February 27, 1969

To the Graduate Council:

I am submitting herewith a thesis written by John Edward Chandler, entitled "Effects of Dose and Dose-Rate of Gamma Radiation on Follicular Development and Reproductive Capacity of the Rat." I recommend that it be accepted for nine quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Husbandry.

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We have read this thesis and
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# EFFECTS OF DOSE AND DOSE-RATE OF GAMMA RADIATION ON FOLLICULAR DEVELOPMENT AND REPRODUCTIVE CAPACITY OF THE RAT

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A Thesis

Presented to

the Graduate Council of

The University of Tennessee

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

---

by

John Edward Chandler

March 1969

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ABSTRACT

The objectives of this study were to determine both the effects of $\gamma$-radiation on the oocyte of the rat and the relationship between numbers of viable oocytes at various times postirradiation and long-term reproductive performance.

Two hundred animals, initial age 30 days, were used in this study. Using 40 control animals, the remaining 160 animals were divided equally into four groups and irradiated with $^{60}Co$ gamma radiation at the following doses and rates: 200 R at 1 R per minute, 200 R at 50 R per minute, 400 R at 1 R per minute, and 400 R at 50 R per minute. Following irradiation, all animals were subjected to continuous breeding until 150 days postirradiation, at which time the experiment was terminated.

At 10, 70, 90, and 110 days postirradiation, 50 animals (10 per treatment group) were unilaterally ovariectomized for histological evaluation of the ovarian population at each postirradiation interval. At the termination of the experiment, the remaining ovary was taken for histological evaluation of the residual population following breeding and irradiation.

The primordial follicle population was drastically reduced by all irradiation levels at 10 days postirradiation. There was also a difference in response to dose and dose rate. The numbers were further reduced by age at 70 days postirradiation. There was, however, no further decline in the primordial follicle population at the 90 and 110 day intervals.

The growing follicle numbers were reduced at 10 days postirradiation by 200 R at 1 R per minute, 400 R at 1 R per minute and 400 R at 50 R per minute, but not by 200 R at 50 R per minute. At 70, 90, and 110 days postirradiation, the growing follicle populations were further depleted by all irradiation treatments.

At 10 days postirradiation, the vesicular follicle numbers were not affected by any of the irradiation levels. However, by 70 days postirradiation, the population was reduced by all treatments and age.

The relationship of follicle numbers surviving irradiation and reproductive performance was tested and a relationship was demonstrated between the number of primordial follicles surviving irradiation and subsequent progeny produced after 70 days postirradiation by both 400 R dose rates ($P < 0.05$).

A relationship also existed between growing follicles and progeny production in the 200 R at 50 R per minute and 400 R at 1 R per minute groups at 70 days postirradiation ($P < 0.05$). A relationship was not elucidated between the vesicular follicles and subsequent progeny produced.

Based on the results obtained in this study, there does not appear to be a significant relationship between numbers of germ cells and progeny production in nonirradiated female rats. However, the irradiation treatments which were imposed did significantly reduce the number of germ cells and subsequent pup production.

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# CHAPTER I

## INTRODUCTION

In the early studies of mammalian gametogenesis, the mechanism of oogenesis was thought to be similar to that of spermatogenesis; that is, a continuous proliferation of mature germ cells from gonocytes throughout adult life. In more recent years the question has been resolved by the demonstration that oogenesis is complete in most mammalian species prior to or just after birth, thus the female is endowed with a finite population of germ cells that are available for reproduction in mature life.

After this population is established, the oocytes enter a period of "rest" relative to the chromosomal stage in meiosis which lasts until the initiation of follicular development. During this period of follicular growth, a portion of the follicles undergo atresia, a portion remains static and the remainder develops at differential rates to mature follicles. Following ovulation, the oocyte, if fertilized, develops into a new individual.

The effects of ionizing radiation on the events of oogenesis have been studied extensively; nevertheless, there still remains a vast amount of knowledge to be gained concerning radiation effects on reproduction in the female.

The purpose of this study was to examine the effects of $\gamma$-radiation on the ovary of the puberal white rat as influenced by total dose, dose-rate and the subsequent reproductive capacity as determined at varying intervals postirradiation.

## CHAPTER II

## REVIEW OF LITERATURE

### Quantitative Observations

In early studies of oogenesis, there was much debate as to the origin of the definitive germ cell. Many workers held that the germ cells arose from continuous proliferations of the "germinal" or ovarian epithelium of the ovary (Kingery, 1917; Arai, 1920; Allen, 1923; Swezy and Evans, 1930; Schmidt and Hoffman, 1941; Slater and Dornfeld, 1945), while others maintained the origin was extragonadal (Cowperthwaite, 1925; Kingsbury, 1938).

The latter view was clearly demonstrated by workers in the late 1940's and early 1950's. In 1948, Witschi traced in the human the migration of the primordial germ cell from the yolk sac, where they are first distinguishable, through the dorsal mesentery and finally to the genital ridges. His work was later confirmed by other workers (human--McKay et al., 1953; mouse--Chiquoine, 1954; Mintz, 1957). The development of the mature mammalian oocytes from the oogonia, the descendants of the primordial germ cells, was established by several workers (Jones, 1949; Mandl and Zuckerman, 1951; Zuckerman, 1951). Jones (1949) used a vital stain of the ovarian epithelium and showed that there were no inclusions in the oocytes. Mandl and Zuckerman (1951) destroyed the ovarian (germinal) epithelium with tannic acid and found that oocytes were still present a year after treatment. Zuckerman (1951) used vital staining, destruction of the epithelium, x-irradiation

and ischemia to demonstrate the non-prolific character of the "germinal" or ovarian epithelium.

Subsequently, oogenesis, ovarian development and senesence have been described in a number of mammalian species (rat--Beaumont and Mandl, 1962; human--Baker, 1963; guinea pig--Ioannou, 1964; rabbit--Peters et al., 1965; monkey--Baker, 1966; cow--Erickson, 1966; pig--Black and Erickson, 1968).

Effects of Radiation on the Postnatal Ovary

The first description of the destructive effects of ionizing irradiation on the ovary was made by Halberstaeder (1905) and Bergonié et al. (1905). The rabbit was used in both of these studies. Subsequently, the destructive effects were described in a number of species (guinea pig--Lengfellner, 1906; mouse--Krause and Ziegler, 1906; rat--Ford and Drips, 1929; human--Reifferscheid, 1910).

Lacassagne (1913) reported that the primary follicles were the most radiosensitive in the rabbit. Parkers (1926) and Brambell et al. (1927) reported the first effect of irradiation was the complete destruction of all the small oocytes by the first week postirradiation in the mouse.

Drips and Ford (1932) reported that from 7 days of age onward after 430 and 570 R of X-rays, follicular degeneration occurred in the rat; the mature Graafian follicles were the most radiosensitive.

Murray (1931), in studying the effects of different doses of X-rays on the ovary of the adult mouse, reported that changes occurred following a dose of 54 R; after 150 R all primary follicles rapidly disappeared and the ovary was completely devoid of all follicles by 43 days postirradiation.

Fels (1935) reported that the rat is considerably more radioresistant than the mouse, in that doses of over 2,200 R are required to induce complete destruction of oocytes. Irradiation with doses between 60 and 420 R has been reported to be followed by a decrease in fertility dependent upon the dose, functional sterility followed exposure of the adult female rat to 570 R (Drips and Ford, 1932).

Ingram (1958) confirmed and extended these earlier findings. This same worker reported that there was a clear-cut relationship between litter-size and number of oocytes surviving in the ovary at autopsy; the latter was also correlated with the ability of the female to give birth to more than one or two litters following irradiation. These variables were correlated also with the dose to which the animal had been exposed.

A thorough review of the early work on the effects of ionizing irradiation on the ovary of various species has been compiled by Lacassagne et al. (1962). Also, Erickson (1965) has reviewed work involving domestic livestock. Consequently, the remainder of this review will deal primarily with the more recent studies.

## Effects of Radiation on the Prepuberal Ovary

Russell et al. (1959) suggested that in the mouse the germ cell population of the relatively young animal was more sensitive to radiation than that of the adult female. Peters (1961) stated that the radiation sensitivity of oocytes in the prepuberal mouse does not seem to be uniform but depends on the stage of development at the time of irradiation. A dose of 20 R of X-rays given on the day of birth resulted in less damage to the oocytes in primordial follicles than the same

dose administered at 7, 14 or 21 days of age. The growing follicles in the ovaries during the later ages were not immediately affected by this dose, however, the later the age at irradiation the smaller the proportion of surviving oocytes in primordial follicles.

Oakberg (1962) irradiated 10-day-old mice with doses of 1 to 25 R of $^{60}Co$ $\gamma$-rays and concluded that the primordial follicle oocytes are highly radiosensitive while the oocytes in later stages of follicular development are relatively radioresistant. Peters and Levy (1964) found that mice which received 200 R of X-rays at 3 weeks of age had only one percent of the normal complement of oocytes at maturity.

Mandl (1959) reported that there was some evidence that the portion of primordial oocytes which are destroyed by a given dose of X-rays is higher in immature than in adult rats. Beaumont (1962) concluded that the most radiosensitive postnatal period for the ovary of the rat is at 5 days of age.

The primary oocytes of the neonatal monkey appear to be more radioresistant than those of the mouse and rat (Baker and Beaumont, 1967). These workers reported that a minimum dose of 2,000 R of X-rays was required to sterilize the newborn monkey.

Erickson (1967a) in his study of the prepuberal bovine ovary, concluded that the dose of $\gamma$-radiation necessary to cause the immediate death and dissolution of the majority of primordial follicles (900 R) is far in excess of the lethal dose for that species (less than 400 R). This same worker (Erickson, 1967b) reported the lethal dose for 50 percent of the porcine primordial follicles was about 500 R.

Effects of Radiation on the Postpuberal Ovary

Mandl (1959) stated that in the mature rat the oocytes in the primordial follicles are more radiosensitive than those in the later stages of follicular development. Mandl (1963) concluded that in the rat the oocytes in the "median-sized" follicles are highly radioresistant. She estimated the lethal-dose for 50 percent of the oocytes of this stage to be about three times higher than that for pre-ovulatory oocytes (14 to 15 hours before expected ovulation).

Oakberg (1960) reconfirmed the early works of Brambell and Parks (1927) and Murray (1931) in reporting that the young oocytes of the adult mouse are very radiosensitive, while the oocytes of larger follicles are rather resistant to ionizing radiation.

Moawad et al. (1965) found no evidence of destruction of the primordial follicles with a low dose of 50 R of X-rays administered to adult rabbits.

Effects of Radiation on Reproductive Capacity

Murphree and Pace (1960) found no difference based on first litters between control and prenatally irradiated females in (1) percent of fertile females, (2) percent of corpora lutea as represented by living fetuses, or (3) fetal deaths. However, ovulation was approximately 20 percent lower in the irradiated than in the control rats.

Chu and Lu (1963), in their investigation on 7-day-old rats exposed to a single whole-body dose of X-rays of 200 to 400 R, found the oocytes in the primordial follicle were more sensitive than those in growing follicles, which developed normally and accounted for the short period of fecundity in the adult.

Peters and Levy (1963), in studying the effect of irradiation on the mouse ovary, reported that ovarian sensitivity varied markedly between birth and maturity. Sensitivity increased during the first 3 weeks of life, followed by a decrease as maturity was attained. The number of offspring an animal produced had no relation to the total number of oocytes still present at the age of maturity. However, they found a correlation between the number of growing and large oocytes at puberty and subsequent number of young produced.

Oakberg (1966) indicated that 25 R of X-rays given to 10-day-old mice reduced the total productivity to 35 percent of controls. The primary factor in reduction of fertility was radiation-induced degeneration of oocytes. He also concluded that irradiated females became sterile because the oocyte population was exhausted, whereas control females became sterile with an ample supply of oocytes still in the ovary.

Oakberg (1960) stated that after an acute exposure to 400 R of X-rays, adult female mice produced an average of one and four tenths litters before they became sterile. With a dose of 50 R, they produced an average of four litters before the onset of sterility. Fertility of female mice following exposure to 50 R of X-rays was demonstrated to be strain dependent (Ehling, 1964).

Petrova (1962), in studying the irradiation effect on the adult mouse, rat, golden hamster and guinea pig, found that a single dose of whole-body irradiation interrupted the estrual cycle, decreased or destroyed fertility and caused changes in ovarian weight and ovarian follicle numbers. Fertility of mice was more sensitive than the

estrual cycle; 15 to 25 R decreased the number of progeny produced by mice while the cycle was unaffected. A dose of 800 R of X-rays to adult rats decreased implantations in the second and third pregnancies (Krehbiel and Plagge, 1963).

Effects of Dose Rate on the Ovary

Very little work has been done on the effects of dose rate on the anatomy and physiology of the mammalian ovary.

Russell et al. (1959) reported that adult female mice exposed to continuous irradiation were considerably less affected in breeding performance than those which received fractionated doses, both groups received the same total dose. They also concluded that, at least, at total doses above 86 R of $^{137}Cs$ gamma rays, the lower the dose rate the smaller is the deleterious effects on fertility with dose rates of 0.009 R and 0.002 R per minute.

Oakberg and Clark (1961) reported that a reduction of dose rate (from 2.85 R per minute to 0.009 R per minute), in both young and adult mice, significantly decreased cell death in the primordial oocyte population.

## CHAPTER III

## EXPERIMENTAL PROCEDURE

### Animals

Two hundred female rats of the Sprague-Dawley strain were assigned to this study at 30 days of age. A commercial pelleted ration[1] and water were supplied ad libitum to all animals. The temperature was maintained at about 75°F in the colony room.

### Irradiation Conditions

The animals were randomly divided into five groups of 40 animals each and received the following treatment: no irradiation (control), 200 R at 1 R per minute, 200 R at 50 R per minute, 400 R at 1 R per minute, and 400 R at 50 R per minute. The irradiated groups will hereafter be referred to as 200-1R, 200-50R, 400-1R and 400-50R, respectively.

Irradiation of rats was accomplished by exposure of the animals to a cobalt-60 ($\gamma$-ray) variable dose rate facility. During exposure, the rats were confined individually in 8 x 13 x 20 cm perforated 3 mm Plexiglas cages.

After irradiation, each treatment group was divided into four subgroups of 10 animals each. The right ovaries were excised from a subgroup within each treatment group at 10, 70, 90 or 110 days postirradiation. At 150 days postirradiation, all females were sacrificed and the remaining (left) ovary removed.

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[1] Purina Laboratory Chow.

Twenty days postirradiation, all of the females were placed with males of proven fertility, five females and one male per cage. Approximately 2 days prior to expected parturition, the females were placed in individual maternity cages. At birth the number of pups was recorded and they were sacrificed. The females were returned to their breeding cages after parturition and breeding was continued until termination of the experiment at 150 days postirradiation.

Ovaries were fixed in Bouin's solution, embedded in parafin, serially sectioned at 7 μ and stained with iron hematoxylin and orange G. Histological classification of follicles was similar to that described by Erickson (1965); i.e., primordial (oocytes encompassed by zero to one layer of follicle cells), growing (oocytes with two or more layers of follicle cells, but without fully-formed vesicles) and vesicular follicles (a fully-formed vesicle with a distinct cumulus oophorus). Primordial follicles were enumerated in every 10th section, while growing and vesicular follicles were counted in every section. Duplication was avoided by counting only those oocytes in which the major part of the nucleolus was present. Total number of oocytes in primordial follicles were determined by multiplication of the number of oocytes counted per section by section interval.

## Methods of Data Analyses

Statistical analyses of the data were conducted as described by Steel and Torrie (1960). Kramer's (1957) modification of Duncan's multiple range test for significant differences among means was used when analyses of variance so warranted.

In view of the experimental objectives, certain preliminary analyses were conducted to determine if the assumptions of the analysis of variance were true when applied to these experimental data. Bartlett's Test of the homogeneity of variance (Steel and Torrie, 1960) indicated that the assumption of homogeneity was not justified when the variation in follicle numbers from nonirradiated animals were compared to that of the irradiated animals. Thus, for the comparison of primordial follicle means between control and irradiated rats, a logarithmic transformation of the data was performed. However, since the inferences made from the transformed data were the same as were made from the non-transformed data, the latter were used to report the results of this study (Snedecor and Cochran, 1967).

The data for the comparison of controls with irradiated animals concerning both growing and vesicular follicle classes were not transformed because the magnitude of the heterogeneity of subclass variances appeared to be smaller than that of the primordial follicle class. Thus, if the heterogeneity of variances that existed in the primordial follicle population did not affect the inferences made there, it was assumed that it would not affect any inference made concerning the other two populations.

The effect of the different dose-dose rate treatment combinations was measured in conjunction with post-treatment intervals by excluding the control groups from the primordial follicle population and performing an analysis with unweighted means to discount the slight disproportionality involved in the subgroup means.

The correlation and regression coefficients are reported on a within subgroup basis to eliminate the pooling of heterogeneous

variances. However, since these individual estimates of correlation and regression were based on fewer observations (approximately 10) than a pooled estimate would be (about 200), the possibility is increased of declaring a coefficient not different from zero when it is actually different.

# CHAPTER IV

## RESULTS

### Effects of Irradiation on the Number of Primordial Follicles (Right Ovary)

An analysis of the data (Table 1) demonstrates a marked reduction of the primordial follicle numbers for all irradiation treatments when compared to the control ($P < 0.01$). However, when the data were analyzed excluding the control group, a dose and dose-rate effect was exhibited at 10 days postirradiation as shown in Table 1 ($P < 0.01$). Table 1 also shows a drastic decline from the individual 10-day means of the 200-1R, 200-50R and 400-50R groups to the later postirradiation intervals ($P < 0.01$). The components of the analysis of the irradiated groups are shown in Table 2.

Figure 1 illustrates the actual values for the control and the irradiated groups expressed as percentages of the control values.

### Effects of Irradiation on the Number of Growing Follicles (Right Ovary)

The data on growing follicles are summarized in Table 3 and the components of the analysis of variance are shown in Table 4. The data are shown graphically in Figure 2. When Kramer's Multiple Range Test (1957) was applied to the means, it was demonstrated that the controls decreased significantly with time ($P < 0.01$). The control 10-day mean differed from all other means ($P < 0.01$) except that of the 200-50R group. However, the 200-50R group was different from the control ($P < 0.10$). It should be noted that the decline in the number of growing follicles at 10 days postirradiation was not as drastic as it was in the primordial follicles.

TABLE 1

PRIMORDIAL FOLLICLE MEANS (RIGHT OVARY)

| Treatment | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|
| | 10 | 70 | 90 | 110 |
| Control | 2735 | 1918 | 2208 | 1849 |
| $S_{\bar{x}}$ | ±302 | ±405 | ±195 | ±339 |
| 200 R at 1 R/min. | 197[b] | 15[d] | 23[d] | 16[d] |
| | ±42 | ±8 | ±8 | ±3 |
| 200 R at 50 R/min. | 291[a] | 49[d] | 43[d] | 25[d] |
| | ±35 | ±17 | ±10 | ±10 |
| 400 R at 1 R/min. | 29[d] | 2[d] | 5[d] | 7[d] |
| | ±7 | ±1 | ±2 | ±4 |
| 400 R at 50 R/min. | 133[c] | 15[d] | 13[d] | 8[d] |
| | ±17 | ±6 | ±4 | ±3 |

[a-d]Irradiated follicle means with the same superscript are not significantly different from each other ($P < 0.01$).

TABLE 2

ANALYSIS OF VARIANCE FOR PRIMORDIAL FOLLICLES IN IRRADIATED ANIMALS

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Total | 148 | 1,348,015 | | |
| Treatment (T) | 3 | 166,405 | 55,468 | 21.03** |
| Day (D) | 3 | 626,928 | 208,976 | 79.25** |
| T x D | 9 | 204,008 | 22,668 | 8.60** |
| Residual | 133 | 350,674 | 2,637 | |

**$P < 0.01$.

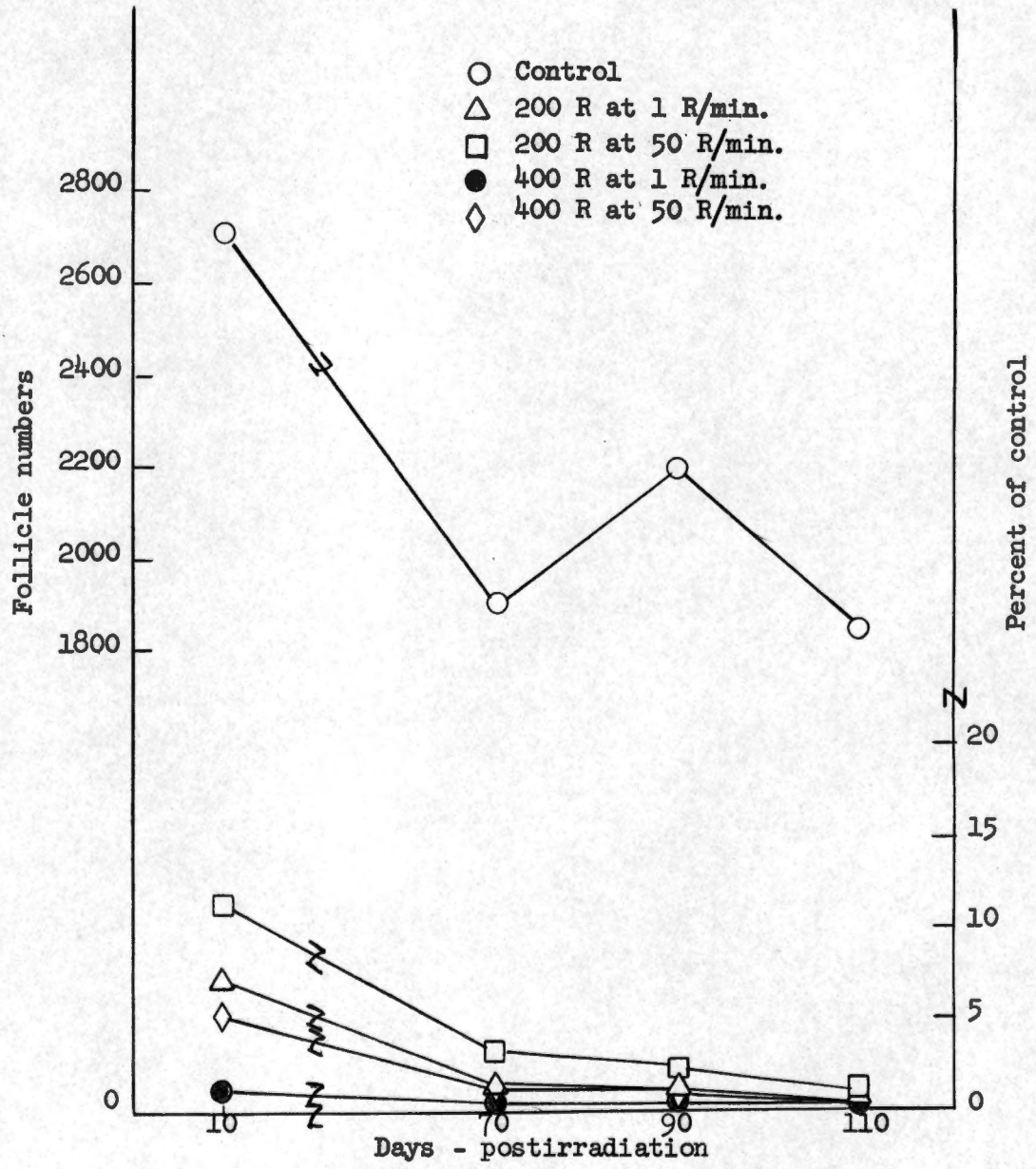



Figure 1. Right ovary primordial follicle means (percent of control).

TABLE 3

GROWING FOLLICLE MEANS (RIGHT OVARY)

| Treatment | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|
| | 10 | 70 | 90 | 110 |
| Control | $245^{a}$ | $122^{cd}$ | $149^{cd}$ | $112^{d}$ |
| $S_{\bar{x}}$ | ±33 | ±10 | ±11 | ±11 |
| 200 R at 1 R/min. | $177^{bc}$ | $20^{e}$ | $12^{e}$ | $6^{e}$ |
| | ±13 | ±3 | ±2 | ±2 |
| 200 R at 50 R/min. | $213^{ab}$ | $26^{e}$ | $21^{e}$ | $6^{e}$ |
| | ±24 | ±5 | ±4 | ±1 |
| 400 R at 1 R/min. | $169^{bc}$ | $5^{e}$ | $2^{e}$ | $1^{e}$ |
| | ±16 | ±1 | ±1 | ±0.3 |
| 400 R at 50 R/min. | $165^{bc}$ | $18^{e}$ | $8^{e}$ | $3^{e}$ |
| | ±6 | ±3 | ±2 | ±1 |

[a-e]Means with the same superscript are not significantly different from each other ($P < 0.01$).

TABLE 4

ANALYSIS OF VARIANCE FOR GROWING FOLLICLES (RIGHT OVARY)

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Total | 187 | 1,520,099 | | |
| Treatment (T) | 4 | 334,812 | 86,203 | 64.57** |
| Day (D) | 3 | 924,130 | 308,043 | 230.74** |
| T x D | 12 | 36,874 | 3,073 | 2.30* |
| Residual | 168 | 224,283 | 1,335 | |

*$P < 0.05$.

**$P < 0.01$.

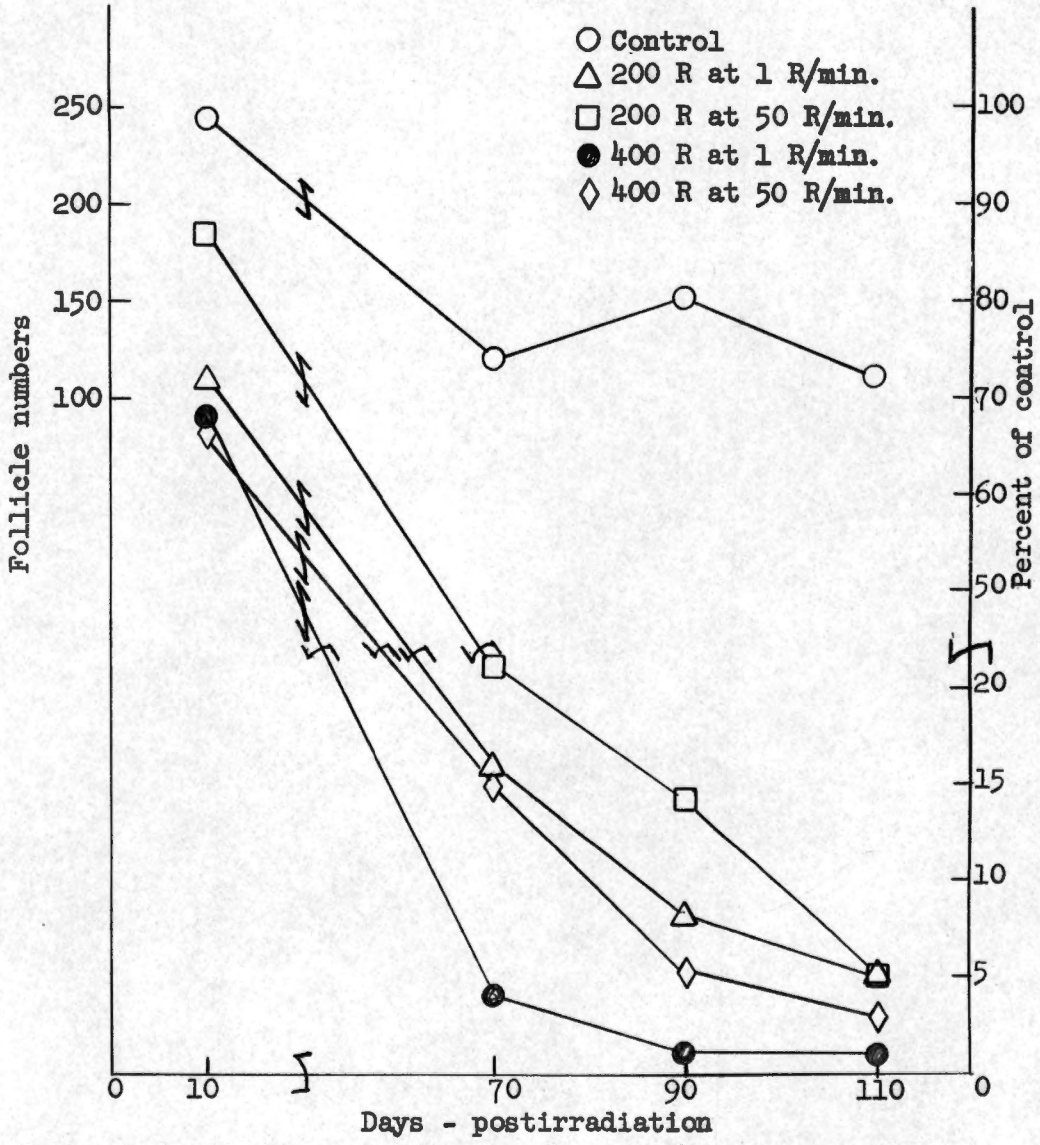



Figure 2. Right ovary growing follicle means (percent of control).

Average number of growing follicles at 70, 90 and 110 days post-irradiation in all irradiated groups, while differing from those of the control and the 10-day mean of each group, are not different from each other ($P < 0.01$). The interaction shown in Table 4 is due to a more rapid decline of growing follicles within the irradiated groups than in the control group.

Figure 2 illustrates the actual control means and the irradiated groups as percentages of the control means.

Effects of Irradiation on the Number of Vesicular Follicles (Right Ovary)

As shown in Table 5, the vesicular follicle means of the control group differed only at 110 days postirradiation ($P < 0.01$). The 70, 90 and 110 day means of each of the irradiated groups differed from their corresponding 10-day and control means ($P < 0.01$). There was a difference in dose response between the 200-1R and 400-1R 10-day means ($P < 0.01$).

The components of the analysis of variance are shown in Table 6. The interaction shown in this table is due to the more rapid decline of vesicular follicles within the irradiated groups than in the controls. Figure 3 depicts the subsequent decline in vesicular follicles as percentages of the controls for each irradiated group.

Effects of Irradiation of the Combined Growing and Vesicular Follicle Numbers (Right Ovary)

It was thought that the combining of the growing and vesicular follicle numbers would give a more accurate estimate of the ovarian population available for reproduction following irradiation. The data

TABLE 5

VESICULAR FOLLICLE MEANS (RIGHT OVARY)

| Treatment | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|
| | 10 | 70 | 90 | 110 |
| Control | $29^{ab}$ | $22^{ab}$ | $24^{ab}$ | $15^{c}$ |
| $S_{\bar{x}}$ | ±2 | ±2 | ±2 | ±2 |
| 200 R at 1 R/min. | $32^{a}$ | $8^{cde}$ | $6^{de}$ | $4^{de}$ |
| | ±2 | ±1 | ±1 | ±1 |
| 200 R at 50 R/min. | $26^{ab}$ | $10^{cd}$ | $9^{cde}$ | $5^{de}$ |
| | ±2 | ±1 | ±2 | ±1 |
| 400 R at 1 R/min. | $22^{b}$ | $3^{de}$ | $2^{e}$ | $2^{e}$ |
| | ±2 | ±1 | ±0.4 | ±0.4 |
| 400 R at 50 R/min. | $29^{ab}$ | $7^{de}$ | $4^{de}$ | $3^{de}$ |
| | ±2 | ±1 | ±1 | ±1 |

$^{a-e}$Means with the same superscript are not significantly different from each other ($P < 0.01$).

TABLE 6

ANALYSIS OF VARIANCE FOR VESICULAR FOLLICLES (RIGHT OVARY)

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Total | 187 | 24,840 | | |
| Treatment (T) | 4 | 5,696 | 1,424 | 63.01** |
| Day (D) | 3 | 13,245 | 4,415 | 195.35** |
| T x D | 12 | 2,103 | 175 | 7.76** |
| Residual | 168 | 3,796 | 23 | |

**$P < 0.01$.

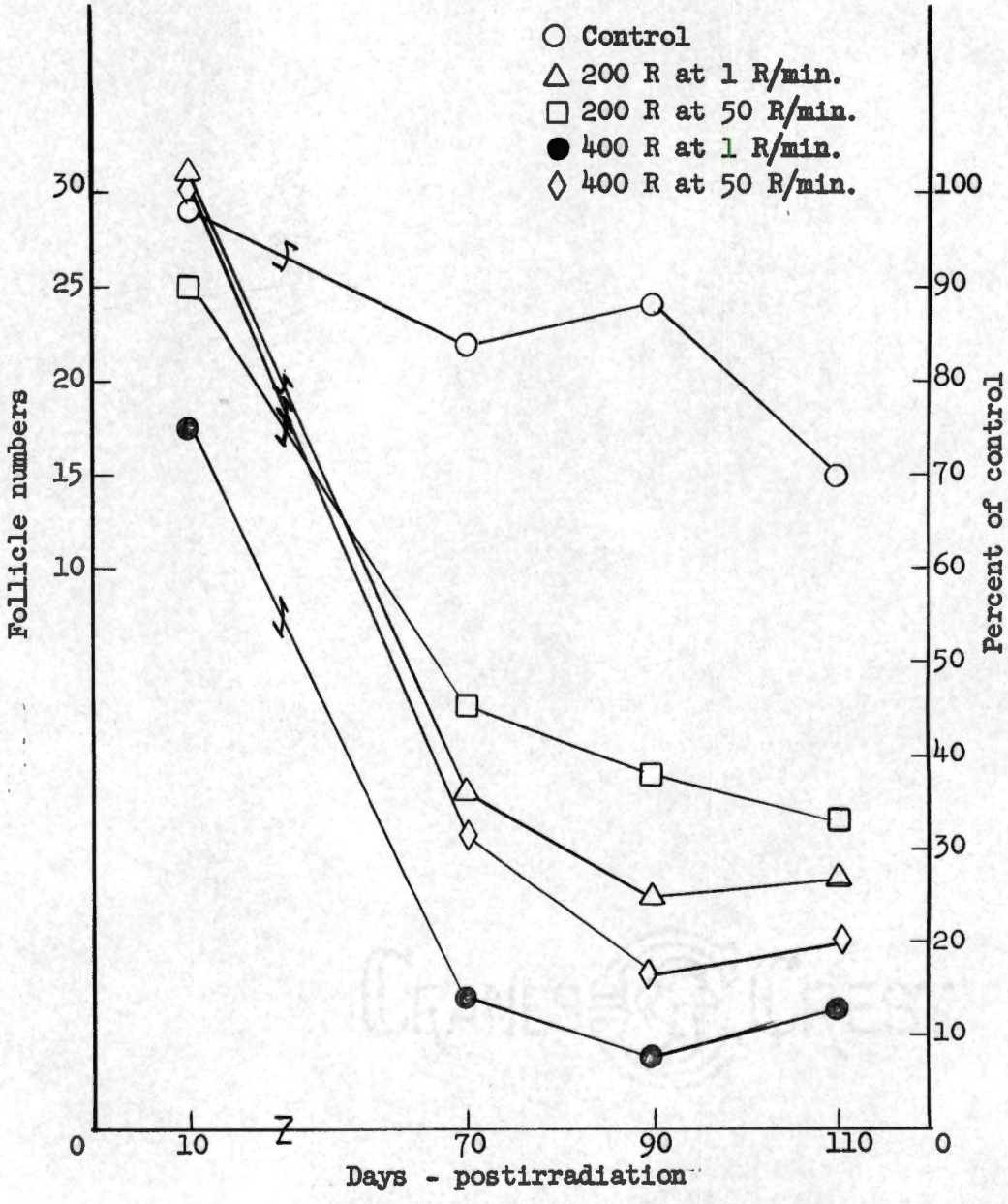



Figure 3. Right ovary vesicular follicle means (percent of control).

are summarized in Table 7, and the components of the analysis are shown in Table 8.

Comparison of the table of the combined numbers with the tables of the individual populations does not change the conclusions drawn from the separate values of growing and vesicular follicles.

Figure 4 shows graphically the control value for each age and the irradiated groups expressed as percentages of these values.

Effects of Irradiation on Subsequent Reproductive Performance

The functional response of the ovary was measured by progeny production after different postirradiation intervals. A summary of the mean number of pups produced by the control and irradiated groups subsequent to unilateral ovariectomy (right ovary) at 10, 70, 90 and 110 days postirradiation is shown in Table 9. Components of the analysis of variance are shown in Table 10.

Those animals ovariectomized at 10 days postirradiation, were different from the controls in number of pups produced by the 200-50R, 400-1R, 400-50R groups ($P < 0.05$) and the 200-1R group ($P < 0.10$). Pup production was less affected by both 200-1R and 200-50R than it was by 400-1R and 400-50R ($P < 0.10$).

In those animals ovariectomized at 70 days postirradiation, as compared with the controls, there was a reduction in pups produced in all irradiated groups; however, the 200-50R was least affected ($P < 0.05$).

There was no difference in numbers of progeny produced among all irradiated groups after ovariectomy at 90 days postirradiation; this was also true for the animals ovariectomized at 110 days postirradiation ($P < 0.05$).

TABLE 7

COMBINED GROWING AND VESICULAR FOLLICLE MEANS (RIGHT OVARY)

| Treatment | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|
| | 10 | 70 | 90 | 110 |
| Control | $274^{a}$ | $147^{d}$ | $174^{cd}$ | $127^{d}$ |
| $S_{\bar{x}}$ | ±34 | ±11 | ±11 | ±11 |
| 200 R at 1 R/min. | $209^{bc}$ | $28^{e}$ | $18^{e}$ | $9^{e}$ |
| | ±14 | ±4 | ±3 | ±2 |
| 200 R at 50 R/min. | $239^{ab}$ | $36^{e}$ | $30^{e}$ | $11^{e}$ |
| | ±25 | ±5 | ±5 | ±2 |
| 400 R at 1 R/min. | $191^{bc}$ | $8^{e}$ | $4^{e}$ | $3^{e}$ |
| | ±18 | ±2 | ±1 | ±1 |
| 400 R at 50 R/min. | $194^{bc}$ | $24^{e}$ | $12^{e}$ | $5^{e}$ |
| | ±7 | ±4 | ±2 | ±2 |

$^{a-e}$Means with the same superscript are not significantly different from each other ($P < 0.01$).

TABLE 8

ANALYSIS OF VARIANCE FOR COMBINED GROWING AND VESICULAR FOLLICIES (RIGHT OVARY)

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Total | 187 | 1,877,375 | | |
| Treatment (T) | 4 | 424,450 | 106,113 | 71.75** |
| Days (D) | 3 | 1,155,917 | 385,306 | 260.52** |
| T x D | 12 | 48,532 | 4,044 | 2.73** |
| Residual | 168 | 248,476 | 1,479 | |

**$P < 0.01$.

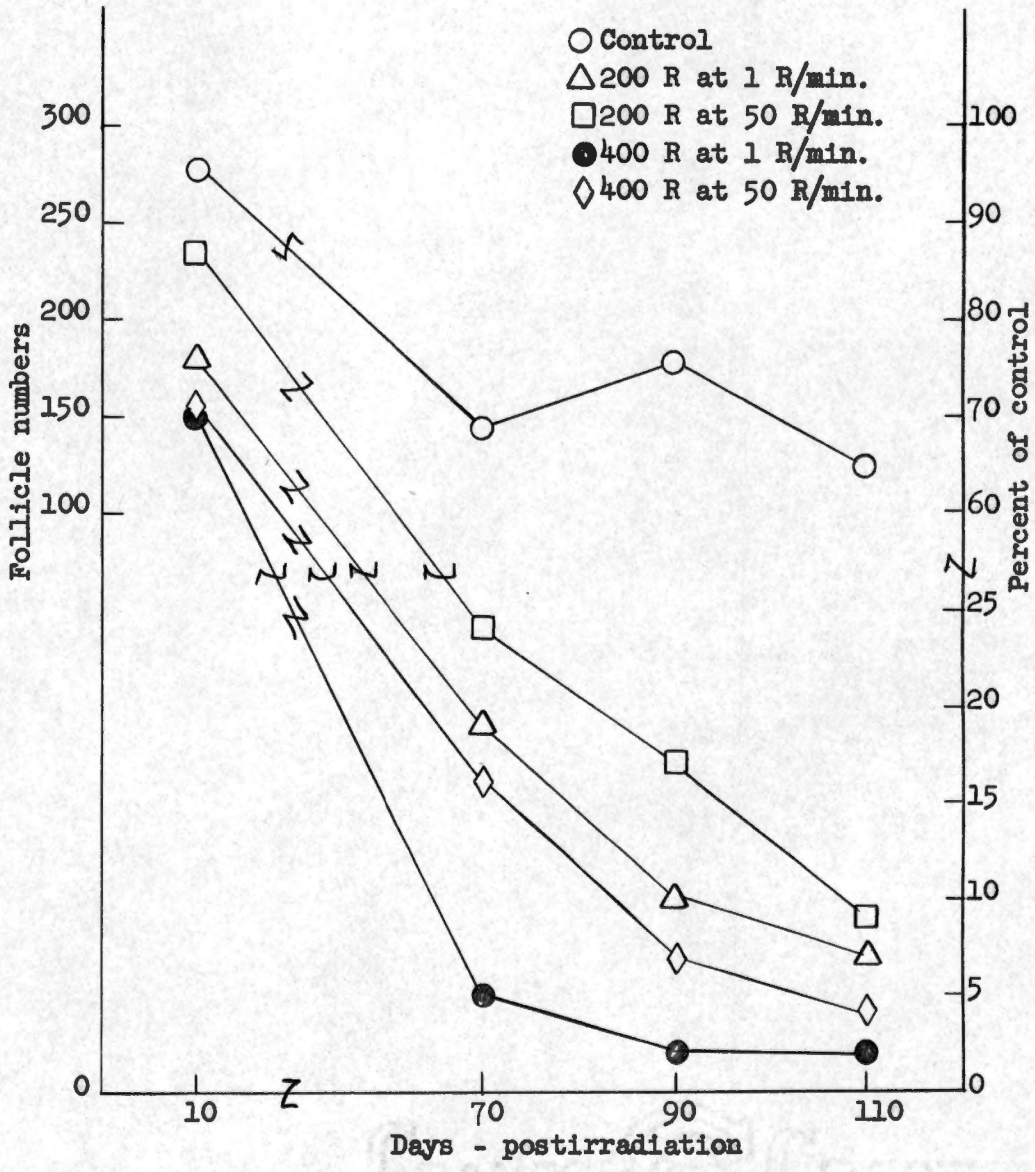



Figure 4. Right ovary combined growing and vesicular means (percent of control).

TABLE 9

PUP MEANS, POSTOVARIECTOMY, COMPARED WITHIN EACH POSTIRRADIATION INTERVAL

| Ovariectomy days postirradiation | Treatment | | | | |
|---|---|---|---|---|---|
| | Control | 200-1R | 200-50R | 400-1R | 400-50R |
| 10 | $27^{a}$ | $22^{ab}$ | $19^{bc}$ | $10^{cd}$ | $14^{d}$ |
| $S_{\bar{x}}$ | ±2 | ±2 | ±1 | ±2 | ±2 |
| 70 | $15^{a}$ | $6^{bc}$ | $8^{bc}$ | $2^{c}$ | $5^{bc}$ |
| | ±2 | ±2 | ±2 | ±2 | ±2 |
| 90 | $11^{a}$ | $1^{b}$ | $2^{b}$ | $0.0^{b}$ | $0.3^{b}$ |
| | ±1 | ±0.5 | ±0.6 | ±0.0 | ±0.2 |
| 110 | $5^{a}$ | $1^{b}$ | $0.4^{b}$ | $0.2^{b}$ | $0.1^{b}$ |
| | ±1 | ±0.5 | ±0.3 | ±0.2 | ±0.1 |

$^{a-d}$Within each postirradiation interval means, with the same superscript are not significantly different from each other ($P < 0.05$).

TABLE 10

ANALYSIS OF VARIANCE FOR PUP NUMBERS - POSTOVARIECTOMY

| Source | df | SS | MS | F |
|---|---|---|---|---|
| | | 10 Days Postirradiation | | |
| Total | 49 | 3670 | | |
| Treatment | 4 | 2043 | 510.75 | 14.12** |
| Residual | 45 | 1627 | 36.16 | |
| | | 70 Days Postirradiation | | |
| Total | 45 | 1963 | | |
| Treatment | 4 | 816 | 203.98 | 7.29** |
| Residual | 41 | 1147 | 27.97 | |
| | | 90 Days Postirradiation | | |
| Total | 48 | 1521 | | |
| Treatment | 4 | 1174 | 293.65 | 37.28** |
| Residual | 44 | 347 | 7.88 | |
| | | 110 Days Postirradiation | | |
| Total | 42 | 1272 | | |
| Treatment | 4 | 930 | 232.60 | 25.84** |
| Residual | 38 | 341 | 9.00 | |

**P < 0.01.

Figure 5 illustrates the mean pup production of each treatment group from the day of ovariectomy until the termination of the experiment at 150 days postirradiation. Figure 6 demonstrates the number of litters and the mean number of pups per litter following the four castration intervals for each treatment.

## Relationship of Follicle Numbers to Postovariectomy Reproductive Performance

The number of progeny produced by rats following exposure to $\gamma$-radiation of different doses and dose rates was expressed as a function of the different follicle populations at 10, 70, 90 and 110 days postirradiation.

The correlation coefficients between the primordial, growing and vesicular follicle numbers and progeny produced for each postirradiation interval were calculated. Also, the regression coefficients were calculated for the regression of pup numbers on each of these follicular classifications. These coefficients are shown in Tables 11, 12 and 13.

In Table 11, the correlation coefficients between primordial follicles surviving irradiation and subsequent reproductive capacity are shown to be different from zero in both 400 R groups ($P < 0.05$) and in all irradiated groups ($P < 0.10$) at 70 days postirradiation. The coefficient of correlation in the 200-1R group was different from zero at 90 days ($P < 0.10$). Also, at 110 days postirradiation, the magnitude of the 200-1R group was comparable, though not different from zero. The estimations of correlation in the 200-50R group at the later posttreatment intervals were also of such a magnitude as to merit cognizance. That is to say, given more observations, these estimates could

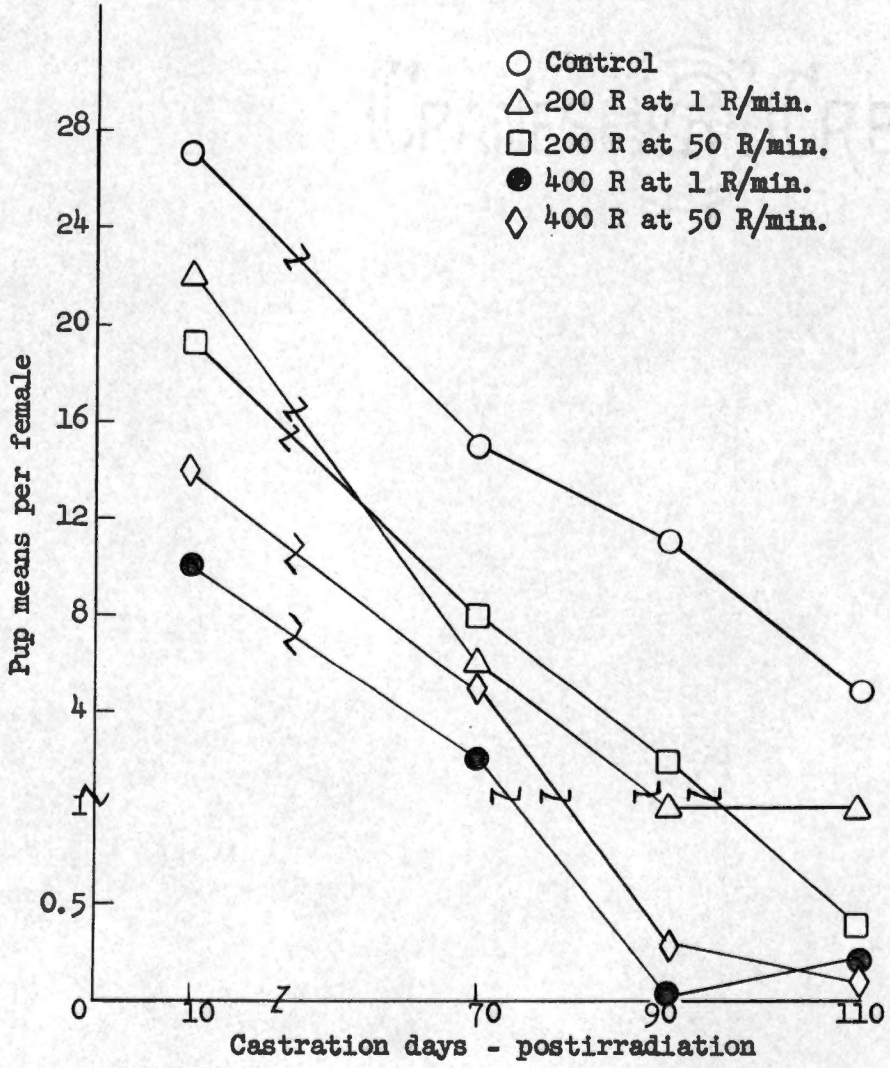



Figure 5. Mean postcastration pup production per female for each treatment.

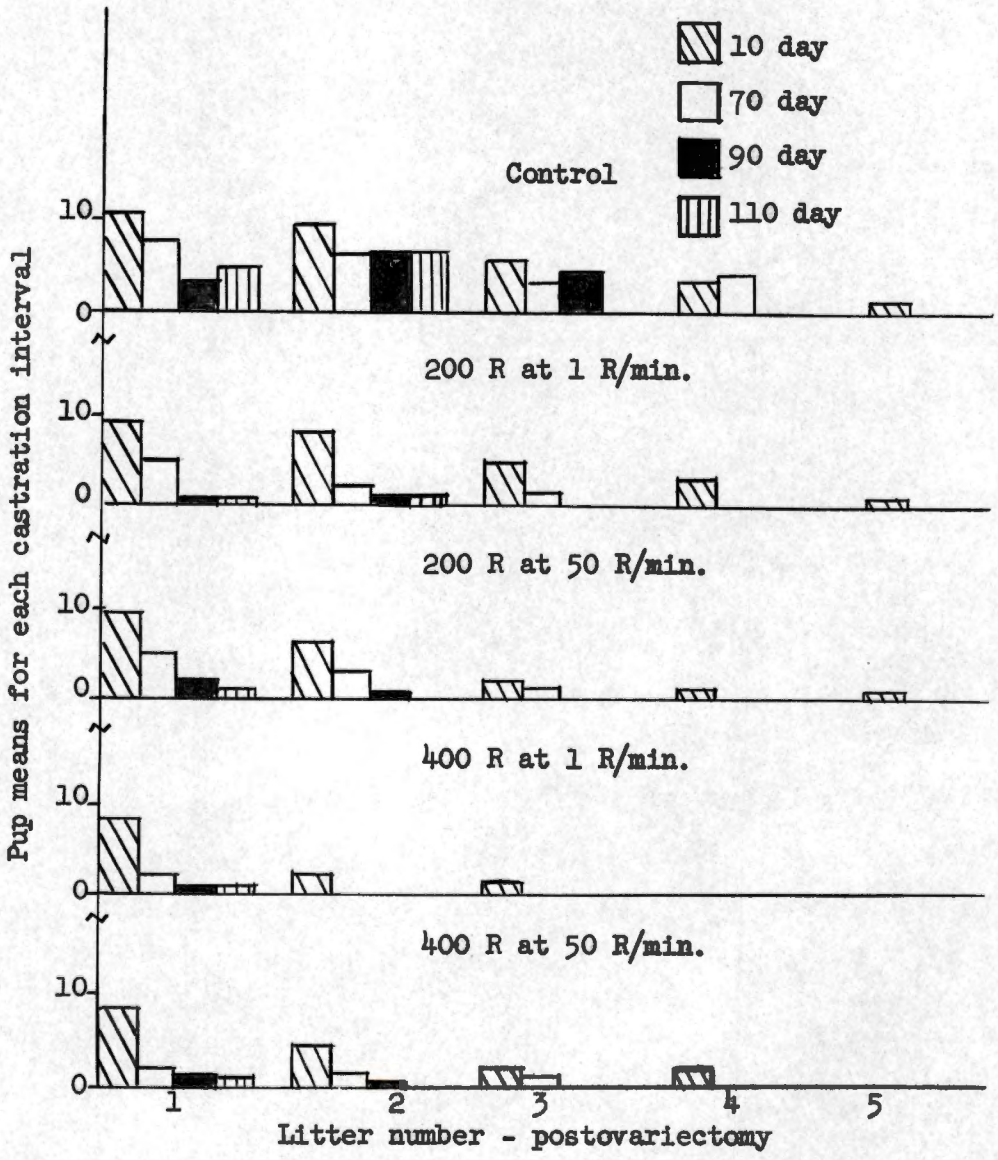



Figure 6. Pups per litter means for each treatment.

TABLE 11

CORRELATION (r) AND REGRESSION (b) COEFFICIENTS OF RIGHT OVARY PRIMORDIAL FOLLICLE NUMBERS WITH THE NUMBER OF POSTCASTRATION PUPS

| Treatment | | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|---|
| | | 10 | 70 | 90 | 110 |
| Control | r | -0.43 | 0.09 | 0.13 | 0.35 |
| | b | -0.002 | 0.0003 | 0.001 | 0.001 |
| 200 R at | r | 0.14 | 0.61 | 0.61 | 0.54 |
| 1 R/min. | b | 00006 | 0.145 | 0.043 | 0.083 |
| 200 R at | r | -0.21 | 0.62 | 0.38 | 0.43 |
| 50 R/min. | b | -0.008 | 0.074 | 0.023 | 0.012 |
| 400 R at | r | -0.03 | 0.69* | -0.0 | 0.66 |
| 1 R/min. | b | -0.008 | 0.250 | -0.0 | 0.033 |
| 400 R at | r | 0.11 | 0.72* | -0.43 | 0.14 |
| 50 R/min. | b | 0.014 | 0.273 | -0.025 | 0.007 |

*$P < 0.05$.

TABLE 12

CORRELATION (r) AND REGRESSION (b) COEFFICIENTS OF RIGHT OVARY GROWING FOLLICLE NUMBERS WITH THE NUMBER OF POSTCASTRATION PUPS

| Treatment | | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|---|
| | | 10 | 70 | 90 | 110 |
| Control | r | -0.46 | -0.34 | 0.28 | -0.14 |
| | b | -0.021 | -0.046 | 0.033 | -0.015 |
| 200 R at | r | -0.12 | 0.47 | 0.33 | 0.74* |
| 1 R/min. | b | -0.019 | 0.264 | 0.075 | 0.218 |
| 200 R at | r | -0.14 | 0.88** | 0.47 | 0.39 |
| 50 R/min. | b | -0.008 | 0.354 | 0.074 | 0.075 |
| 400 R at | r | -0.55 | 0.83** | 0.0 | 0.30 |
| 1 R/min. | b | -0.071 | 0.288 | -0.0 | 0.206 |
| 400 R at | r | -0.26 | 0.41 | -0.10 | 0.34 |
| 50 R/min. | b | -0.104 | 0.327 | -0.015 | 0.047 |

*P < 0.05.

**P < 0.01.

TABLE 13

CORRELATION (r) AND REGRESSION (b) COEFFICIENTS OF RIGHT OVARY VESICULAR FOLLICLE NUMBERS WITH THE NUMBER OF POSTCASTRATION PUPS

| Treatment | | Ovariectomy - Days Postirradiation | | | |
|---|---|---|---|---|---|
| | | 10 | 70 | 90 | 110 |
| Control | r | -0.26 | -0.26 | 0.23 | 0.17 |
| | b | -0.191 | -0.190 | 0.148 | 0.110 |
| 200 R at | r | 0.13 | 0.02 | 0.29 | 0.94** |
| 1 R/min. | b | 0.156 | 0.022 | 0.339 | 0.44** |
| 200 R at | r | 0.53 | 0.29 | 0.18 | -0.19 |
| 50 R/min. | b | 0.290 | 0.604 | 0.058 | -0.070 |
| 400 R at | r | -0.53 | -0.09 | 0.0 | 0.06 |
| 1 R/min. | b | -0.565 | -0.056 | -0.0 | 0.029 |
| 400 R at | r | -0.12 | 0.16 | 0.50 | -0.24 |
| 50 R/min. | b | -0.122 | 0.269 | 0.098 | -0.033 |

**P < 0.01.

possibly have been declared different from zero. In the 400-1R group the correlation coefficient was different from zero ($P < 0.10$) at 110 days postirradiation. Evidently, the 400-50R treatment was severe enough to affect the reproductive capacity of the animals so as to show no particular trend at the later post-treatment intervals.

The increasing magnitude of the correlation coefficients at the different postcastration intervals in the control (Table 11) suggests that, given more time postirradiation and more observations, the measure of the relationship possibly might have approached significance.

The regression coefficients of both 400 R groups at 70 days postirradiation (Table 11) were different from zero ($P < 0.05$). All of the coefficients in the irradiated groups at 70 days postirradiation were different from zero ($P < 0.10$). They were also different from zero in the 200-1R group at 90 days and in the 400-1R group at 110 days postirradiation ($P < 0.10$).

The correlation coefficients of growing follicle numbers with numbers of postcastration pups are shown in Table 12. The estimates of correlation were declared different from zero at 70 days postirradiation in the 200-50R and 400-1R groups ($P < 0.01$). Also, this declaration was made concerning the 200-1R estimate at 110 days ($P < 0.05$). The comparable magnitude of the coefficients in the 200-1R group at 70 and 90 days, the 200-50R group at 90 and 110 days and in both 400-1R groups at 110 days suggests that if more observation were made, the estimates of the correlation might have been asserted as significant divergents from zero.

The regression coefficients (Table 12) were different from zero in both the 200-50R and 400-1R groups at 70 days postirradiation

($P < 0.01$). It was also found that the coefficients of the 200-1R group at 110 days was different from zero ($P < 0.05$).

The joint relationships between vesicular follicle numbers and post-castration pup numbers are shown in Table 13. It was found that these estimates of the relationship were not of sufficient magnitude in any instance, except that of the coefficient of the 200-1R group at 110 days ($P < 0.01$), to be declared significantly different from zero.

Only the regression coefficient of the 200-1R group at 110 days postirradiation in Table 13 was different from zero ($P < 0.01$).

Ovarian Follicle Numbers of Fertile and Sterile Rats

The fertility at sacrifice of each rat was determined by the presence or absence of fetuses in the uterine horn and whether or not the animal had littered within 30 days pre-sacrifice. A sterile animal would have no fetuses in her uterus and would not have littered within 30 days prior to sacrifice.

The mean number of follicles in each classification for each treatment are shown in Table 14 in conjunction with the number of fertile and sterile animals per treatment.

Table 15 summarizes the number of fertile and sterile animals in each treatment classified as to the total number of follicles present at 150 days postirradiation. In both the 200-1R and 200-50R groups, there was a greater frequency of fertile animals with high follicle populations than there were sterile animals ($P < 0.01$). The same was true for the 400-50R group ($P < 0.05$).

The control group was not used in this analysis because all of the animals were fertile. The 400-1R group also was not used due to the fact that all these animals were sterile at 150 days postirradiation.

TABLE 14

MEAN NUMBER OF FOLLICLES PRESENT IN THE LEFT OVARY AT 150 DAYS POSTIRRADIATION AND NUMBER OF FERTILE AND STERILE FEMALES PER TREATMENT

| Treatment | | Primordial follicle $\bar{x}$ | Growing follicle $\bar{x}$ | Vesicular follicle $\bar{x}$ | Number of animals |
|---|---|---|---|---|---|
| Control | Fert. | 1462 | 129 | 23 | 37 |
| | Ster. | ---- | --- | -- | 0 |
| 200 R at | Fert. | 16 | 6 | 5 | 19 |
| 1 R/min. | Ster. | 3 | 3 | 2 | 16 |
| 200 R at | Fert. | 40 | 10 | 5 | 22 |
| 50 R/min. | Ster. | 14 | 5 | 2 | 16 |
| 400 R at | Fert. | ---- | --- | -- | 0 |
| 1 R/min. | Ster. | 2 | 1 | 0.5 | 34 |
| 400 R at | Fert. | 20 | 5 | 2 | 7 |
| 50 R/min. | Ster. | 8 | 3 | 1 | 27 |

TABLE 15

NUMBER OF FERTILE AND STERILE ANIMALS AT 150 DAYS POSTIRRADIATION CLASSIFIED AS TO SIZE OF TOTAL FOLLICLE POPULATION

| Treatment | | Number of animals: High follicle number[a] | Number of animals: Low follicle number | $\chi^2$ |
|---|---|---|---|---|
| 200-R at 1 R/min. | Fert. | 15 | 4 | 15.35** |
| | Ster. | 2 | 14 | |
| 200-R at 50 R/min. | Fert. | 15 | 7 | 6.91** |
| | Ster. | 4 | 12 | |
| 400 R at 50 R/min. | Fert. | 6 | 1 | 4.50* |
| | Ster. | 11 | 16 | |

*P < 0.05.

**P < 0.01.

[a]Animals were ranked according to follicle number; those individuals with follicular numbers above the median were designated as the high group.

# CHAPTER V

## DISCUSSION

The results of this study agree in the most part with the well documented review by Lacassagne et al. (1962) on the effects of ionizing radiation on the different follicle populations of the rat ovary. Mandl (1959) reported that a drastic reduction in primordial follicle numbers occurs within 18 hours after irradiation. The results of this study deal with the viability of the surviving primordial follicle population since the first observation was made 10 days after treatment. The observation concerning the number of primordial follicles and their reduction with increasing time agrees with those made by Zuckerman (1951) and Mandl (1959).

Lacassagne et al. (1962) reported that the growing follicle is relatively radioresistant. However, in this study, resistance of the growing follicles to ionizing irradiation was not made so lucid since a significant decrease was seen in three of the four irradiated groups (Table 3, page 16) at 10 days postirradiation ($P < 0.01$).

The significant decline in the number of growing follicles of the controls with increasing age is the result of normal ovarian physiology, i.e., atresia and maturation of the follicles. The reduction observed in the irradiated groups is due not only to normal function, but also, to the fact that the number and quality of the primordial follicles were so affected by irradiation that the process of replenishment is negligible.

The vesicular follicle is relatively radioresistant up until 14 to 15 hours prior to expected ovulation at which time it becomes very sensitive (Mandl, 1963). Since, in this study, the vesicular follicles were exposed to irradiation when they were not near ovulation, it would follow that exposure had no effect on the number of vesicular follicles observed at 10 days postirradiation. The results shown in Table 5 (page 19) confirm this assumption. However, irradiation does have an indirect influence on the vesicular follicle numbers at later post-treatment intervals. That is, as a consequence of the initial reduction in numbers and lowered quality of primordial follicles, there is a paucity of growing follicles for development into vesicular follicles. This is evidenced in the 70, 90 and 110 days means of all irradiated groups (Table 5, page 19).

Numbers of growing and vesicular follicles were combined to view the majority of the postirradiation ovarian follicle population and the influence asserted on it by irradiation. The results of this combination are similar to those for the growing follicle numbers because the growing follicle effects were great enough to mask those of the vesicular follicles.

Decreased fertility following irradiation has been reported in the mouse, rat and other species (Lacassagne et al., 1962). Peters and Levy (1964) state that radiation given at any age altered the normal physiology of the ovary in the mouse. The results of this study are consistant with these previous works.

Russell et al. (1959) states that, in the mouse, the lower the dose rate, the smaller the deleterious effects on fertility. In this

this is in agreement only with the 200-1R 10 day group (Table 9, page 24). However, it is possible that the 400 R total dose was so massive that this effect was overshadowed in the 400-1R 10 day group.

It should be noted that unilateral ovariectomy, while shortening the reproductive life-span by approximately one-half, does not affect the total production of viable germ cells per ovulation (Mandl and Zuckerman, 1951). These workers showed that the number of large follicles in the unilaterally spayed rat is about twice that found in the single ovary of intact females. This has been further substantiated in the mouse by McLaren (1963) and in the guinea pig by Hermreck and Greenwald (1964).

Since the number of follicles in the right ovary is not statistically different from that in the left (Zuckerman, 1951), the right ovary was used in this experiment as an index of the left ovary population at 10, 70, 90 and 110 days postirradiation. From this, establishment of a relationship between follicle numbers at these post-treatment intervals and subsequent reproductive capacity was attempted.

A relationship between surviving primordial follicle numbers and postcastration pup numbers was observed at 70 days in all of the irradiated groups ($P < 0.10$). The apparent reason is that irradiation reduced the follicular population to the extent of elucidating this relationship. The relatively large correlation coefficients in the 200-1R group at 90 and 110 days is evidently a result of the sustained pup production in this group at these later intervals while it diminishes in the other groups; the follicular populations remained relatively constant.

The joint relationship of growing follicle numbers with postcastration pup numbers was evidenced only in the 200-50R and 400-1R groups at 70 days after irradiation.

The relationship of vesicular follicle numbers at various intervals postirradiation with total pup production after these specified intervals is not meaningful because the observed populations at these intervals, plus additional growing follicles which mature into this population, contribute to the total progeny produced. Thus, a vesicular follicle population that has been limited by irradiation would possibly show a definite relationship with the number of progeny produced in the first litter after observation of that population. This possibly is the case for the 200-1R group at 110 days; however, the high correlation coefficient of this group at this day might also be due to the fact that one would normally expect one coefficient in twenty to be significant ($P < 0.05$) due to chance alone. It should be noted that there were no significant correlation coefficients between any of the follicle classes and progeny production in the control group.

Ingram (1958) suggested that impaired fertility in rats is, in part, due to a depletion in the number of oocytes by irradiation. This worker also suggested that a relationship existed between surviving oocytes and reproductive capacity, however, this relationship was established between the pup production and residual oocyte number at autopsy. He, also, reported a decline in number of oocytes following irradiation.

Peters and Levy (1964) reported that in mice a relationship exists between the number of growing and large follicles present when an animal enters maturity and subsequent reproductive performance.

The findings of the present study agree with the previous work in that a reduction of numbers in the different follicle classes does occur. However, since the occasional occurrence of significant coefficients in the growing and vesicular follicle classes ($P < 0.05$) could be due to chance alone, a definite relationship between these follicle classes and subsequent reproductive capacity could not be established from these data. Yet, the correlation between surviving primordial follicles at 70 days postirradiation and subsequent progeny produced was shown to exist at a lower level of probability ($P < 0.10$).

At the termination of the experiment, it was found that the frequency of sterile animals with a low total number of follicles was greater than that of the fertile animals. Thus, it appears that a minimum number of follicles must be present for an animal to be fertile. Mandl and Zuckerman (1951) state that the normal rat aged 24 months may have as many as 2,000 oocytes in its ovaries, but is almost invariably infertile.

# CHAPTER VI

## SUMMARY

The objectives of this study were to determine both the effects of $\gamma$-radiation on the oocyte of the rat and the relationship between numbers of viable oocytes at various times postirradiation and long-term reproductive performance.

Two hundred animals, initial age 30 days, were used in this study. Using 40 control animals, the remaining 160 animals were divided equally into four groups and irradiated with $^{60}Co$ gamma radiation at the following doses and rates: 200 R at 1 R per minute, 200 R at 50 R per minute, 400 R at 1 R per minute and 400 R at 50 R per minute. Following irradiation, all animals were subjected to continuous breeding until 150 days postirradiation, at which time the experiment was terminated.

At 10, 70, 90 and 110 days postirradiation, 50 animals (10 per treatment group) were unilaterally ovariectomized for histological evaluation of the ovarian population at each postirradiation interval. At the termination of the experiment, the remaining ovary was taken for histological evaluation of the residual population following breeding and irradiation.

The primordial follicle population was drastically reduced by all irradiation levels at 10 days postirradiation. There was also a difference in response to dose and dose rate. The numbers were further reduced by age at 70 days postirradiation. There was, however, no further decline in the primordial follicle population at the 90 and 110 day intervals.

The growing follicle numbers were reduced at 10 days postirradiation by 200 R at 1 R per minute, 400 R at 1 R per minute and 400 R at 50 R per minute, but not by 200 R at 50 R per minute. At 70, 90 and 110 days postirradiation, the growing follicle populations were further depleted by all irradiation treatments.

At 10 days postirradiation, the vesicular follicle numbers were not affected by any of the irradiation levels. However, by 70 days post-irradiation, the population was reduced by all treatments and age.

The relationship of follicle numbers surviving irradiation and reproductive performance was tested and a relationship was demonstrated between the number of primordial follicles surviving irradiation and subsequent progeny produced after 70 days postirradiation by both 400 R dose rates ($P < 0.05$).

A relationship also existed between growing follicles and progeny production in the 200 R at 50 R per minute and 400 R at 1 R per minute groups at 70 days postirradiation ($P < 0.05$). A relationship was not elucidated between the vesicular follicles and subsequent progeny produced.

Based on the results obtained in this study, there does not appear to be a significant relationship between number of germ cells and progeny production in nonirradiated female rats. However, the irradiation treatments which were imposed did significantly reduce the number of germ cells and subsequent pup production.

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## LITERATURE CITED


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# VITA

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