermatids, and spermatozoa are quite different. Oakberg (1960), in the mouse, reported that radiation doses of as much as 1000R were required to cause significant harm to primary spermatocytes, while no observable damage to spermatids and spermatozoa was detected after exposure to doses of as much as 1500R. In the human, Rowley et al. (1974) observed the radioresistance of the spermatocyte to be ten times that of the spermatogonium. The spermatid was found to be even more resistant than the spermatocyte.

CHAPTER III

MATERIALS AND METHODS

Irradiation

A total of 129 gravid cows were used for this study. While 10 of these females served as controls, the remaining 119 cows were exposed continuously to whole-body doses of gamma radiation at approximate rates of either 8 or 15 roentgens/day, each for 10 days. The term roentgen (R) refers to a unit of exposure to ionizing radiation, and is that amount of gamma or X rays required to produce ions carrying one electrostatic unit of electrical charge in one cubic centimeter of dry air. Any use of the term rad in this study will be in reference to that amount of the roentgen dose that was absorbed. Total doses were 80 and 150R, respectively.

Irradiations were carried out in a multipoint ^{60}Co field as described by Wilding et al. (1952) and occurred at 18 different stages of gestation: (1) 30-40 days; (2) 60-70 days; (3) 70-80 days; (4) 80-90 days; (5) 100-110 days; (6) 110-120 days; (7) 130-140 days; (8) 140-150 days; (9) 150-160 days; (10) 160-170 days; (11) 170-180 days; (12) 180-190 days; (13) 190-200 days; (14) 200-210 days; (15) 210-220 days; (16) 220-230 days; (17) 230-240 days; (18) 260-270 days. The dose to the fetus was estimated from readings of thermoluminescent dosimeters (lithium fluoride) placed in the bladders of the gravid cows. Approximately one-third of the air dose was absorbed by the fetus.

Ovarian Histology

Fifty-seven prenatally irradiated heifers and five control heifers were slaughtered between 15 and 18 months of age and the ovaries from each animal removed. All tissue was fixed in Bouin's fluid and embedded in paraffin. Each ovary was serially sectioned at 10 and every twentieth section was mounted on glass slides and stained with Harrison's Haemotoxylin and Orange G.

The ovarian cross-sections were viewed microscopically (X250). Response of the prenatally irradiated germ cell was determined by evaluations of follicular development and number. Follicles were categorized and counted with respect to specific stages of development. The three main categories established were primary follicles, growing follicles, and vesicular follicles. A primary follicle was defined as an oocyte encompassed by a single layer of granulosa cells. These follicles were further subdivided into four types according to granulosa-cell differentiation and condition of oocyte chromatin. The four types were as follows: (1) oocyte with condensed chromatin and squamous-type granulosa cells (Fig. 1); (2) oocyte with diffused chromatin and squamous-type granulosa cells (Fig. 2); (3) oocyte with condensed chromatin and cuboidal-type granulosa cells (Fig. 3); and (4) oocyte with diffused chromatin and cuboidal-type granulosa cells (Fig. 4). A single class of growing follicles was established and contained those oocytes encompassed by a multi-layer of granulosa cells without a fully-formed vesicle (Fig. 5). Those follicles with fully-formed vesicles were classified

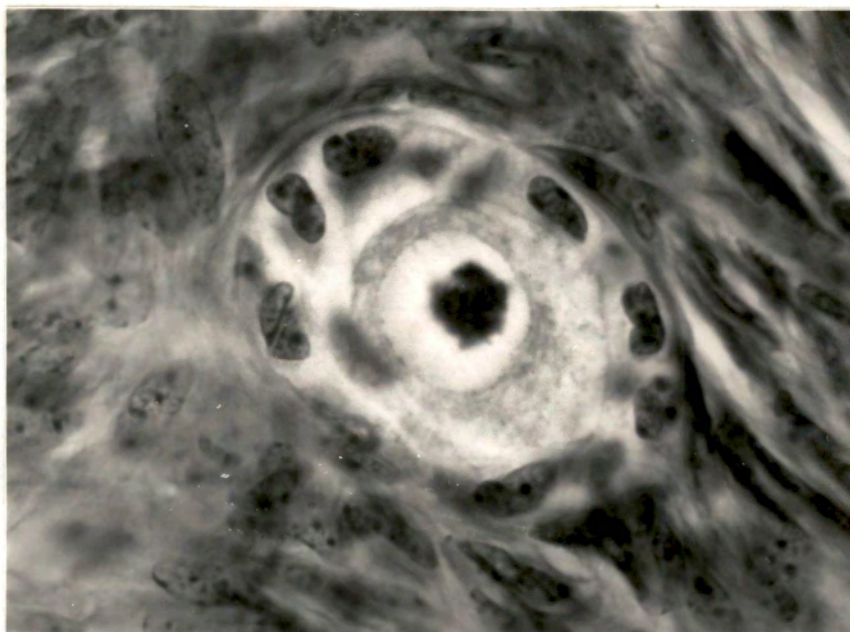


Fig. 1. Cross-section of a type 1 primary follicle. Note condensed chromatin of oocyte. X1350.

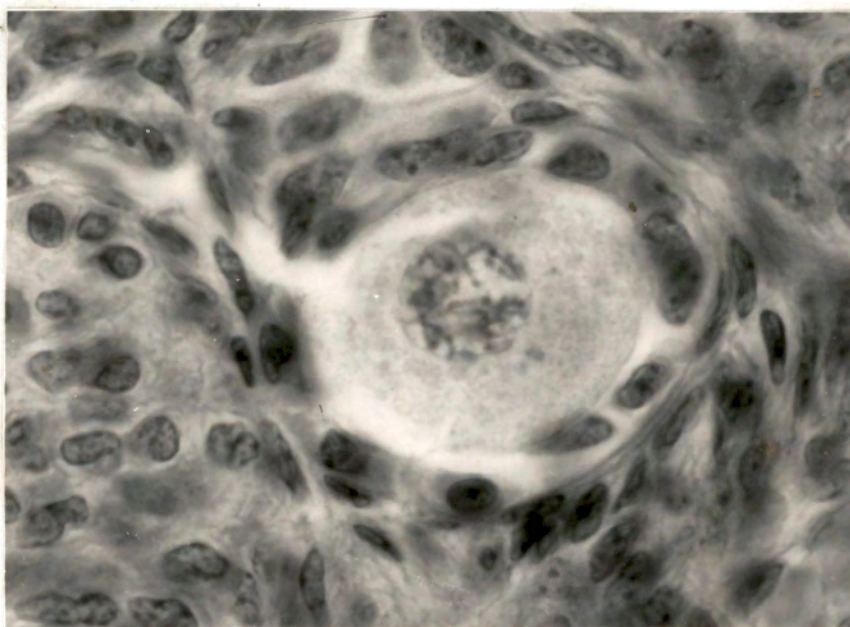


Fig. 2. Cross-section of a type 2 primary follicle. Note diffused chromatin of oocyte. X1350

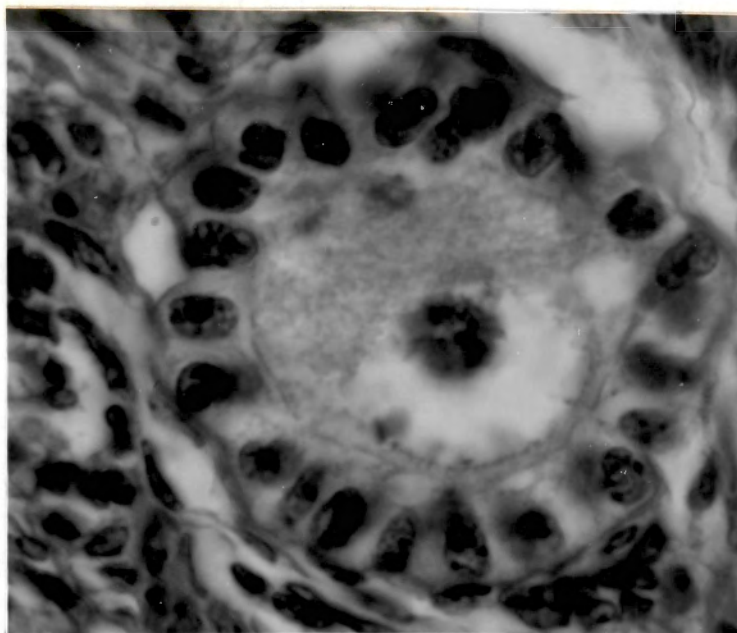


Fig. 3. Cross-section of a type 3 primary follicle. Note condensed chromatin of oocyte. X1350

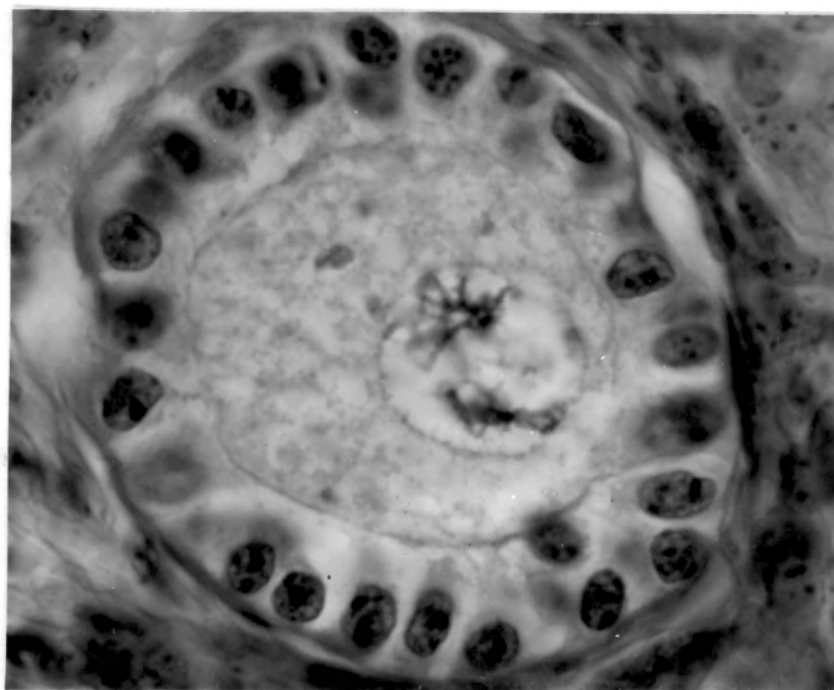


Fig. 4. Cross-section of a type 4 primary follicle. Note diffused chromatin of oocyte. X1350

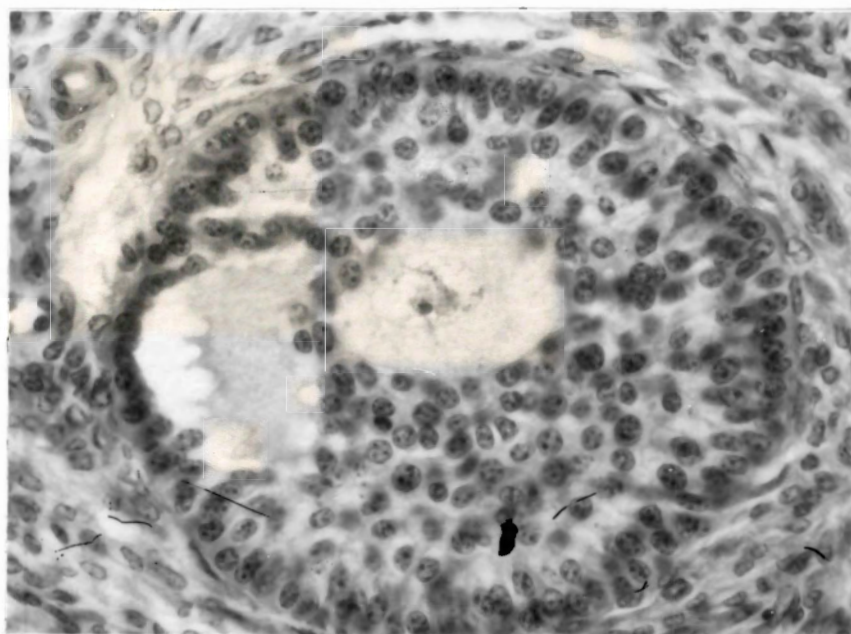


Fig. 5. Cross-section of a growing follicle. X500

as vesicular follicles in either a normal state (Fig. 6) or an atretic state (Fig. 7). A vesicular follicle was determined atretic if the granulosa cells were either absent or degenerating. Total follicular counts were estimated through multiplication of counts derived from every twentieth section of each of the serially sectioned ovaries.

Testicular Histology

Sixty-two prenatally irradiated newborn bull calves and five newborn bull calf controls were orchidectomized. Preparation of testicular tissue for observation was the same as that described in ovarian tissue preparation. The testes were sectioned at 5 μ and representative sections from the middle and both ends were obtained.

The testicular cross-sections were scanned microscopically (x400) and approximately 275 seminiferous tubule cross-sections per testis were scored randomly. Germ cells were enumerated. Those tubule cross-sections containing germ cells were scored as fertile (Fig. 8) and those without germ cells were scored as sterile (Fig. 9). With these data, percent tubular sterility was determined as was number of germ cells per tubule cross-section. Dead germ cells (Fig. 10) were also enumerated and percent dead germ cells determined.

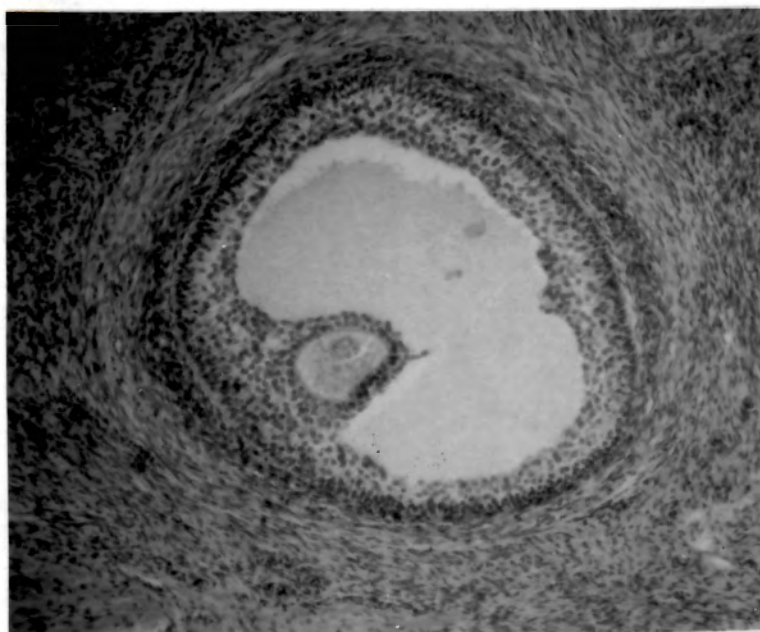


Fig. 6. Cross-section of a normal vesicular follicle. X140

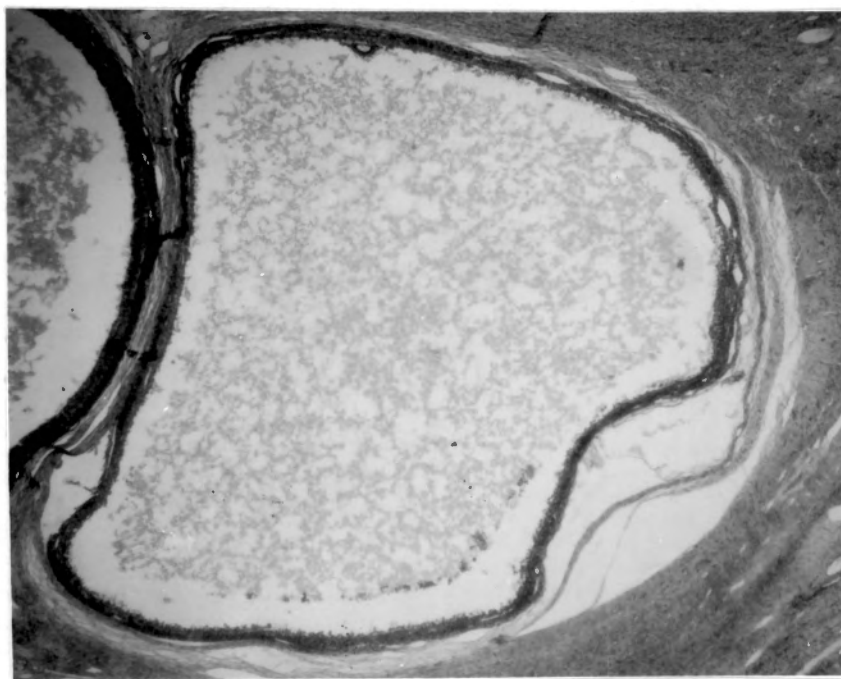


Fig. 7. Cross-section of an atretic vesicular follicle. Note degenerating granulosa layer. X40.

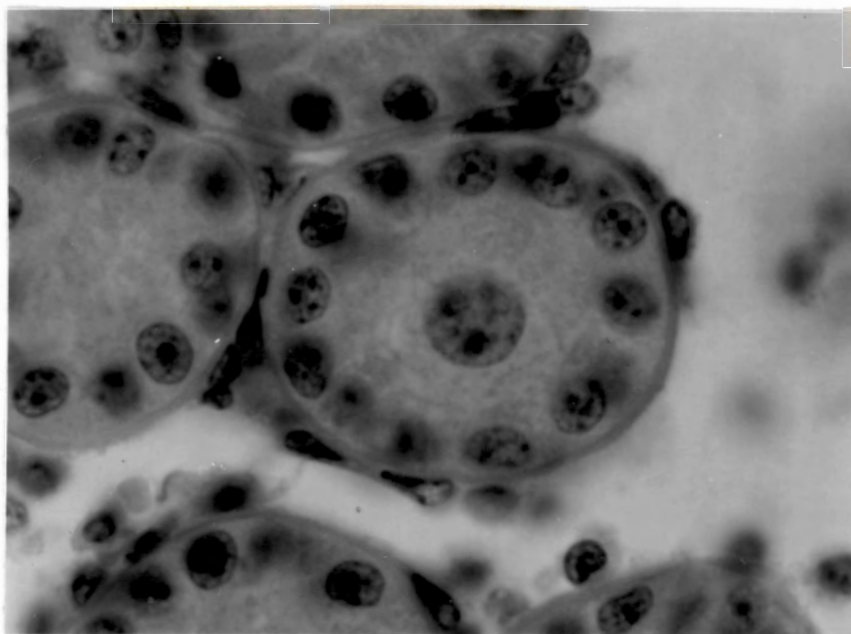


Fig. 8. Seminiferous tubule cross-section (fertile). Note germ cell encompassed by a layer of sertoli cells. X1350

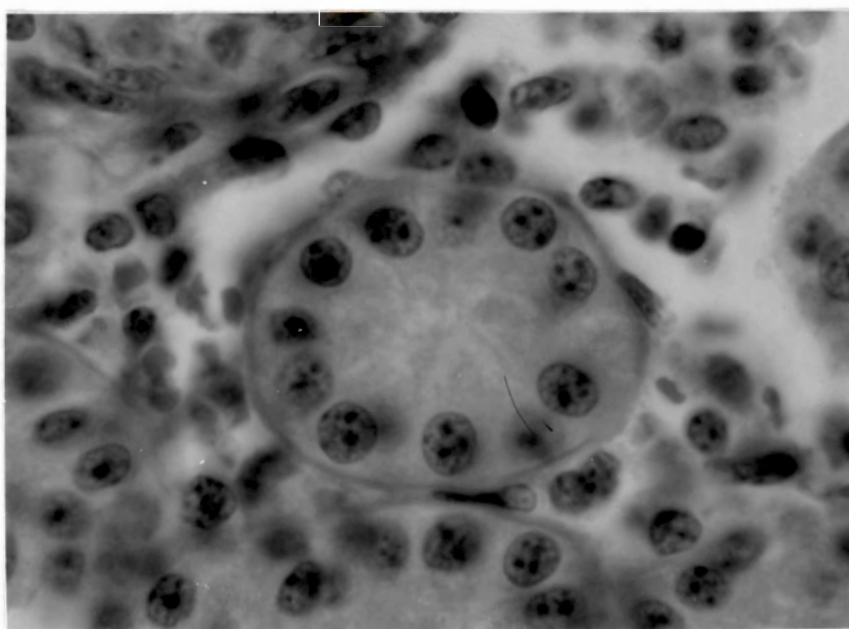


Fig. 9. Seminiferous tubule cross-section (sterile). Note absence of germ cell in tubule. X1350

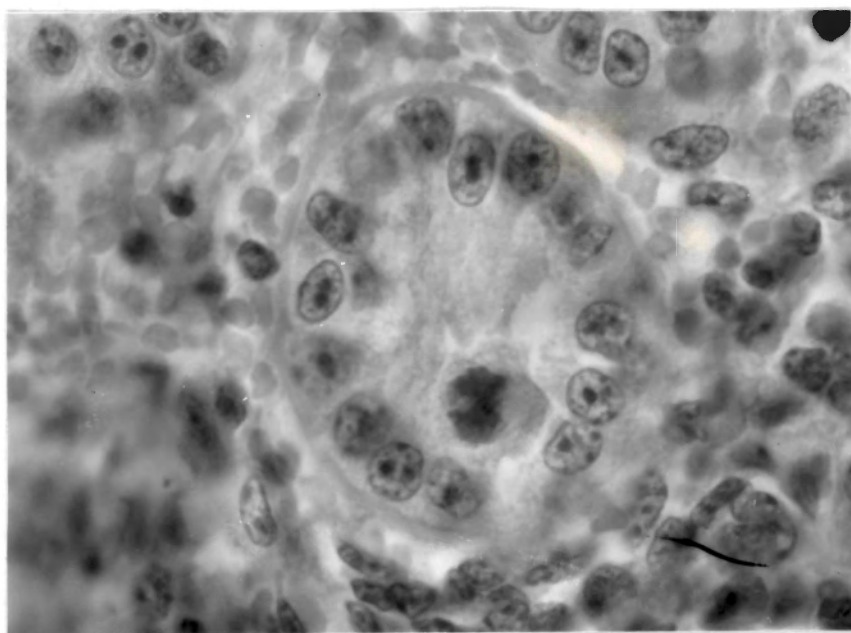


Fig. 10. Seminiferous tubule cross-section (dead germ cell).
Note pyknotic nucleus. X1350

Statistical Analysis

The number of observations in the various treatment periods was not sufficient for proper analysis, therefore, the 18 prenatal periods were grouped into four major periods representative of specific stages of germ cell development. The four periods and characteristics of each for the female are as follows: (1) 30-40 days--represents the oogonial population during their period of limited mitoses; (2) 60-110 days--characterized by mitotically active oogonia; (3) 110-170 days--represents the early stages of meiosis; (4) 170-230 days--typified by diplotene or resting oocytes incorporated into primary follicles. The four major periods of the male and their features are as follows: (1) 30-40 days--early stages of gonadal differentiation; (2) 60-90 days--characterized by mitotically active gonocytes; (3) 100-190 days--mitotically arrested gonocytes representing the majority of the germ cell population; (4) 190-270 days--characterized by a limited renewal of gonocyte mitosis.

The general linears model procedure (SAS, 1979) was used to perform an analysis of variance on all data collected as well as to predict significant differences between means. In the female, correlations between number of follicles of the various follicle types were determined. Correlations between percent tubular sterility, percent dead germ cells per testis, and number of germ cells per tubule in the male were calculated.

CHAPTER IV

RESULTS

Germ-Cell Radioresponse in the Female

The response of the germ cell of the female to continuous ionizing radiation was determined by quantitative and qualitative analysis of ovarian follicles. This was under the assumption that any radiation effects on the prenatal germ cells would be seen in later developmental stages.

Primary Follicles (80R)

Although no significant decrease in number of primary follicles in heifers aged 15 to 18 months was seen when the oogonia were continuously irradiated between 30 and 40 days of gestation (Table 1), a reduction in primary follicles of 65% of control was noted with the 80R dose (Fig. 11). The greatest decrease in the primary follicle population was seen when irradiation occurred between 60 and 110 days of gestation. The dose of 80R significantly reduced primary follicle numbers to 21% of control when administered continuously during this period ($P < 0.01$). Beyond day 110, the germ cells became increasingly resistant to radiation. In those animals prenatally irradiated between 110 and 170 days, the primary follicle population was reduced to 67% of control by the 80R dose, and when irradiated between 170 and 230 days of gestation, follicle counts were only reduced to 77% of control. Neither reduction was significant ($P < 0.05$).

TABLE 1

EFFECTS OF CONTINUOUS ^{60}Co γ RADIATION (80R, 8R/DAY FOR 10 DAYS)
ON THE GERM CELLS OF THE PRENATAL BOVINE OVARY AS
REFLECTED BY POSTNATAL FOLLICULAR DEVELOPMENT.^a

Prenatal Age at Irradiation (Days)	Animals (No.)	Follicle Number ($\bar{X} \pm \text{SE}$)			
		Primary ^b	Growing ^c	Vesicular	
				Normal	Atretic
Control	5	165,346 $\pm$ 34,365	2,082 $\pm$ 525	69 $\pm$ 14	38 $\pm$ 9
30-40	2	107,910 $\pm$ 54,337	1,457 $\pm$ 830	36 $\pm$ 22	26 $\pm$ 14
60-110	9	35,326 $\pm$ 25,615 ^e	1,304 $\pm$ 391	33 $\pm$ 10	13 $\pm$ 7 ^d
110-170	10	111,173 $\pm$ 24,300	1,450 $\pm$ 371	51 $\pm$ 10	33 $\pm$ 6
170-230	9	127,246 $\pm$ 25,615	2,264 $\pm$ 391	76 $\pm$ 10	52 $\pm$ 7

^aData collected from ovaries of heifers aged 15 to 18 months.

^bOocyte encompassed by a single layer of squamous-type granulosa cells (Fig. 2).

^cOocyte encompassed by a multi-layer of granulosa cells but without a fully-formed vesicle (Fig. 5).

^dSignificantly different from control ($P < 0.05$).

^eSignificantly different from control ($P < 0.01$).

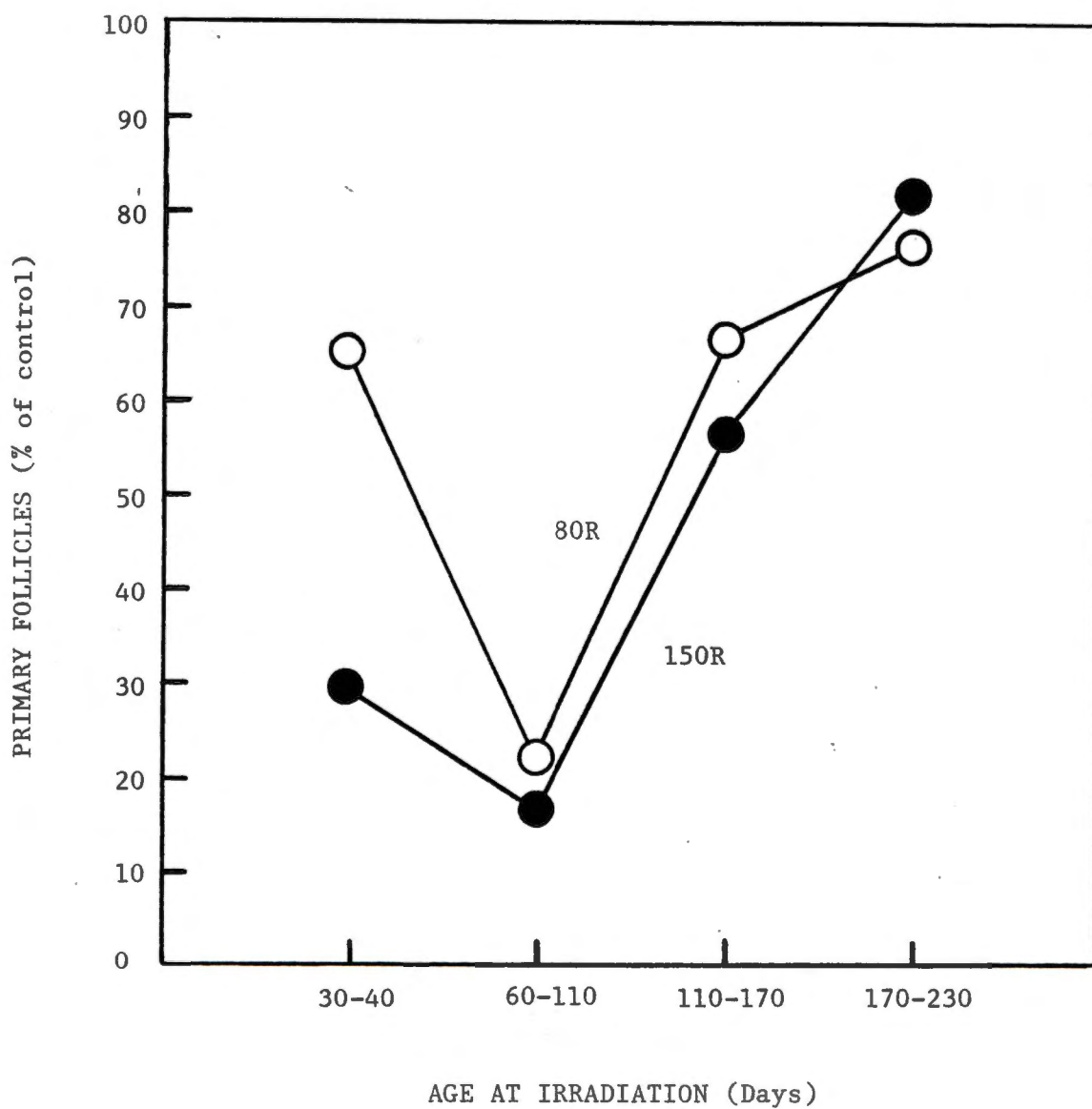


Figure 11. Effect of Continuous Prenatal Irradiation on Number of Primary Follicles.

Primary Follicles (150R)

No significant differences were found between the dose of 80R and the larger dose of 150R when administered continuously ($P < 0.05$); however, there was a tendency for the higher dose to be more destructive to the germ cell. The continuous administration of 150R of gamma radiation between 30 and 40 days of gestation greatly reduced subsequent primary follicle numbers to 30% of control (Fig. 11), although no significant differences were determined (Table 2). This observation is probably misleading in view of the fact that only one animal was irradiated with 150R during this period. The reductions in primary follicle numbers found in ovaries irradiated at more advanced stages of development were very similar to those noted for the dose of 80R. Again, the greatest germ cell sensitivity appeared to be between 60 and 110 days of gestation. A reduction in the primary follicle population to 18% of control was observed when the continuous dose of 150R was administered between 60 and 110 days of gestation ($P < 0.01$). Follicle counts for the prenatal period of 110 to 170 days and 170 to 230 days were 57% and 82% of control, respectively. Neither values differed significantly from controls ($P < 0.05$).

Growing and Vesicular Follicles

It appears that the ability of primary follicles to develop into growing and vesicular follicles was minimally impaired by irradiation (substantiated by calculation of correlation coefficients for each period and discussed later in further detail).

TABLE 2

EFFECTS OF CONTINUOUS ^{60}Co γ RADIATION (150R, 15R/DAY FOR 10 DAYS) ON THE GERM CELLS OF THE PRENATAL BOVINE OVARY AS REFLECTED BY POSTNATAL FOLLICULAR DEVELOPMENT.

Prenatal Age at Irradiation (Days)	Animals (No.)	Follicle Number ($\bar{X} \pm \text{SE}$)			
		Primary	Growing	Normal	Vesicular Atretic
Control	5	165,346 $\pm$ 34,365	2,082 $\pm$ 525	69 $\pm$ 14	38 $\pm$ 9
30-40	1	49,806 $\pm$ 76,844	1,618 $\pm$ 1,174	92 $\pm$ 31	54 $\pm$ 20
60-110	9	30,122 $\pm$ 25,615 ^a	794 $\pm$ 391	36 $\pm$ 10	24 $\pm$ 7
110-170	11	94,565 $\pm$ 23,169	868 $\pm$ 354	48 $\pm$ 9	31 $\pm$ 6
170-230	6	136,160 $\pm$ 31,371	733 $\pm$ 479	27 $\pm$ 13	19 $\pm$ 8

^aOnly value which differed significantly from control ($P < 0.01$).

The average number of growing follicles ranged from 0.5% to 3.7% of the primary follicle population. The smallest number of growing follicles were found mainly in those animals that were irradiated continuously with either 80R or 150R between 60 and 110 days of gestation (Fig. 12); however, during no period did the number of growing follicles differ from the control ($P < 0.05$). A significant difference was found between the two doses in animals irradiated between 170 and 230 days of gestation ($P < 0.05$). While the largest number of growing follicles were observed in the animals irradiated during this period with the dose of 80R (109% of control), those continuously irradiated with 150R contained the smallest number of growing follicles (35% of control).

No significant differences were found between normal vesicular follicle numbers and controls ($P < 0.05$), though reduced follicular counts were seen at stages in which primary and growing follicular numbers were reduced (Fig. 13). The only group that differed significantly from controls with respect to number of atretic vesicular follicles were those irradiated between 60 and 110 days of gestation with a dose of 80R (Fig. 14). An interesting determination was that the ovaries analyzed from animals irradiated continuously with 150R between 30 and 40 days and 60 and 110 days contained relatively greater numbers of both normal and atretic vesicular follicles than those irradiated with 80R of radiation. This did not follow the patterns seen in the primary and growing follicles where the higher dose was associated with lower numbers of follicles.

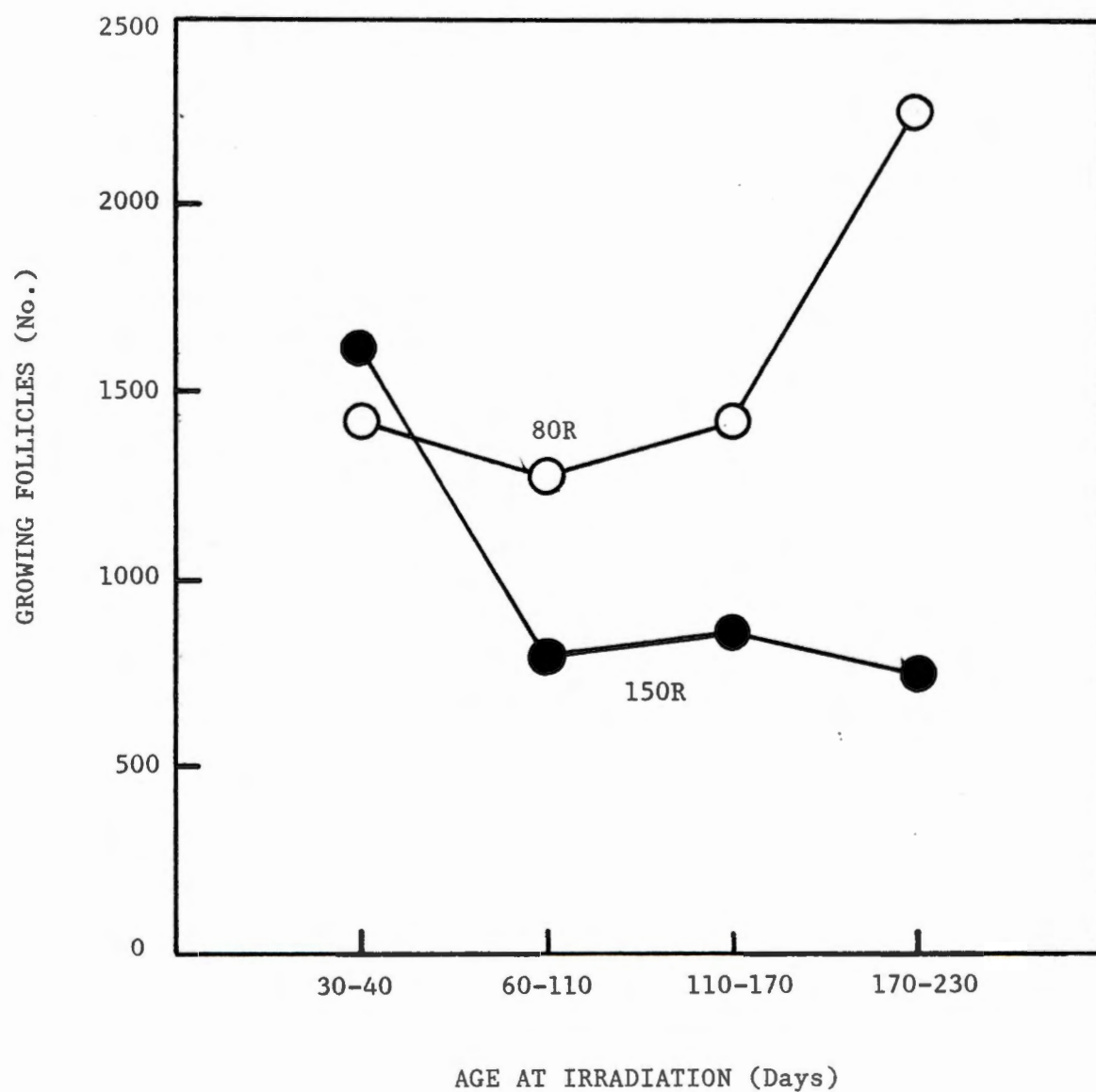


Figure 12. Effect of Continuous Prenatal Irradiation on Number of Growing Follicles.

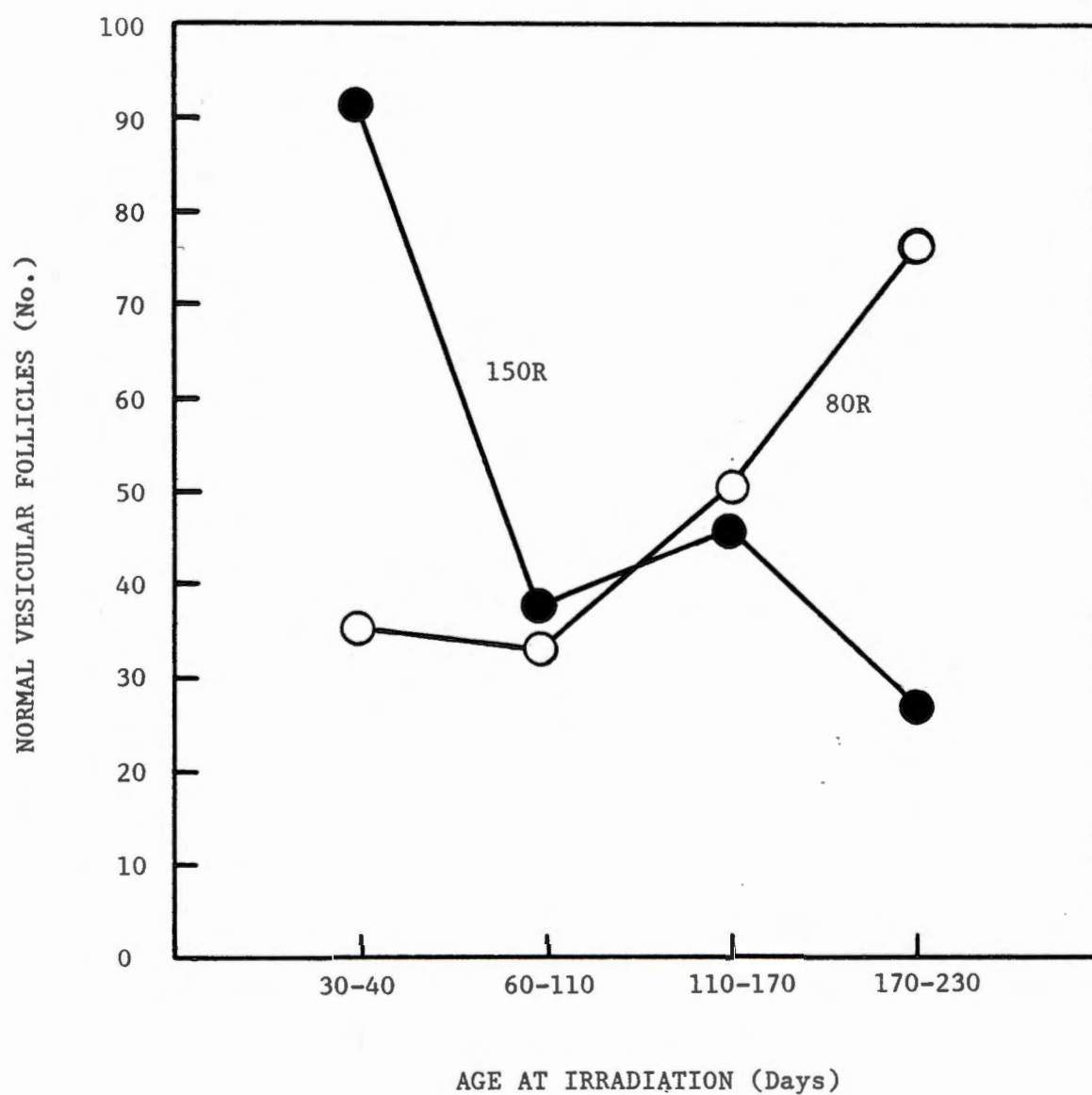


Figure 13. Effect of Continuous Prenatal Irradiation on Number of Normal Vesicular Follicles.

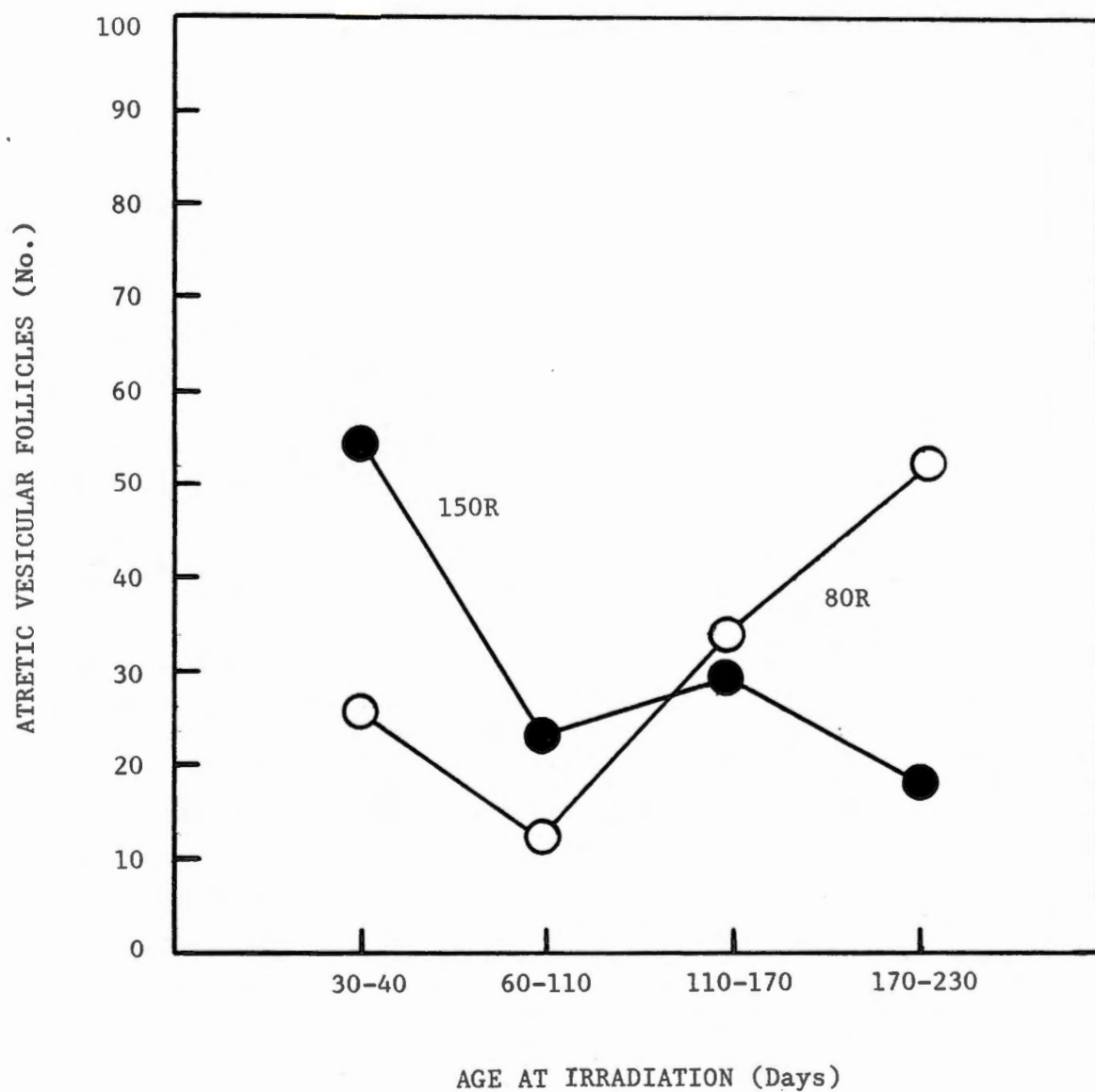


Figure 14. Effect of Continuous Prenatal Irradiation on Number of Atretic Vesicular Follicles.

Combined Effects

Data collected from all prenatally irradiated animals were pooled with respect to treatment and tested for period effects (Table 3). Since differences between the two doses of 80R and 150R were seldom significant, it would be expected that combining doses would not indicate a substantially different response. Similar responses were, in fact, found when data from both doses were combined. The only value that differed significantly from the controls was the number of primary follicles when irradiation occurred between 60 and 110 days of gestation ($P < 0.01$). Primary follicle number was reduced to 20% of controls in these animals.

Follicle Class Correlation

Correlations between the different follicle types were essentially all positive (Table 4). Generally, the magnitude of the associations was greatest between normal and atretic vesicular follicles. Correlation coefficients were consistently high ranging from .71 to .85. Correlations between the number of growing follicles and vesicular follicles were more varied. While growing and atretic vesicular follicles were significantly correlated in all prenatal periods as well as in the controls (.51 to .91), slightly diminished association existed between the number of growing follicles and normal vesicular follicles. Coefficients for these two follicle types ranged from .52 to .78 and no significant correlations were observed in the control animals nor those animals irradiated between

TABLE 3

COMBINED EFFECTS OF 80R AND 150R OF CONTINUOUS ^{60}Co γ RADIATION ON THE
GERM CELLS OF THE PRENATAL BOVINE OVARY AS REFLECTED BY
POSTNATAL FOLLICULAR DEVELOPMENT.

Prenatal Age at Irradiation (Days)	Animal (No.)	Follicle Number ($\bar{X} \pm \text{SE}$)			
		Primary	Growing	Normal	Vesicular Atretic
Control	5	165,346 $\pm$ 33,532	2,082 $\pm$ 541	69 $\pm$ 14	38 $\pm$ 9
30-40	3	88,542 $\pm$ 43,290	1,511 $\pm$ 699	55 $\pm$ 19	35 $\pm$ 12
60-110	18	32,724 $\pm$ 17,673 ^a	1,049 $\pm$ 285	35 $\pm$ 8	18 $\pm$ 5
110-170	21	102,474 $\pm$ 16,362	1,145 $\pm$ 264	49 $\pm$ 7	32 $\pm$ 5
170-230	15	130,812 $\pm$ 19,360	1,651 $\pm$ 312	56 $\pm$ 8	39 $\pm$ 5

^aOnly value which differs significantly from controls ($P < 0.01$).

TABLE 4
CORRELATION COEFFICIENTS FOR THE DIFFERENT
FOLLICLE CLASSES BY PERIOD^{ab}

Follicle Class	Growing	<u>CONTROL</u>	
		Normal Vesicular	Atretic Vesicular
Primary	-.13	.34	.19
Growing		.69	.91 ^c
Normal Vesicular			.74

Follicle Class	Growing	<u>30-40 DAYS</u>	
		Normal Vesicular	Atretic Vesicular
Primary	.28	.22	.09
Growing		.66	.78 ^c
Normal Vesicular			.81 ^c

Follicle Class	Growing	<u>60-110 DAYS</u>	
		Normal Vesicular	Atretic Vesicular
Primary	.61 ^c	.65 ^c	.60 ^c
Growing		.76 ^c	.59 ^c
Normal Vesicular			.85 ^c

Follicle Class	Growing	<u>110-170 DAYS</u>	
		Normal Vesicular	Atretic Vesicular
Primary	.36	.50 ^c	.59 ^c
Growing		.52 ^c	.51 ^c
Normal Vesicular			.71 ^c

Follicle Class	Growing	<u>170-230 DAYS</u>	
		Normal Vesicular	Atretic Vesicular
Primary	.47 ^c	.54 ^c	.55 ^c
Growing		.78	.73 ^c
Normal Vesicular			.84 ^c

^aCorrelation coefficients determined for follicle numbers.

^bData from both irradiation treatments were pooled in each period.

^cSignificant correlations ($P < 0.05$).

30 and 40 days. Relationships observed between primary follicle counts and growing and vesicular follicle counts were quite varied from minimal to moderate. While the least amount of correlation was observed between primary and growing follicles ranging from $-.13$ to $.61$, correlations between primary and vesicular follicle numbers were similar with coefficients ranging from $.09$ to $.65$.

Germ-Cell Radioresponse in the Male

Histological analysis of unirradiated newborn bovine testes revealed that approximately 40% of the seminiferous tubule cross sections examined were sterile, although the average number of germ cells per tubule was more than one (Table 5).

Continuous irradiation of fetuses between days 30 and 40 of gestation with a dose of 80R resulted in an increased amount of tubular sterility of 52%, while prenatal exposure to 150R during this same period rendered only 38% of the tubules sterile (Fig. 15). As shown in Fig. 16, the number of germ cells per tubule were reduced in these same animals to 60% and 84% of control with the doses of 80R and 150R, respectively, although no significant differences were found ($P < 0.05$). The occurrence of a relatively large proportion of necrotic germ cells in the tubules of those animals irradiated with 80R during this same period was unexpected since this was not seen in previous studies.

At approximately 60 days, the gonocyte of the prenatal bovine testis apparently becomes increasingly sensitive to the effects of continuous irradiation. Exposure of fetuses between 60 and 90 days

TABLE 5

RESPONSE OF THE GERM CELLS OF THE PRENATAL BOVINE TESTES TO CONTINUOUS
⁶⁰Co γ RADIATION AS DETERMINED AT BIRTH.

Prenatal Age at Irradiation (Days)	Dose (R)	Animals (No.)	% Tubule Sterility	% Dead Germ ^a Cells/Testes	No. Germ ^a Cells/Tubule
Control	--	5	40.0±8.5	0.0±2.2	1.0±0.2
30-40	80 ^b	3	52.0±11.0	13.0±2.8 ^e	0.6±0.2
	150 ^c	3	37.6±11.0	0.2±2.8	0.9±0.2
60-90	80	5	72.7±8.5 ^e	0	0.3±0.2 ^e
	150	6	77.2±7.8 ^e	0.1±2.0	0.3±0.2 ^e
100-190	80	9	78.2±6.4 ^e	1.7±1.6	0.3±6.1 ^e
	150	18	87.9±4.5 ^e	2.6±1.1	0.1±0.1 ^e
190-270	80	8	64.6±6.7 ^d	5.8±1.7 ^d	0.4±0.1 ^e
	150	10	72.9±6.0 ^e	3.0±1.5	0.3±0.1 ^e

^a Determined via microscopic observation of a minimum of 275 seminiferous tubule cross-sections per testis.

^b Dose-rate of 8R/day for 10 days.

^c Dose-rate of 15R/day for 10 days.

^d Significantly different from controls (P<0.05).

^e Significantly different from controls (P<0.01).

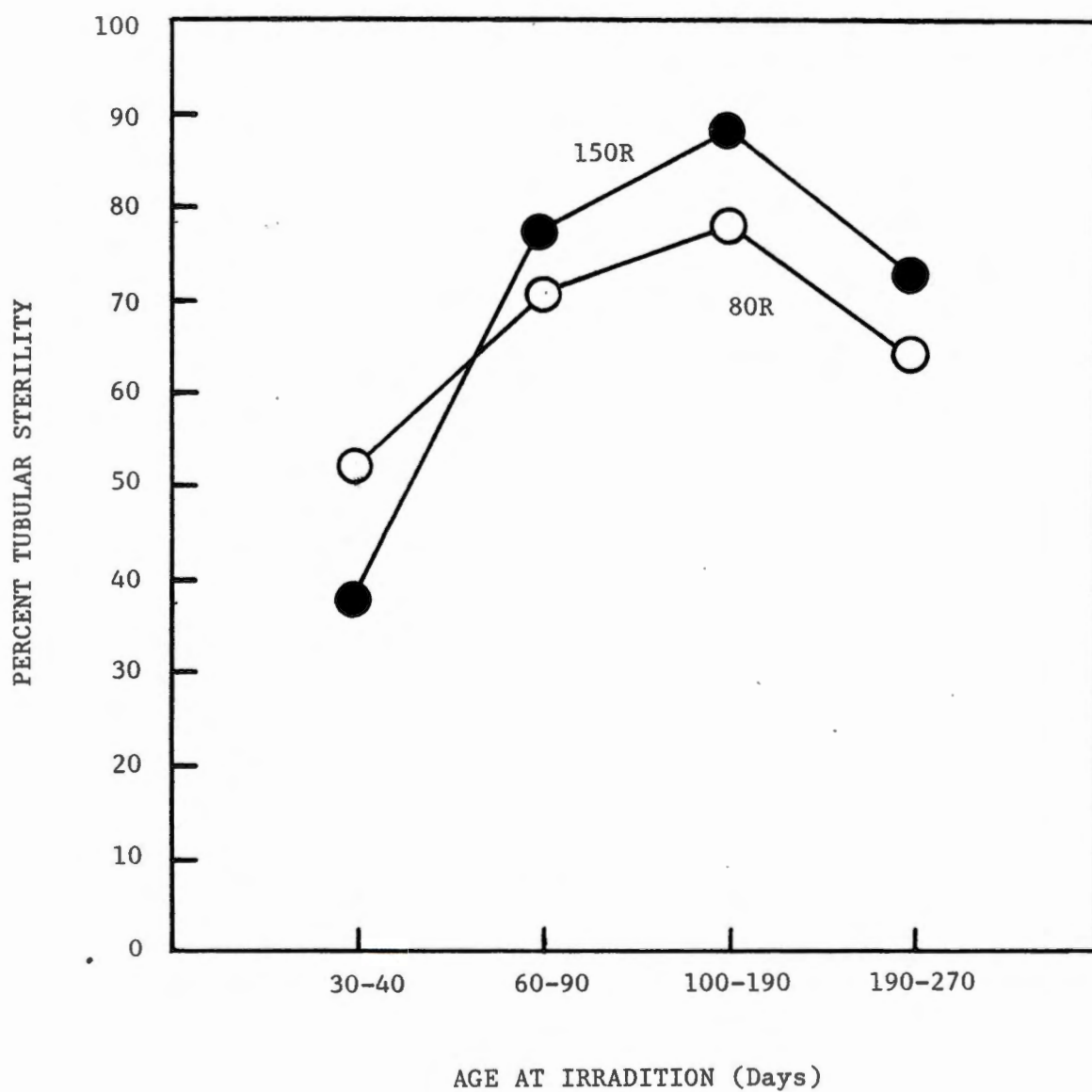


Figure 15. Sterilizing Effects of Continuous Radiation Administered to the Prenatal Bovine Testis.

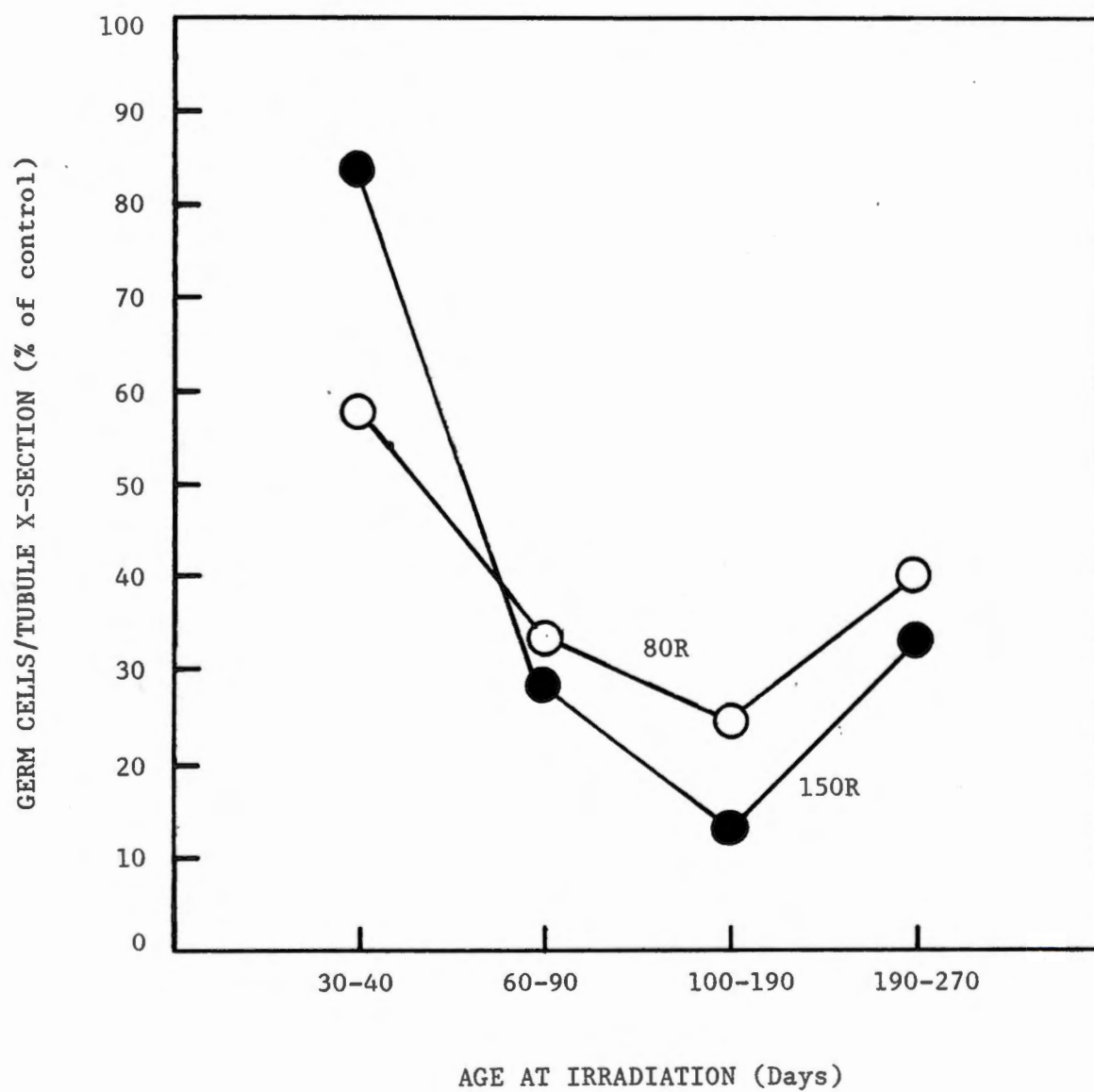


Figure 16. Continuous Radiation Effects on the Germ Cells of the Prenatal Bovine Testis.

of gestation to a continuous dose of 80R resulted in 73% tubular sterility which was significantly different from controls ($P < 0.01$). The number of germ cells per tubule was significantly reduced to 32% of control ($P < 0.01$). Administration of a dose of 150R was slightly more detrimental to the gonocyte resulting in 77% tubular sterility and reduction of germ cells per tubule to 30% of control. Very few necrotic germ cells, if any, were observed in the testes of animals irradiated during this same prenatal period.

The period of greatest germ cell radiosensitivity was observed between 100 and 190 days of gestation. Tubular sterility was significantly greater than controls during this period with values of 78% and 88% following continuous exposures of 80R and 150R, respectively ($P < 0.01$), while the number of germ cells per tubule were at their lowest levels. An increase in necrosis was also observed although values did not differ significantly from controls ($P < 0.05$). Susceptibility of the germ cell to irradiation was also determined between 190 and 270 days of gestation; however, the magnitude of the effect was diminished. Tubular sterility in those animals irradiated during this period with a dose of 80R was 64%. While still significantly higher than controls ($P < 0.05$), the response was comparatively less than that found in the periods between 60 and 90 days and 100 and 190 days. A dose of 150R increased tubular sterility to 74%. The number of germ cells per tubule was reduced to 40% and 32% of controls respectively following exposures of 80R and 150R. A significant amount of germ-cell death was observed in those animals

irradiated with 80R ($P < 0.05$) as almost 9% of the cells viewed were necrotic. No significant amount of germ cell death was noted following the 150R exposure however ($P < 0.05$).

As was found in the female, differences between the lower dose of 80R and the higher dose of 150R were negligible. Pooling data from both doses and statistically testing for effects resulted in little variation in response from that already reported (Table 6).

Correlations

The associations found between the testicular traits measured in the male were variable with respect to period (Table 7). Correlations between percent tubular sterility and percent dead germ cells ranged from .49 in the period of 30 to 40 days to -.46 between 60 and 90 days. Varied correlations were also observed between percent dead germ cells and number of germ cells per tubule. While a moderately negative correlation was observed in the prenatal period between 30 and 40 days (-.43), a positive correlation coefficient of .33 was determined for the period between 60 and 90 days. The only variables which were significantly correlated consistently throughout all prenatal periods studied were percent tubular sterility and number of germ cells per tubule ($P < 0.05$). Large negative correlations were observed between these testicular traits in all periods as well as in the controls.

TABLE 6

RESPONSE OF GERM CELLS OF THE PRENATAL BOVINE TESTES TO COMBINED DOSES
OF 80R (8R/DAY FOR 10 DAYS) AND 150R (15R/DAY FOR 10 DAYS)
OF CONTINUOUS ^{60}Co γ RADIATION AS DETERMINED AT BIRTH.

Prenatal Age at Irradiation (Days)	Animals (No.)	% Tubular Sterility ^a	% Dead Germ ^a Cells/Testes	No. Germ Cells/Tubule ^a
Control	5	40.0 $\pm$ 8.5	0.1 $\pm$ 2.3	1.0 $\pm$ 0.2
30-40	6	44.8 $\pm$ 7.8	6.6 $\pm$ 2.1 ^b	0.7 $\pm$ 0.2
60-90	11	75.1 $\pm$ 5.7 ^c	0.1 $\pm$ 1.5	0.3 $\pm$ 0.1 ^c
100-190	27	84.7 $\pm$ 3.7 ^c	2.3 $\pm$ 1.0	0.2 $\pm$ 0.1 ^c
190-270	18	69.2 $\pm$ 4.5 ^c	4.3 $\pm$ 1.2	0.4 $\pm$ 0.1 ^c

^aDetermined via microscopic observation of 275 seminiferous tubule cross-sections per testis.

^bValues which differ significantly from controls ($P < 0.05$).

^cValues which differ significantly from controls ($P < 0.01$).

TABLE 7
CORRELATION COEFFICIENTS FOR TESTICULAR
CHARACTERISTICS BY PERIOD^a

<u>CONTROLS</u>		
Characteristics	% Dead Germ Cells/Testis	No. Germ Cells/Tubule
% Tubular Sterility	-.43	-.91 ^b
% Dead Germ Cells/Testis		.24
<u>30-40 DAYS</u>		
Characteristics	% Dead Germ Cells/Testis	No. Germ Cells/Tubule
% Tubular Sterility	.49	-.91 ^b
% Dead Germ Cells/Testis		-.43
<u>60-90 DAYS</u>		
Characteristics	% Dead Germ Cells/Testis	No. Germ Cells/Tubule
% Tubular Sterility	-.46	-.94 ^b
% Dead Germ Cells/Testis		.33
<u>100-190 DAYS</u>		
Characteristics	% Dead Germ Cells/Testis	No. Germ Cells/Tubule
% Tubular Sterility	.19	-.95 ^b
% Dead Germ Cells/Testis		-.17
<u>190-270 DAYS</u>		
Characteristics	% Dead Germ Cells/Testis	No. Germ Cells/Tubule
% Tubular Sterility	.10	-.92 ^b
% Dead Germ Cells/Testis		-.16

^aData from both irradiation treatments were pooled in each period.

^bSignificant correlations ($P < 0.05$).

CHAPTER V

DISCUSSION

Female

The results from this study indicate that the germ cells of the prenatal bovine ovary are highly sensitive to the effects of continuous irradiation only during a relatively brief period. This period extends from approximately Day 60 to Day 110 of gestation which is coincidental with a large proportion of the oogonial population undergoing mitosis (Erickson, 1966). While reports have indicated that the mitotically active oogonia of the long-lived species is relatively sensitive to acute doses of radiation (Erickson, 1977, in the porcine; Erickson, 1966 and Erickson and Reynolds, 1978, in the bovine; Baker and Beaumont, 1967, in the monkey; Lindop, 1969, in the human) a similar response of the mitotically active oogonia has been seen in the continuously irradiated prenatal pig. Erickson and Martin (1976) found that gilts were sterile after being continuously irradiated between 45 and 66 days of gestation, a period in which oogonial mitosis is high.

Germ cell mitosis during the first 60 days of gestation is limited in the bovine (Erickson, 1966). The oogonia during this period of low mitosis appear to be inherently refractory to irradiation, and coupled with the fact that the surviving oogonia have ample time between irradiation and the end of mitosis to compensate for

losses, the absence of a significant effect in this study during the period between 30 and 40 days was expected. Both Erickson (1966) and Erickson and Reynolds (1978) reported an unresponsiveness of the bovine oogonia to acute doses of radiation when administered prior to Day 60 of gestation.

The increase in germ cell radioresistance between Days 110 and 170 of gestation, as determined by increased follicle counts for animals irradiated during this period, is due to several factors. In the bovine, an increasing proportion of the oogonia are differentiating into oocytes during this period (Erickson, 1966). Oocytes, in general, are considered less sensitive to irradiation than oogonia (Beaumont, 1961), thus radiation effects during this period would expectedly be decreased. Also, the complete cessation of oogonial mitosis occurs at approximately 150 days of gestation (Erickson, 1966) and would further explain the increased follicle counts found in animals irradiated between 110 and 170 days of gestation.

The near absence of radiation effects observed in animals irradiated between 170 and 230 days is primarily due to a major morphological change in the oocyte and associated somatic cells. By this time, the oocyte has reached its resting stage of development (diplotene) in which radioresistance is high, and envelopment of the oocyte by a single layer of granulosa cells is also taking place (formation of primary follicle). Erickson (1967) determined in the prepuberal bovine that a dose of 900R of gamma radiation was required to bring about major dissolution of the primary follicle populations. To further emphasize this resistance, a dose of only 300 to 400R was regarded as lethal to the animal itself.

While the continuous dose of 150R was generally more destructive than the dose of 80R, a significant difference in response to dose was not determined. The large variation that was found between animals and the fact that each treatment and period was represented by a relatively small number of animals could be at least partially responsible for the apparent absence of difference. Alternatively, the dose of 80R could have been sufficient to destroy the majority of the germ cells during sensitive periods while the periods of germ cell resistance were such that an additional 70R was insufficient to elicit greater damage.

Previous studies in the bovine have suggested that a reduction in germ cell numbers of approximately 36% would not dramatically affect the reproductive capacity of the female (Erickson and Reynolds, 1978). The reductions in germ cell numbers observed in this study due to continuous irradiation, however, are of much greater magnitude. Only 18% of the germ cells survived the dose of 150R when administered between 60 and 110 days of gestation. Although not measured, it would appear that this magnitude of a reduction in germ cell numbers would be considerably more detrimental to the subsequent fertility of the cow. Since the period of greatest risk in the bovine is when the oogonia are mitotically active, and the duration of this period is similar to that seen in the human (Baker, 1963), it would be reasonable to assume that the reproductive capacity of the human female would likewise be affected by continuous prenatal irradiation.

Follicle Class Correlation

Since primary follicles develop into growing follicles, which in turn develop into vesicular follicles, a certain amount of positive correlation would normally be expected between the various follicle types. Although unexplained low correlations were found between the number of primary follicles and the number of growing and vesicular follicles in both the control animals and those irradiated between 30 and 40 days, associations determined in this study were mostly positive and moderate to high. This would tend to indicate that continuous irradiation during the prenatal period has little effect on the subsequent development or growth of the ovarian follicle.

Male

Unlike the female, the prenatal male appears to experience a longer period of germ cell susceptibility to continuous irradiation extending from Day 60 of gestation through much of the prenatal period. A refractoriness of the gonocyte to irradiation was apparent during the early stages of gestation as neither doses of 80R and 150R, administered between 30 and 40 days, significantly affected germ-cell survival. Similar radioresistance in the bovine has been observed when radiation was acutely administered. Matschke and Erickson (1969) reported no adverse effects of 150 rads of gamma radiation acutely administered prior to 80 days of gestation. These researchers also observed the gonocyte to be mitotically active during this period. In contrast to the female, where the mitotically active

germ cell is sensitive to irradiation, the germ cell of the male is apparently less responsive during periods of mitosis. The surviving oogonia of the female, however, are capable of repeated mitosis in an attempt to replenish the germ cell population (Baker, 1971). The gonocyte of the male, on the other hand, does not experience this burst of mitotic activity in an effort to compensate for losses, thus, time for recovery is extended (Erickson et al. 1963).

The increase in sensitivity of the gonocyte observed at approximately 60 days corresponds to the period when cessation of mitotic activity of the gonocyte is occurring. Numerous studies have established the arrested gonocyte as one of the most sensitive cells to irradiation. Both Beaumont (1960) and Hughes (1962) sterilized the rat with acute doses of X-rays administered during this period. While the arrested gonocyte of the long-lived species is less adversely affected by acute doses of radiation (Matschke and Erickson, 1969; Erickson et al., 1963), the effects of continuous irradiation have been found to be quite detrimental. Erickson and Martin (1976) reduced gonocyte survival in the pig to 12% of control with a continuous dose of 7 rads applied daily during the stage of mitotic arrest.

The gonocyte of the prenatal bovine experiences this period of low mitotic activity until approximately 230 days of gestation (Matschke and Erickson, 1969). The results of this study indicate that the effects of continuous irradiation would remain high during

this period with the greatest amount of damage occurring between 100 and 190 days of gestation. This would be the period when the majority of the gonocytes are experiencing mitotic arrest and are, thus, susceptible to greater amounts of damage.

The slight increase in gonocyte survival observed in animals irradiated between 190 and 270 days is probably due to reinitiation of gonocyte mitosis. The gonocyte of the bovine experiences an upturn in mitosis at approximately 230 days along with an increased radioresistance. Erickson et al. (1972) observed this in the acutely irradiated prenatal bull also.

The extent of germ-cell death observed in the newborn male was relatively low, however. Instances of significant levels of necrosis were determined and appeared to be associated with the periods in which mitosis of the gonocyte was occurring. Only between 30 and 40 days and 190 and 270 days, when the gonocyte is mitotically active, was a significant amount of necrosis determined. Since these periods were found in this study, as well as others, to be more refractory to irradiation effects, the observed increase in germ cell death is most probably due to the wide variation that exists between these animals. Also, the fact that this increased necrosis was found only in animals irradiated with the dose of 80R would tend to substantiate this view. The possibility exists, however, that the mitotically active gonocyte of the bovine is in some way affected by continuous irradiation which has not been explained.

An attempt to associate increased sterility with cell death was unsuccessful due to these reasons noted above. Extremely high negative correlation between percent sterility and germ cell numbers was expected.

Although not measured, subsequent fertility of the male would not appear to be affected to a great extent by continuous prenatal irradiation even though reductions in germ cell numbers were large. This can be explained by the kinetics of the postnatal germ cell. Those gonocytes that survive irradiation will eventually differentiate into spermatogonia in the prepuberal animal. As described earlier, two types of spermatogonia are formed. First, there are those spermatogonia that continue to differentiate, ultimately forming viable spermatozoa. The other type of spermatogonia (stem spermatogonia) will not undergo differentiation, but rather will divide to form other stem spermatogonia, thus, to a considerable extent, repopulating the stem cell pool. By this process, losses, with respect to the gonocyte, can be at least partially restored. Matschke and Erickson (1969) reported that a sufficient number of spermatozoa for optimum fertility were subsequently produced in bulls following depletion of approximately 91% of the gonocyte population due to acute prenatal irradiation. The fact that the greatest amount of gonocyte depletion observed in this study was of similar levels would seem to indicate that the fertility of these animals would not be altered to a great degree. Though the response of the male germ

cell of the bovine to continuous prenatal irradiation might not be permanently damaging, it is evident that the gonocyte during the period of rest is very sensitive. Since this same period in man is much more protracted (approximately 10 years, Charney et al., 1952), it is likely that the detrimental effects of continuous irradiation would also be much greater.

CHAPTER VI

CONCLUSIONS

1. During prenatal development, the mitotically active oogonium and the mitotically arrested gonocyte are the most vulnerable to the effects of continuous irradiation.
2. The period of germ-cell vulnerability is longer in the male (approximately 210 days) than in the female (approximately 50 days).
3. 80R of continuous gamma radiation was as deleterious to the germ cell as was 150R.
4. Follicular development was not impaired by continuous irradiation.
5. The oocyte of the primordial follicle and the mitotically active gonocyte are relatively resistant to continuous irradiation.
6. While the male has the ability to regenerate germ cells through spermatogonial stem cell renewal, the female cannot compensate for germ cell loss, thus, the effects of continuous irradiation are more detrimental to the subsequent fertility of the female than the male.

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