Journal of Applied and Natural Science 6 (1): 57-61 (2014)

JANS

Quantitative variations in ovarian follicles of female Sprague Dawley rats after exposure to low dose gamma radiation

S. Singh[1]*, J. Sharma[2] and P. K. Goyal[3]

[1]Department of Zoology, Nari Shiksha Niketan P.G. College, Lucknow, INDIA
[2,3] Radiation and Cancer Biology Laboratory, Department of Zoology, University of Rajasthan, Jaipur, INDIA
*Corresponding author. E-mail drseemasingh_23@yahoo.co.in

Received: January 12, 2014; .Revised received:February 30, 2014; Accepted: March 8, 2014

Abstract: In the present experiment, an attempt was made to assess the genetic risk of low dose radiations in mammals. For this purpose female Sprague Dawley rats, 11-12 weeks old, were irradiated with whole body Co 60 gamma rays in three fractionated doses of 0.10 Gy (cumulative dose 0.30 Gy) given at an interval of one month at two different dose-rates (0.00368 Gy/min. and 0.0589 Gy/min.). Ovaries were studied for quantitative evaluation of follicles at 1, 4, 12, 28 and 52 weeks after last fractionated exposure. Quantitation revealed lower number of ovarian follicles in irradiated animals than in controls. The follicular number decreased with the advancement of time after last exposure (i.e. 2 months) and reached a peak level on 28 weeks. After that the recovery was evident but the number remained below 25% of total follicles even at 52 weeks autopsy interval, which indicated an irreversible damage in ovarian tissue. Primary follicles were found to be the most radiosensitive among the various types of follicles. The highest loss in these follicles was noted at 12 weeks after exposure with the high dose-rate, where only 10.61% of them were scored. Dose-rate exhibited an inverse relationship with the number of surviving follicles. At the higher dose-rate (0.0589 Gy / min.), depletion in the total follicular number was significantly higher than at the low dose-rate (0.00368 Gy/min.) used.

Keywords: Dose-rate, Gamma radiation, Late effects, Ovarian follicles, Sprague Dawley rats

INTRODUCTION

Despite an expanding base of knowledge concerning effects of exposure to high dose radiations, many issues still remain unresolved that are essential in understanding the impact of low dose and dose-rate radiations. Human epidemiologic data on quantitative risk of ovarian failure following exposure to ionizing radiation are scarce and based either on accidental exposure or medical exposure for radiotherapy and radiological diagnosis. Therefore, the most comprehensive data on the genetic hazard of ionizing radiation come from experiments with the laboratory animals which can be used to estimate relative risks of low vs high dose-rate exposure especially at the low doses.

Gonads have been found to be quite radiosensitive organ in the body of living beings. Female fertility in mammals is comparatively a more sensitive parameter because the total number of follicles is fixed at the time of birth and damage to the system cannot be overcome by the division of surviving cells as there is no regenerative potential in ovaries (Greenwald and Roy, 1994, Bristol-Goull *et al*., 2006).

Radiotherapy is now a well-known cause of ovarian damage (Agarwal *et al.*, 2004, Sonmezer and Oktay, 2008, Andersen *et al.*, 2012). Differences in radio sensitivity of germ cells in different stages of development are more difficult to account for the female than the male, since no new oocytes are formed in the mammalian ovary after puberty. It is clear that only a quantitative study would provide a meaningful answer to the problem. The object of this work was to identify those stages of follicular development in female Sprague Dawley rats which are markedly affected by the known variation in dose-rates of radiation.

MATERIALS AND METHODS

Female Sprague Dawley rats, 11-12 weeks old, were selected from the inbred colony and divided into two groups. One group was exposed to Co 60 gamma rays of 0.10 Gy at the dose-rate of 0.0589 Gy/min. and further exposed to same respective dose and dose- rate at an interval of one month. Total dose delivered in three fractionated doses to the groups was therefore 0.30 Gy. The other group was exposed as above but at the dose- rate $1/16^{th}$ of the above i.e. 0.00368 Gy/min. A Sham-irradiated control animals were maintained for the comparison. Ovaries were taken out after 1, 4, 12, 28 and 52 weeks after the last fractionated exposure and were studied for quantitative studies. The total number of follicles was computed according to the method described by Mandl and Zuckerman (1951) and Pedersen (1969). Statistical analysis was carried

out by the Students t-test. The animal care and handling were approved by institutional ethical committee and was done according to guidelines of World Health Organization, Geneva, Switzerland and Indian National Science Academy, New Delhi, India.

RESULTS AND DISCUSSION

It is a widely held view that the mammalian neonatal ovary contains a finite stockpile of non-growing primordial follicles each of which encloses an oocyte arrested at the diplotene step of meiotic prophase (Kerr *et al.,* 2006, Albertini, 2004, Greenfeld and Flaws 2004, Telfer *et al.,* 2005). Ionizing radiation possess a threat to oocytes leading to their destruction (Crisp, 1992). The degree of ovarian impairment is related to the volume treated, total irradiation dose, fractionation schedule and age at time of treatment (Meirow and Nugent, 2001; Wallace *et al.*, 2005).

The radiation sensitivity of ovaries depend on several factors like the developing stage of cell, animal species involved, dose of irradiation, dose-rate used and on post irradiation time (Aurora *et al.*, 2012, Adriaens *et al.,* 2009). Quantitation of the follicles in the present experiment showed that they have varied radio sensitivity. Primary follicles were found to be most sensitive among the various types of follicles. The highest loss in these follicles was noted at 12 weeks after exposure with higher dose-rate where only 10.61% of them were visualized (Fig. 1). In type II follicles, the maximum loss was noticed at 4 weeks of autopsy interval, when exposed at the rate of 0.0589 Gy/min. Here, 9.67% of these follicles were noticed. At 28 weeks minimum percentage of survival follicles (10.15%) was computed with the higher dose-rate (0.0589 Gy/min.) of radiation (Fig. 2).

Present results indicated that oocytes contained in primordial follicles were particularly sensitive to ionizing radiation, whereas growing oocytes were comparatively radio resistant. These findings are in agreement with Said *et al.* (2012) who found that irradiation induce almost complete deletion of the primordial follicle pool within a time period of 24-hrs in contrast to the other growing antral follicles.

The decreased number of growing follicles was also noted in the current experiment (Table 1). In type IV follicles, the maximum loss (65.71%) was noted at 1 week autopsy interval and for type V the maximum loss was (57.87%) at 1 week autopsy interval in the high dose-rate group (0.0589 Gy/min.) (Table 2). This might be due to the depletion of primary follicles which would have otherwise grown to multilayered follicles. Similarly, minimum percent survival for type VI was observed in the high dose-rate group but at the 52 weeks autopsy interval (20.05%). The decrease in the percent survival reached the peak level at 28 weeks for all the follicles in the ovary and then the recovery was obtained but the number remained below 25% of the control (Fig. 3).

The present results showed that radio sensitivity of oocytes during growth from primordial follicle to graafian follicle decreased steadily up to stage V but after which it again increased. As the oocytes increased in volume, the size of follicle also increased with the formation of the layer of granulosa cells. A connective tissue theca surrounded the oocytes and the basal lamina separated the follicle from the surrounding stroma (Anderson, 1979). The development of theca reduced the radiation injury to the oocytes and the antric fluid present in graafian follicle made the oocyte more sensitive due to the free radical formation in the antral fluid. It in turn might cause pronounced damage to oocyte. Ionizing radiation reduces preantral follicle numbers in rodents and humans in a dose-dependent manner (Mark-Kappeler *et al.*, 2011). In female rats exposed *in- utero* to 1.5 Gy gamma-irradiation, severe depletion in oocytes was evidenced by premature ovarian failure from 6 months by Mazaud *et al.* (2002). Various other studies have also demonstrated radiation-induced loss of ovarian follicles (Ataya *et al.*, 1995, Lee and Yoon, 2005, Pesty *et al.*, 2010).

In the present study, dose-rate was inversely related to the survival of follicles. It was noticed for all the post-irradiation intervals. The dependence of the survival of small oocytes on the dose-rate and the corresponding total accumulated dose had an exponential character

Table 1. Number of ovarian follicles (Mean ± SE) at various autopsy intervals after exposure to cumulative dose 0.30 Gy gamma rays at the dose-rate of 0.00368 Gy/min. (Mean ± SE of 12 replicates).

A I	Type I	Type II	Type III	Type IV	Type V	Type VI	Total Follicles
1	984.67±127.5 (46.60)*	534.00±55.33 (31.43)#	161.33±20.60 (33.38)#	112.00±18.52 (48.38)	82.66±7.57 (58.04)#	62.33±8.32 (49.49)#	1937.00±145.66 (39.56)#
4	492.33±17.09 (24.49)*	216.00±44.64 (16.26)#	122.66±10.40 (30.21)#	80.33±6.02 (49.66)	73.33±8.50 (67.36)#	56.00±9.84 (63.21)*	1040.66±61.49 (25.27)#
12	212.66±17.55 (11.68)#	177.33±11.59 (17.37)#	86.33±7.09 (23.21)#	70.66±12.85 (52.18)	65.33±15.04 (70.03)o	40.66±13.57 (49.56)*	653.66±17.62 (18.36)#
28	175.66±30.82 (13.19)#	130.33±17.47 (14.69)#	57.66±7.09 (20.37)#	50.33±2.08 (59.25)	37.00±2.64 (67.30)o	30.00±3.00 (73.95)o	481.00±23.81 (17.95)#
52	112.00±12.76 (20.05)#	88.00±18.69 (19.26)#	50.33±4.04 (26.11)#	39.00±6.56 (63.1)	36.33±3.06 (81.37)o	27.66±3.05 (71.33)	353.33±32.25 (25.72)#

#: P<0.001; *: p< 0.01; º: P< 0.05, AI: Autopsy interval in weeks; Values in brackets are the percent survival of follicles

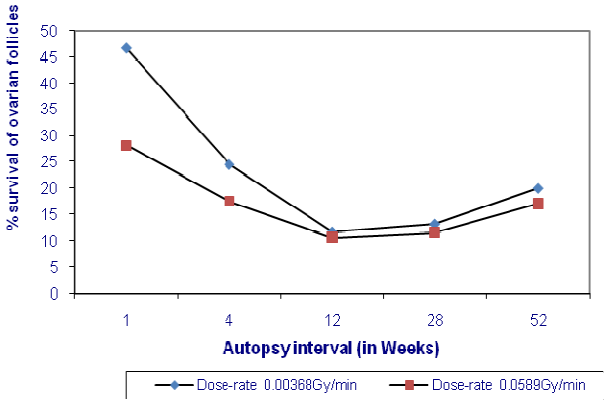

Fig. 1. *Percent survival of type 1 follicles at various autopsy intervals after gamma irradiation exposure to female Sprague Dawley rats.*

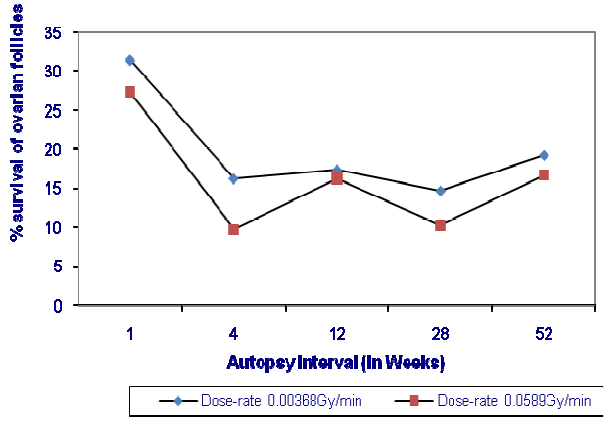

Fig. 2. *Percent survival of type II follicles at various autopsy intervals after gamma irradiation exposure to female Sprague Dawley rats.*

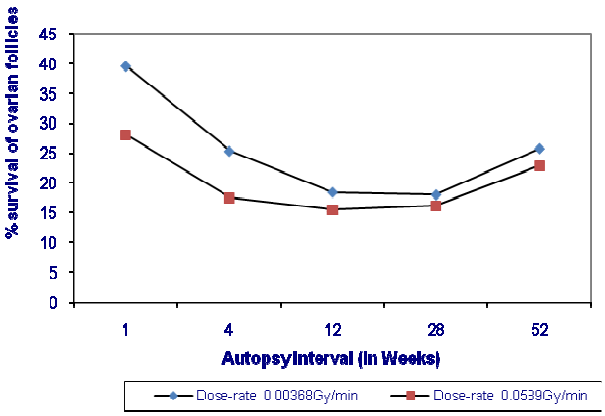

Fig.3. *Percent survival of total follicles at various autopsy intervals after gamma irradiation exposure to female Sprague Dawley rats.*

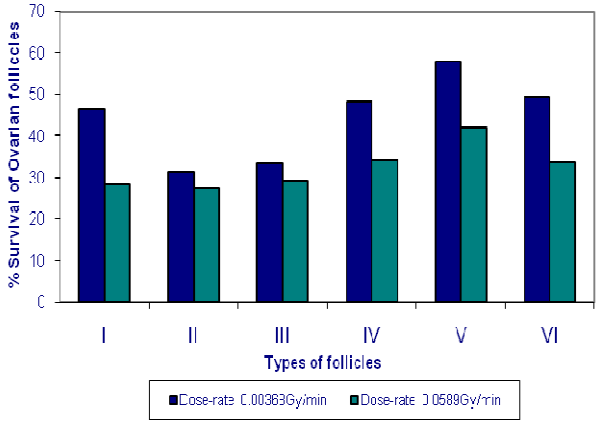

Fig. 4. *Percent survival of various types of follicles at 1 week autopsy interval after gamma irradiation.*

(Pistrzakflis and Gomulka, 1984). A dose-rate effect was most clearly marked in the rats (Benova *et al*, 1985). In the present experiment, the total no of surviving follicles decreased with the advent of dose rate and the time after irradiation. After 1 week of the last fractionated exposure, at the higher dose-rate (0.0589 Gy/min.), depletion of the follicle type I, V, VI was significantly higher than the low dose rate 0.00368Gy/min.(Fig. 4).

In females, the response to dose-rate was quite different from that in the males. It is suggested that changes in the chromosomal configuration of the cells were responsible for the marked differences in radiosensitivity. The primordial oocytes of mice and rats contain irregular masses of heterochromatin. The chromosomes at the dictyate stage consist of a diffused lamp brush condition in which the loops are highly extended oocytes in which the lamp brush loops are condensed and surrounded by a dense sheath of ribonucleoprotien are resistant to radiation induced cell death but the cells having highly extended chromosomes surrounded by a thin sheath of ribo nucleoprotien as in the primary oocytes of rodents.

In mature and maturing oocytes, dose- rate change from 1000 r/min. to0.009 r/min. resulted in a continuous drop in mutation frequency. Large total doses of high dose-rate irradiation given in small fractions at appropriate intervals had reduced effects as compared to single dose (Nias, 1998, Hall and Giacia, 2008). When the dose-rate was low enough, the process of recovery could take place. This replenished the cell population which was lost through damage. Repair of damage within the nucleus of injured cells reduced the total amount of damage that existed within the cells at any given moment during exposures. High dose-rate radiations are mutagenic in nature. Maturing oocytes with induced mutation do not recover from radiation arrested oocytes. The efficient repair processes have been invoked to explain latter finding. The protracted dose drastically reduced mutation yield from mature and maturing oocytes. A dose protraction effect was demonstrated at an early developmental stage when the nuclear morphology of mice oocytes resemble with human (Russell and Russell, 1992).

It has been testified that the oocytes die by apoptosis after irradiation (Hanoux *et al.*, 2007). The marked

Table 2. Number of ovarian follicles at various autopsy intervals after exposure to cumulative dose of 0.30 Gy gamma rays at the dose-rate of 0.0589 Gy/min. (Mean ± SE of 12 replicates).

A I	Type I	Type II	Type III	Type IV	Type V	Type VI	Total Follicles
1	602.33±63.35 (28.13)#	449.00±54.83 (27.32)#	140.00±16.09 (29.37)#	79.67±5.03 (34.29)#	60.33±2.08 (42.13)#	92.67±2.52 (33.63)*	1374.00±77.99 (28.02)#
4	353.67±55.59 (17.45)#	129.00±19.70 (9.67)#	91.00±3.61 (22.29)#	59.33±4.73 (36.70)#	55.33±5.13 (50.55)#	35.67±3.06 (40.35)#	724.00±72.34 (17.51)#
12	194.66±15.27 (10.61)#	163.00±18.64 (16.22)#	60.66±3.57 (16.62)#	53.00±5.00 (40.34)º	40.66±2.51 (46.45)#	37.00±2.00 (44.92)#	549.00±43.86 (15.48)#
28	152.66±10.59 (11.50)#	90.33±10.06 (10.15)#	60.00±4.60 (21.42)#	39.66±8.50 (50.60)º	30.66±2.51 (56.61)*	29.33±4.51 (72.75)º	402.67±25.66 (16.07)#
52	103.00±16.37 (17.95)#	74.67±10.26 (16.66)#	57.33±6.51 (28.74)#	30.33±2.08 (49.71)*	25.00±2.00 (55.53)#	25.00±1.00 (65.67)º	315.33±16.56 (22.88)#

#: P<0.001; *: p< 0.01; º: P< 0.05; AI: Autopsy interval in weeks; Values in brackets are the percent survival of follicles.

reduction in cell killing is observed when the dose-rate is lowered, indicating that the initial radiation damage can be partially repaired before it leads to cell death. Further several minor complexities in the effect of radiation on reproductive life span are present. For example apoptosis occurs at each stage of follicular development and there is a marked reduction in the number of follicles present at birth (Markstrom *et al.*, 2002) in normal unirradiated females and it is possible that radiation induced depletion in the population of oocytes may have some effect on the rate of the process.

The late effects of irradiation then had been traced back to the immediate damage which is simply early cell killing. This suggests that serious consideration should be given to the possibility that the cellular basis for some apparently complex late effects might be traced back to the cell death immediately following irradiation.

Conclusion

From the results, it is concluded that there is a continuous fall in number of ovarian follicles as the post irradiation time is extended. It suggests that the late effects of irradiation could lead to female infertility in mammals.

REFERENCES

Adriaens, I., Smitz, J. and Jacquet, P. (2009). The current knowledge on radiosensitivity of ovarian follicle development stages. *Hum. Reprod. Update*, 15 (3): 359-377.

Agarwal, A., Rangnathan, P., Kattal, N., Pasqualetto, F., Hallak, J., Khayal, S. and Masha, E. (2004). Fertility after cancer: a prospective review of assisted reproductive outcome with banked semen specimens. *Fertil. Steril.,* 81:342-348.

Albertini, D.F. (2004). Micromanagement of the ovarian reserve-do stem cells play into the ledger? *Reproduction*, 127: 513–514.

Andersen, C.Y., Kristensen, S.G., Greve, T. and Schmidt, K.T. (2012). Cryopreservation of ovarian tissue for fertility preservation in young female oncological patients. *Future Oncol*., 8: 595–608.

Anderson (1979). In ovarian follicular development and function. (Midgley, A. R. Jr and Sadler, W.A, eds.) Raven Press, New York. pp, 91-105.

Ataya, K., Pydyn, E., Ramahi-Ataya, A. and Orton, C.G. (1995). Is radiation-induced ovarian failure in rhesus monkeys preventable by luteinizing hormone releasing hormone agonists? preliminary observations. *J. Clin. Endocrinol. Metab*., 80:790–795.

Aurora, R.H., Francisca, G. and Montserrat, G.C. (2012). Radiobiology and Reproduction: What Can We Learn from Mammalian Females? *Gene*s, 3: 521-544.

Benova, D.K., Balrakova, A.K., Vuglenov, A.K., Kusheva R.P. and Rupova, I.M. (1985). Assessment of the genetic risk of radiation by irradiation data from laboratory mammals. *Genetika*., 21: 574-581.

Bristol-Gould, S.K., Kreeger, P.K., Selkirk, C.G., Kilen S.M,. Mayo, K.E, Shea, L.D. and Woodraff, T.K. (2006). Fate of the initial follicle pool : Empirical and mathematical evidence supporting its sufficiency for adult fertility. *Developmental Biology*, 298:149-154.

Crisp T. M. (1992). Organization of the ovarian follicle and events in its biology: oogenesis, ovulation or atresia. *Mutation Res.*, 296: 89-106.

Greenfeld, C. and Flaws, J. A. (2004). Renewed debate over postnatal oogenesis in the mammalian ovary. *Bioessays*, 26: 829-832.

Greenwald, G.S. and Roy, S. K. (1994). Follicular development and its control. (Eds.: E. Knobil, J.D. Neill). The Physiology of reproduction. New York, Raven Press. pp. 629.

Hall, E. J. and Giaccia, A. (2008). Radiobiology for the Radiologist. 6th ed. Philadelphia, PA: Lippincott, Williams and Wilkins.

Hanoux, V., Pairault, C. and Bakalska, M. (2007). Caspase-2 involvement during ionizing radiation induced oocyte death in the mouse ovary. *Cell Death Differ*., 14: 671-681.

Kerr, J. B., Duckett, R., Myers, M., Britt, K.L, Mladenovska, T. and Findlay, J. K. (2006). Quantification of healthy follicles in the neonatal and adult mouse ovary: evidence for maintenance of primordial follicle supply. *Reproduction*, 132: 95-109.

Lee, C.J. and Yoon, Y.D. (2005). Gamma-radiation-induced follicular degeneration in the prepubertal mouse ovary. *Mutat. Res*., 578: 247-255.

Mandl, A. and Zuckerman, S. (1951). The relation of age to number of oocytes. *J. Edocrinol.*, 7:190-193.

Mark-Kappeler, J.C., Hoyer, P.B. and Devine, P.J. (2011). Xenobiotic effects on ovarian preantral follicles. *Biol. of Reprod*., 85: 871–883.

Markstrom, E., Svensson, E.C.H., Shao, R., Svanberg, B. and Billig, H. (2002). Survival factors regulating ovarian apoptosis - dependence on follicle differentiation. *Reproduction*, 123(1): 23-30.

Mazaud,S., Guigon,C.J. and Magre, S. (2002). Establishment of the reproductive function and transient fertility of female rats lacking primordial follicle stock after fetal gamma-irradiation. *Endocrinology*, 143(12): 4775-87

Meirow, D. and Nugent, D. (2001). The effects of radiotherapy and chemotherapy on female reproduction. *Hum. Reprod. Update*., 7: 535–543.

Nias, A.H.W. (1998). An introduction to radiobiology. 2nd ed. New York, NY: Wiley.

Pesty, A., Doussau, M., Lahaye, J.B. and Lefevre, B. (2010). Whole-body or isolated ovary (60)Co irradiation: effects on in vivo and in vitro folliculogenesis and oocyte maturation. *Reprod. Toxicol*., 29: 93–98.

Pedersen, T. (1969). Follicle growth in the immature mouse ovary. *Acta Endocrinol*., 62: 117-32.

Pistrzakflis, S. and Wasilowska Gomulka, M. (1984). Effect of life time intake of organically bound tritium and tritiated water on the oocytes of rats. *Radiat. Environ. Biophys*., 23: 61-68.

Russell, L.B. and Russell, W. L. (1992). Frequency and nature of specific locus mutations induced in female mice by radiations and chemicals: a review. *Mut. Research*, 296: 107-127.

Said, R.S., Nada, A.S. and Demerdash, EI. (2012). Sodium selenite improves folliculogenesis in radiation-induced ovarian failure: a mechanistic approach. *PLoS One* ,7 (12):e50928.

Sonmezer, M. and Oktay, K. (2008). Assisted reproduction and fertility preservation techniques in cancer patients. *Curr. Opin. Endocrino. lDiabetes Obes*., 15: 514-522

Telfer, E. E., Gosden, R. G., Byskov, A. G., Spears, N., Albertini D., Andersen, C.Y., Anderson, R., R.Z. Braw-Tal, R., Clarke, H., Gougeon, A., McLaughtin, E., McLaren, A., McNatly, K., Schatten, G., Silber, S. and Tsafriri, A.(2005). On regenerating the ovary and generating controversy. *Cell*, 122: 821-822.

Wallace, W.H., Thomson, A.B., Saran, F. and Kelsey, T.W. (2005). Predicting age of ovarian failure after radiation to a field that includes the ovaries. *Int. J. Radiat. Oncol. Biol*. *Phys*., 62:738–744.