

# Morphometric and Histological Changes in Ovaries of Prepubertal Rats after Gamma Irradiation

Naila Tawakal<sup>1</sup>, Ferdose Sultana<sup>2</sup>, Naheed Tawakal<sup>3</sup> and Umar Farooq Khan<sup>4</sup>

<sup>1</sup>Department of Anatomy, Services Institute of Medical Sciences Lahore, Pakistan.

<sup>2</sup>Department of Anatomy, Services Institute of Medical Sciences, Lahore, Pakistan.

<sup>3</sup>Akhtar Saeed Medical College, Lahore Pakistan.

<sup>4</sup>Executive District Officer Health, Lahore Pakistan.

## ABSTRACT

**Purpose:** To analyze the morphometric changes, including diameter of ovary and oocytes in ovarian follicles as well as histological changes in these follicles in prepubertal rats after exposure to gamma radiation. **Materials and Methods:** 108 albino rats of 3wks age were the subjects of this study. Out of them, randomly chosen 36 animals received 4.5Gy and next 36 received 8.3Gy of gamma radiation, while rest of them received sham radiation. Ovaries of animals were dissected out 2hrs, 4hrs, 6hrs, one day, 7days and 14days after irradiation. Micrometry was performed for diameter of ovaries and oocytes in ovarian follicles. Histological examination of five random sections of each ovary was performed. **Results:** There was a dose related reduction in mean diameter of ovary and diameter of oocyte in primordial and primary follicles within 7 days. Oocytes in primordial and primary follicles also showed necrotic changes within 7days. Granulosa cells in primary follicles showed intense apoptosis during first six hours, followed by necrosis and development of cystic cavities containing remnants of oocyte and granulosa cells. Apoptosis, necrosis and cyst formation were not observed in granulosa cells of primordial follicles. **Conclusion:** Therefore, it is concluded that gamma ionizing radiation induces reduction in diameter of ovaries and oocytes of primordial and primary follicles. It causes necrosis of oocytes and sequential changes including intense apoptosis and necrotic degeneration of granulosa cells in primary follicles leading to formation of abundant follicular cysts. It induces intense reduction in ovarian follicular reserve.

**Key Words:** Ovary, Rat, Gamma radiation, Apoptosis, Oocyte, Granulosa cells.

## INTRODUCTION

Gamma radiations are high-energy penetrating photons, in form of electromagnetic waves having extremely short wavelength, emitted from nuclei of radioisotopes. Gamma rays photons are 10,000 to 10,000,000 times more energetic than photons of visible light. These rays are capable of traveling long distances through air, biological tissues and other materials. They can be shielded by few inches of lead or several feet of water but there is no such thing as perfect shield<sup>1</sup>.

Radiation is involved in many sectors of human activity, such as in biomedicine, nuclear research and nuclear industry with increased risks of

occupational, medical and accidental exposures<sup>2</sup>. Medical radiation is an essential diagnostic as well as therapeutic tool in medical practice. Therapeutically, radioactive substances, I<sup>131</sup>, P<sup>32</sup> and Co<sup>60</sup> are being utilized for treatment of malignant tumors<sup>3</sup>.

Radiolysis of water produces free radicals, which are highly reactive and unstable, contains excess energy that can disrupt bonds and produce point lesions. During radiolysis, interaction of free radicals with macromolecules is responsible for 99% damage to proteins and development of atherosclerosis<sup>4</sup>.

DNA and rapidly dividing cells are primary targets of ionizing radiation and more vulnerable to

injury by oxidation of guanine bases generating guanine radicals<sup>5</sup>. During cellular division, the cell membrane is dissolved and the nucleus is exposed to harmful substances and becomes more sensitive to radiation. Gonads, stomach, intestinal lining, bone marrow and skin show rapid division and are easily damaged by radiation<sup>6</sup>.

Gamma radiation, with Co<sup>60</sup> has increased longtime survival of young cancer patients by destroying rapidly dividing cancer cells<sup>7</sup>. Radioactive iodine, being used for treatment of thyroid cancer has many oppressing actions on reproductive system and fetal development causing depletion of germ cells and delayed maturity in ovarian follicles during immature period<sup>8,9</sup>.

Human gonads are critically important target organs for radiation. Average gonad dose from natural radiation is about 90 $\mu$ Sv annually<sup>10</sup>. Oogonias reach about 7 millions during mid pregnancy and then declines because of spontaneous degeneration<sup>11</sup>. Primordial follicles in neonates, represents the total number of gametes available to a female throughout her reproductive life<sup>12</sup>.

Since these organs produce germ cells that control fertility and heredity, their response to radiation should be studied extensively. Various physiological effects of gamma radiation have been studied in the past but morphometric and histological sensitivities in different ovarian follicles and their components are not demonstrated. Therefore, the present study was performed to analyze the morphometric and histological changes for a time period of 14days after gamma irradiation.

## MATERIAL AND METHODS

108 female albino rats of 3wks age were obtained from NIH Islamabad. The animals were kept under standard condition of temperature 24 $\pm$ 1°C and relative humidity 55 $\pm$ 5% with regular 12 hour light/dark cycle and providing pellet food and tap water *ad libitum* in animal house of Postgraduate Medical Institute. Animals were randomly selected for distribution in groups A, B and C, each group comprising of 36 rats. All groups were subdivided into six subgroups, comprising of six rats each (Table 1).

**Table 1: Experimental design.**

Main group	Sub-groups	Dose of Radiation	Time
Group A	A1	4.5Gy of Gamma Radiation	A1, 2hours after irradiation
	A2		A2, 4hours after irradiation
	A3		A3, 6hours after irradiation
	A4		A4, one day after irradiation
	A5		A5, 7days after irradiation
	A6		A6, 14days after irradiation
Group B	B1	8.3Gy of Gamma Radiation	B1, 2hours after irradiation
	B2		B2, 4hours after irradiation
	B3		B3, 6hours after irradiation
	B4		B4, one day after irradiation
	B5		B5, 7days after irradiation
	B6		B6, 14days after irradiation
Group C	C1	Sham Irradiation	C1 sacrificed with A1 & B1
	C2		C2 sacrificed with A2 & B2
	C3		C3 sacrificed with A3 & B3
	C4		C4 sacrificed with A4 & B4
	C5		C5 sacrificed with A5 & B5
	C6		C6 sacrificed with A6 & B6

## Irradiation

Rats of experimental groups received whole body radiation from Co 60 isotopic source with a dose rate of 81 .2 cGy/min and source strength of 300 curie. Group “A” was exposed to 4.5 Gy, and group “B” was irradiated with 8.3 Gy of gamma radiation while group “C” was given sham radiation. The animals of these subgroups were sacrificed 2hrs, 4hrs, 6hrs, one day, 7days and 15 days after irradiation respectively.

Ovaries of the animals were dissected, fixed and processed for histological examination. Five random sections of 3-5 $\mu$ m from each ovary were stained with Haematoxylin & Eosin (H&E) and Periodic Acid Schiff (PAS) stains.

## Histological examination of Ovarian follicles

The diameters of ovaries and oocytes was measured by micrometry. Two diameters of each ovary were recorded which were at right angle to each other. The mean of those diameters will give the actual diameter. The same method was adopted for calculation of diameters of oocytes. During micrometry 40x objective was used.

70 eyepiece divisions were equal to 17 stage divisions

100 stage divisions = 1 mm 1000  $\mu$ m  
1 stage division = 10  $\mu$ m

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70 divisions of eyepiece  
micrometer =  
1 division of eyepiece  
micrometer =

17 stage divisions = 50  $\mu$ m  
 $17/70 = 0.242$  stage divisions  
= 2.42  $\mu$ m

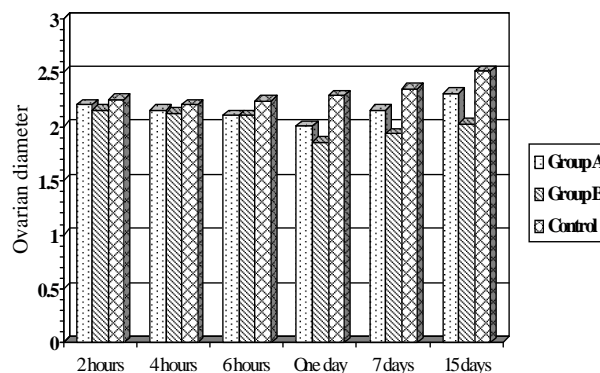
Primordial, primary and secondary follicles with few antral follicles were found at the age of 3wks. Normal and atretic varieties were found among primordial and primary follicles. Normal follicles were having healthy oocyte and normal granulosa cells. Atretic primordial follicles were having necrotic oocytes and relatively normal granulosa cells while primary follicles showed of pyknotic, apoptotic or necrotic granulosa cells and were graded as atretic.

### Statistics

In numeric data, mean and standard deviation was computed and student "t" test was applied. In descriptive data, percentage ratio was generated and "Z" test was applied. Statistical differences between the experimental groups were considered significant when "P" value was smaller than 0.05.

## RESULTS

There was a reduction in ovarian diameter of both experimental groups (A & B) 7-days after irradiation (Tables 2, 3, Fig. 1). Reduction in diameter of oocytes in primordial and primary follicles was noted in experimental groups, especially with high dose experimental group but great reduction in diameter was noted one day, 7-days and 14days after irradiation (Tables 4, 5, 6, 7).



**Fig. 1: Comparison of diameter of ovary in groups A, B and C**

In subgroups of experimental groups (A & B) the nuclei of atretic primordial follicles were necrotic, showing karyorrhexis and karyolysis. Each nucleus became fragmented, hazy (Fig. 3) and disappeared showing its radiosensitivity. Pyknosis, apoptosis and necrosis were not observed in granulosa cells of primordial follicles showing their radio resistance. As soon as the oocyte disappeared from the follicle, the stromal cells started infiltrating into the follicular cavity and it was difficult to differentiate the squamous shaped granulosa cells from stromal cells. The primordial follicles were almost depleted within 24hrs in experimental groups (Fig. 3).

**Fig. 2: Comparison of Cyst formation in Primary follicles in groups A, B, and C**

In atretic primary follicles, the oocyte of primary follicles showed necrosis. The nuclear membrane was indistinct and cytoplasm was not homogenous anymore. The oocyte became fragmented and was not clearly visible (Fig. 5).

In primary follicles degeneration of oocyte was slower than primordial follicles (Fig. 3, 6). Most of the oocytes in primary follicles were healthy while granulosa cells were showing pyknosis and rapid apoptosis at the same time (Fig. 4). Apoptotic bodies gradually increased in their number and reached to their maximum within 6hrs. Abundant necrosis of granulosa cells was observed one day after irradiation (Fig. 5). Gradually the necrotic material was engulfed by macrophages leading to formation of smaller cavities 7-days after exposure. Larger cavities with remnants of necrotic oocyte and granulosa cells, surrounded by theca cells appeared 14 days after irradiation (Fig. 6).

**Table 2:** Comparison of diameter (micrometer) of ovaries in group “A” and “C”.

Time interval	Experimental Group “A” diameter (μm)			Control group “C” diameter (μm)			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	2.20	0.19	6	2.25	0.19	p-value = 0.66
4 hours	6	2.15	0.69	6	2.20	0.13	p-value < 0.01
6 hours	6	2.10	0.30	6	2.23	0.25	p-value = 0.33
One day	6	2.00	0.22	6	2.28	0.18	p-value = 0.97
7 days	5	2.15	0.38	6	2.20	0.10	p-value < 0.01
15 days	6	2.30	0.26	6	2.51	0.20	p-value = 0.15

**Table 3:** Comparison of diameter (μm) of ovary in groups “B” and “C”

Time interval	Experimental Group “B” diameter (μm)			Control group “C” diameter (μm)			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	2.15	0.162	6	2.25	0.19	p-value = 0.34
4 hours	6	2.12	0.188	6	2.20	0.13	p-value = 0.41
6 hours	6	2.10	0.246	6	2.23	0.25	p-value = 0.39
One day	6	1.85	0.143	6	2.28	0.18	p-value < 0.01
7 days	5	1.93	0.144	6	2.20	0.10	p-value < 0.01
15 days	1	2.02	0.000	6	2.51	0.20	p-value = 0.07

**Table 4:** Comparison of diameter (μm) of Oocytes of the primordial follicles in group “A” & “C”

Time interval	Experimental Group “A” diameter (μm)			Control group “C” diameter (μm)			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	13.35	0.75	6	13.08	0.86	p-value = 0.02
4 hours	6	13.0	0.97	6	13.15	0.86	p-value = 0.37
6 hours	6	12.6	1.33	6	13.25	0.93	p-value < 0.01
One day	6	12.45	1.40	6	13.45	0.72	p-value = 0.05
7 days	6	12.20	1.53	6	13.90	1.10	p-value < 0.01
15 days	6	12.0	1.67	6	14.20	0.65	p-value = 0.04

**Table 5:** Comparison of diameter (μm) of Oocytes of the primordial follicles in group “B” and “C”

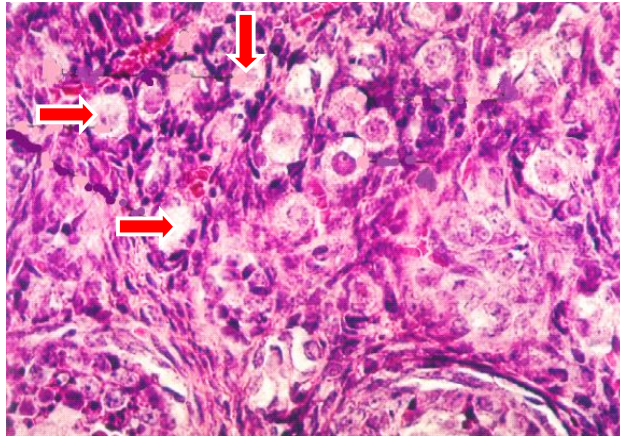
Time interval	Experimental Group “B” diameter (μm)			Control group “C” diameter (μm)			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	13.20	1.09	6	13.08	0.86	p-value = 0.51
4 hours	6	12.80	1.23	6	13.15	0.86	p-value = 0.02
6 hours	6	12.35	0.96	6	13.25	0.93	p-value = 0.03
One day	6	12.05	0.66	6	13.45	0.72	p-value < 0.01
7 days	5	11.70	1.27	6	13.90	1.10	p-value = 0.04
15 days	1	11.40	0.00	6	14.20	0.65	p-value < 0.01

## DISCUSSION

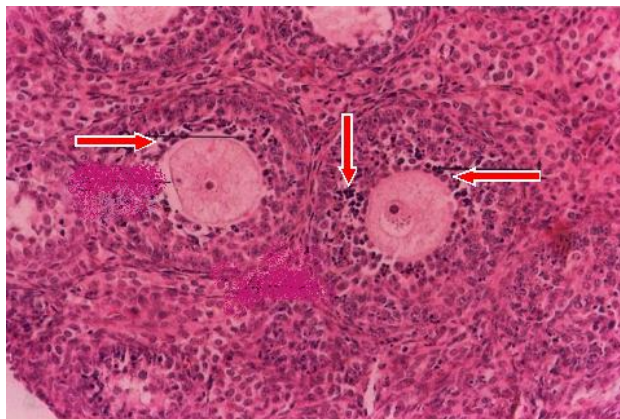
The gross degenerating effects of radiation on

ovarian follicles in mammals have been studied in the past but information regarding morphometric and histological changes in ovarian follicles was

still deficient and limited<sup>9,13,16</sup>. In the present study, light microscopic, morphometric and morphological changes in ovaries after exposure to gamma radiation have been examined for a time period of 15 days.



**Fig 3: Degenerating Primordial follicles with necrotic nuclei.**

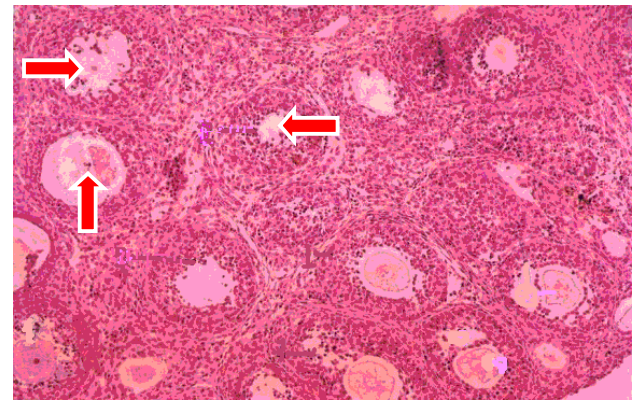


**Fig. 4: Primary follicles with apoptotic bodies.**

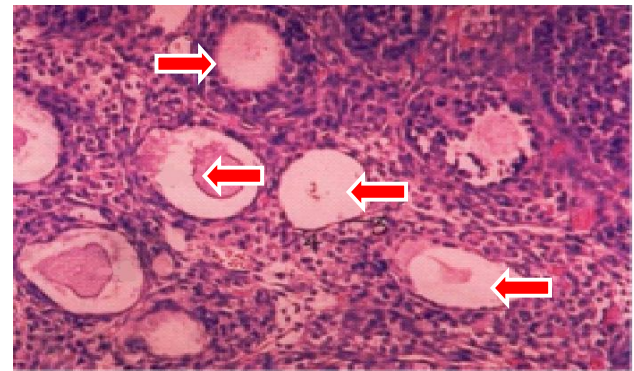
There is a relative decrease in the diameter of ovaries in experimental groups (Fig.1). The results were considerably significant with p value < 0.01, in high dose group sacrificed one day and seven days after irradiation (Table 3).

At 3 weeks age, majority of the ovarian follicles, present in ovaries of experimental and control groups were primordial and primary follicles. There was a relative and gradual decrease in diameter in oocytes of primordial follicles in high dose experimental group with a significant p values ranging between 0.01-0.04 (Table 4).

Relative and gradual decrease in mean diameter of oocytes of primary follicles was significant with P value <0.01, sacrificed one day, 7 days and 15 days after exposure respectively (Tables 6, 7). In high dose group, changes were appreciated earlier than low dose group suggesting early damage by 8.3 Gy of gamma radiation (Table 7).



**Fig. 5: Necrotic granulosa cells and oocytes in primary follicles.**



**Fig. 6: Abundant cyst formation after degeneration of oocyte and granulosa cells.**

Mode of death in oocytes of primordial follicles was by necrotic process, with acute onset after irradiation. Majority of primordial follicles were depleted within 24 h showing their greater susceptibility to gamma radiation. The granulosa cells of primordial follicles were more radioresistant<sup>13</sup>. Degeneration of oocytes in primordial follicles of experimental groups was highly marked however, p value was not significant but it showed a definite decreasing trend. Nucleus and cytoplasm in primordial follicles showed necrotic changes<sup>14</sup>.

**Table 6:** Comparison of diameter ( $\mu\text{m}$ ) of Oocytes of the Primary follicles in group “A” and “C”

Time interval	Experimental Group “A” diameter ( $\mu\text{m}$ )			Control group “C” diameter ( $\mu\text{m}$ )			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	63.88	3.77	6	65.67	5.56	p-value = 0.33
4 hours	6	63.39	3.24	6	65.17	7.08	p-value = 0.59
6 hours	6	63.02	6.87	6	65.35	1.41	p-value = 0.91
One day	6	63.99	4.82	6	67.02	1.56	p-value = 0.02
7 days	6	66.18	4.67	6	69.75	1.79	p-value < 0.01
15 days	1	68.47	8.08	6	71.97	2.78	p-value < 0.01

**Table 7:** Comparison of diameter ( $\mu\text{m}$ ) of Oocytes of the Primary follicles in group “B” and group “C”

Time interval	Experimental Group “B” diameter ( $\mu\text{m}$ )			Control group “C” diameter ( $\mu\text{m}$ )			Significance test
	(n)	Mean	S.D.	(n)	Mean	S.D.	
2 hours	6	64.12	3.23	6	65.67	5.56	p-value = 0.35
4 hours	6	63.01	6.71	6	65.17	7.08	p-value = 0.60
6 hours	6	59.55	3.48	6	65.35	1.41	p-value = 0.01
One day	6	61.20	6.92	6	67.02	1.56	p-value = 0.04
7 days	5	63.04	5.97	6	69.75	1.79	p-value < 0.01
15 days	1	65.05	0.00	6	71.97	2.78	p-value < 0.01

Degeneration of oocyte in primary follicle was by necrotic process. The granulosa cells of primary follicles proved highly radiosensitive. Pyknosis and apoptosis appeared within two hours, before any change was observed. Number of apoptotic bodies increased rapidly and reached to its maximum within 6 hours after irradiation (Fig. 4). The death of granulosa cells during follicular atresia was through the process of apoptosis<sup>15</sup>.

Apoptosis is reported as a classical hallmark of physiological atresia<sup>17</sup>. Remaining granulosa cells under went necrotic process (Fig. 5), which was completed within 24 hours. Rest of the granulosa cells degenerated producing cellular debris. Numerous cystic cavities appeared after lysis of cellular debris 7-days after irradiation<sup>18</sup>. Ovaries showed lots of cystic cavities 14-days after irradiation (Fig. 6). Maximum number of cysts appeared 7 days after exposure to radiation. Number of cystic cavities slightly decreased 15 days after irradiation due to fibrotic scarring of cystic cavities (Figs .2, 6).

## CONCLUSION

In summary, the present study infers that

there is decrease in diameter of ovary as well as diameter of oocytes in primordial and primary follicles after gamma irradiation. The death of oocytes in these follicles is by necrosis. The granulosa cells of primordial follicles are relatively radioresistant. Majority of the primordial follicles depleted within 24 hours. The granulosa cells of primary follicles demonstrated sequential changes of intense and accelerated pyknosis, apoptosis and necrosis followed by cyst formation after gamma irradiation. Similar changes are expected in human ovaries, which may lead to early cessation of menstruation and infertility in future.

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#### **The Authors:**

Naila Tawakal,  
Department of Anatomy,  
Services Institute of Medical Sciences,  
Lahore, Pakistan.

Ferdose Sultana,  
Department of Anatomy,  
Services Institute of Medical Sciences,  
Lahore, Pakistan.

Naheed Tawakal  
Akhtar Saeed Medical College,  
Lahore Pakistan.

Umar Farooq Khan  
Executive District Officer Health,  
Lahore Pakistan.

#### **Corresponding Author:**

Naila Tawakal  
Department of Anatomy,  
Services Institute of Medical Sciences,  
Lahore.  
Email: [nailatawakal@yahoo.co.uk](mailto:nailatawakal@yahoo.co.uk).

