Original Article

GAMMA RADIATION INDUCED CHANGES IN PRIMARY OOCYTE AND ZONA PELLUCIDA

Naila Tawakal, Naheed Tawakal, Attiya Mubari and Umar Farooq

Objective: This study was performed to analyze the morphological changes in primary oocyte and zona pellucida of primary follicle in prepubertal albino rats of three weeks age after exposure to gamma radiation.

Material and Methods: 72 female albino rats of age of three weeks were used. Out of these 24, received 4.5 Gray, another 24 received 8.3 Gray of Gamma radiation, and the rest of them received sham radiation. Ovaries of rats were dissected after one day, 7 days and 14 days after irradiation. Five random histological sections of each ovary were examined.

Results: Intense necrosis was evident in oocyte and granulosa cells of primary follicles after one day- post radiation. The zona pellucida remained in contact with the oocyte and granulosa cells in initial phase of atresia. With progressing atresia, zona pellucida lost its contact with the oocytes and granulosa cells. Disintegration of zona pellucida started after development of apoptotic bodies in primary follicles. Disintegrated zona pellucida disappeared rapidly in experimental groups, particularly in high dose group. The basement membrane was irregular and disrupted in atretic follicles.

Conclusion: Therefore, we can conclude that gamma radiation induces rapid necrosis and cyst formation in primary oocyte. Disintegration of zona pellucida with progression of atresia in ovarian follicle was evident. Gamma radiation also produces irregular, disrupted and thinner basement membrane.

Keywords: ovary, primary oocyte, zona pellucida, basement membrane.

Introduction

FRadiation interaction with biological tissue produces free radicals including hydrogen, hydroxyl, hydroperoxyl radicals and hydrogen peroxide. Radiation induces cellular injury that induces damage to DNA, cell membrane and fatty change.[1] Rapidly dividing cells are primary target of ionizing radiation and are more vulnerable to injury.[2]

Annual exposure to natural environmental radiation is approximately 100 mrads (1mGy) but after adding artificial sources, worldwide average annual exposure is about 3.5 nm Sv. Ionizing radiation is a danger to biological tissue.[3] Ionizing radiation includes electromagnetic waves and particulate radiation, which can remove an electron from atom to cause ionization. Gamma radiation is in the form of electromagnetic waves, produced from nuclei of radioactive elements. They can travel long distances through biological tissues, air and other materials.[4]

Unilaminar follicles consisting of primary oocytes surrounded by a single granulosa layer, composed either of flattened, cuboidal or columnar cells, have been studied in normal prepubertal hamsters[5].The nucleoli of the young oocytes are large and complex. The zona pellucida is an extracellular matrix of glycoproteins which surrounds the mammalian oocyte and preimplantation embryo and is formed during follicular development in the ovary, persists at the time of fertilization within the oviduct, and then surrounds the embryo until implantation in the uterus. Although the structure and chemical properties of the ZP have been extensively studied, the precise site of origin of the ZP remains a matter of controversy[6]

The mammalian zona pellucida is an egg extracellular matrix to which sperm bind. Mouse zonae are composed of three glycoproteins (ZP1, ZP2, and ZP3), while rat zonae contain four (ZP1, ZP2, ZP3, and ZP4/ZPB). Mouse sperm bind to zonae comprised solely of mouse ZP2 and ZP3.[8]

The initial steps in the formation of the zona pellucida take place in follicles with a single layer of cuboidal granulosa cell[9]. By the time the oocyte is surrounded by a single layer of columnar granulosa cells a continuous zona pellucida within which lies oocyte microvilli and granulosa cell processes are present.[10]

It has been reported that degeneration of ovarian follicles is caused by gamma radiation. However, radiation sensitivities to different components of ovarian follicles, including oocyte and zona pellucida, over a wide range of time have not been

morphologically demonstrated. Therefore, the present study was performed to analyze the morphological changes in oocyte and zona pellucida after exposure to gamma radiation.

Material and Method

This descriptive case series of subject were selected from department of Anatomy over a period of 6 months from June to December. Random 72 Female Albino rats of 3wks age were included in the study. They were maintained in a 23▫C controlled animal care room with light/dark (12/12hr). The animals had free access to water and commercial diet during experiment. Animals were distributed into two experimental groups 'A' and 'B' and one control group 'C', having 24 animals each. These groups were further subdivided into three subgroups, each subgroup comprised of eight prepubertal female albino rats **(Table.1).**

Experimental groups received whole body gamma radiation from Co 60 istopic source with a dose rate of 8.12 cGy/min and source strength of 300 curie at Institute of Nuclear Medicine and Oncology Lahore (INMOL. Animal of group "A" received 4.5 Gy and group "B" received 8.3 Gy of gamma radiation. Animals were sacrificed on day one, day 7 and day 14 after radiation **(Table 1).** The ovaries were dissected, fixed and processed for histological examination. The sections of 3-5 µm were stained with Haomatoxylin and Eosin (H&E) and Periodic Acid Schiff (PAS) Stains

.Histological examination of ovarian follicle:

Primary oocytes were examined for histological changes in primary follicles. Healthy oocytes were observed in normal follicles and degenerative changes were observed in atretic oocytes.

Statistics Analysis

SPSS version 20 was used to analyzed the date. Categorical variables were analyzed by frequency percentage.

Table-1: Experimental design.

Groups (24 rate each)	Subgroups (8 rats each)	Dose of Radiation	Schedule of sacrifice
Experimental Group A	A1	4.5 Gy of gamma radiation	A1, one day after radiation
	A2		A2 7 days after radiation
	A3		A3. 14 days after radiation
Experimental Group B	B1	8.3 Gy of gamma radiation	B1, one day after radiation
	B2		B2, 7 days after radiation
	B3		B3, 14 days after radiation
Experimental Group C	C1	Sham radiation (received no radiation)	C1, sacrificed with A1 and B1
	C2		C2, sacrificed with A2 and B2
	C3		C3, sacrificed with A3 and B3

Table-2: Necrotic degeneration in oocytes of primary follicles.

Groups	One Day Post Radiation	7 Days Post Radiation	14 Days Post Radiation	P-Value
Group A	73.6%	78.2%	81.5%	0.021
Group B	80.4%	85.7%	86.5%	0.52
Group C	35.1%	38.2%	43.1 %	0.032

Results

In normal primary follicles, healthy oocytes with 3 to 4 layers of normal looking granulosa cells were present and oocyte was surrouneded by zona pellucida **(Fig-1).** The oocyte was shrunken, nucleus was fragmented and nuclear membrane was indistinct in the irradiated group **(Fig-2).**

There waskaryorrhexis and karyolysis of nuclear material. The cytoplasm was not clear. The oocyte appeared segmented during advanced atresia **(Fig-3).** Abandoned follicular cystic cavities were visible in the ovaries of experimental group seven days after radiation. A few necrotic remnants of oocytes were still present in cystic cavities. These cystic cavities

were lined with disrupted basement membrane and theca cells **(Fig-4).**

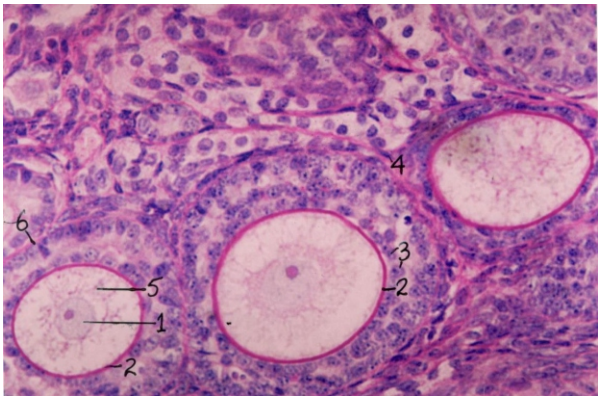

Fig-1: Primary oocytes surrounded by intact zona pellucida.

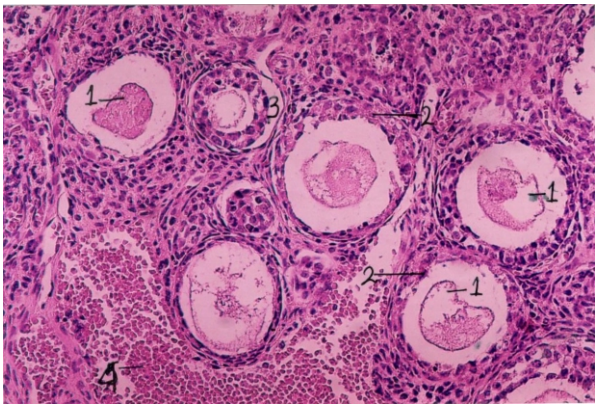

Fig-2: Oocytes are shrunken, nuclei are fragmented, nuclear membranes are irregular in the irradiated group.

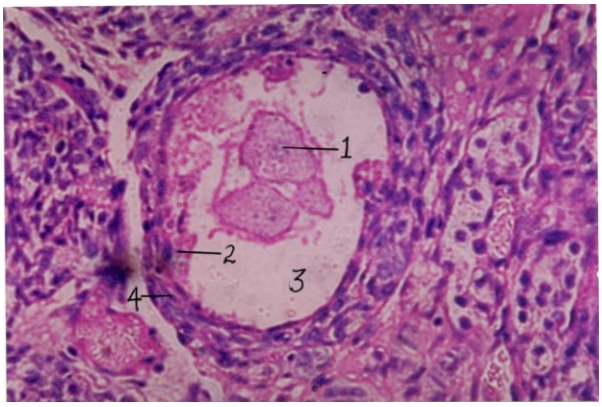

Fig-3: The oocyte appeared segmented during advanced atresia.

The percentage follicular cyst formation was visible. Zona pellucida was intact in normal follicles while it was disintegrated in atretic follicles. The basement

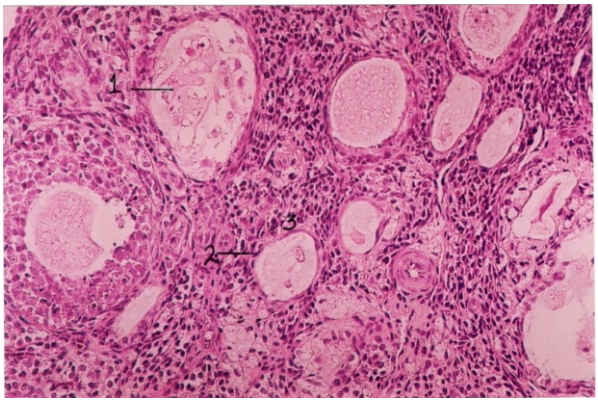

Fig-4: Necrotic remnants of oocytes are still visible in cystic cavities lined with disrupted membranes.

membrane was disrupted during advanced cyst formation. The zona pellucida remained in contact with the oocyte in initial phase of atresia. With progressing atresia zona pellucida lost its contact with oocytes and granulosa cells. Disintegration of zona pellucida started with appearance of apoptopic bodies. It was disintegrated and disappeared rapidly in experimental groups. Basement membrane was irregular and disrupted in atretic follicles.

Discussion

The impact of radiation on ovarian follicles was studied in the past but the knowledge about the morphological changes in different components of ovarian follicles for a longer duration was not explored fully. This study demonstrates the presence of necrosis in ovarian follicles during prepubertal age after exposure to gamma radiation.[6] The death of oocytes in primary follicles appeared a necrotic death with acute onset after irradiation. The demise of oocytes in primary follicles was also through necrotic process.[8] The follicular cavities were filled with cellular debris. Numerous cell fragments were present in atretic follicles. Cystic follicular appearance of ovary was a classical feature of atresia.[9] Normal to atretic perenctage ratio of primary follicles fall sharply with time showing p-value < 0.05. In control ovaries necrosis was absent.

Conclusion

Hence to conclude; gamma radiation induces rapid degeneration of primary follicles and rapid disintegration of zona pellucida. In summary, the present study showed the sequential changes in ovarian follicles during follicular atresia after irradiation. There was death of oocyte, caused by speedy and acute necrosis in primordial and primary

ovarian follicles. The demise of granulosa cells in primary follicles was by intense and acute necrosis followed by follicular cyst formation after irradiation. Cystic follicular appearance of ovary was a classical feature of atresia by gamma irradiation that may lead to infertility in future.

Department of Anatomy
Gujranwala Medical College, Gujranwala Pakistan
www.esculapio.pk

References

1. Masse R,Ionizing radiation. How to put an aplphabetical order C R Aced Sci. 2000. 111, 323 (7): 633-40.
2. Lambert HE the principals of radiotherapy and chemo-therapy. In: Shaw RW, Soutter WP, Stanton SL. eds. Gynaecology. 1st ed. Churchill Livingstone; 1992. 461-468.
3. Albenese J, Dainiak N, Modulation of intercellular communication mediated at cell surface and on extracellular, plasma membrane drived vesicles by ionizing radiation. Exp. Hematol;2003. 31(6); 455-64.
4. Kumar V, Cotran RS, Robbins LS, eds, 2003. Environmental Diseases. In: Robbins Basic Pathology, 7th ed. Saunders. 2003; 287-288.
5. Mazaud S. Guigon CJ. Lozach A , Coudouel N. Forest MG, Coffigny H. 2002, Establishment of the reproduction function and transient fertility of female rats lacking primordial follicles stock after fetal gamma irradiation. Endocrinology, 143 (12): 4775-4787.
6. Rosales-Torres AM, Avalos-Rodriguez A, Vergara Onofre M, Hernandez-Perez O, Ballesteros LM, Garcia-Marcedo R. 2000, Multiparametric study of atresia in ewe antral follicle: histology flow cytometry, internucleosomal DNA fragmentation, and lyssomal enzyme activities in granulose cells and follicular fluid. Mol. Reprod Dev, 55 (3): 270-81.
7. Lee CJ, Yoon YD. 2005, Gamma radiation-induced follicular degeneration in the prepubertal mouse ovary. Mutant Res, 15; 578 (1-2): 247-55.
8. Tilly JL, Kowalski KI, Johnson AL, Hsueh AJW. 1992, Involvement of apoptosis and ovarian follicular atresia and post ovulatory regression. Endocrinology, 6: 1942-1950.
9. Eriksson M. 1981. The development of the ovary in the wood mouse. Apodemus sylvaticus. Acta Morphol Nerl Scand 19 (1): 35-40.
10. Structural Conservation of Mouse and Rat Zona Pellucida Glycoproteins. Probing the Native Rat Zona Pellucida Proteome by Mass Spectrometry. Emily S. Boja , Tanya Hoodbhoy , Mark Garfield and Henry M. Fales.
11. The ultrastructure of unilaminar follicles of the hamster ovary. D. Louise Odor