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# CHANGES IN SEMEN QUALITY AND QUANTITY AND DOMINANT LETHAL MUTATIONS IN RABBITS FOLLOWING EXTERNAL GAMMA IRRADIATION

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**Summary**

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Sexually mature male rabbits (3 groups of 6 animals in each) were irradiated with gamma rays in the dose range 0.5-2.5 Gy with dose density of 24 cGy/min. One groups of 6 non-irradiated rabbits served as control. The quantitative and qualitative parameters of sperm were determined prior to irradiation and up to post irradiation day 60 at 10-day intervals. The induction of dominant lethal mutations (DLM) in differentiating germinative cells was determined according to the schedule ♀ non-irradiated × ♂ irradiated.

The results showed a dose-dependent decrease in spermatozoa concentration during the entire experimental period. The lowest concentration was that at post irradiation day 60 in the 2.5 Gy group ($11\pm0.8 \times 10^6$/mL). A statistically significant increase in the percentage of abnormal spermatozoa was established after irradiation at 1.5 and 2.5 Gy. The DLM test showed highest DLM percentages between post irradiation days 30 and 40, i.e. in fertilization with sperm irradiated at the spermatid stage.

The data from the study showed a reduced reproductive potential in rabbits irradiated in the tested dose range.

**Key words**: rabbits, radiation mutagenesis, reproduction, spermatogenesis

## INTRODUCTION

The reproductive system, responsible for the continuation of the species, is highly sensitive to the multiple geno- and cytotoxic factors, including ionized radiation.

Male sexual glands, being a complex system with endocrine and germinative function, are known to be highly sensitive to the cyto- and genotoxic activity of ionized radiation. Despite the fact, that the spermatogonial cell population in male individuals is continuously renovated and possesses a big potential for regeneration from damages, the restoration requires time and sometimes is not complete (Ellis, 1970; Oakberg, 1975; Meistrich, 1993).

The effects of ionized radiation upon the reproductive potential of individuals from one hand, and the offsprings of irradiated parents, from the other, are negative.

Depending on the parameters of irradiation, the level of injuries of the spermatogenesis, spermatozoa concentration, motility and the nuclear chromatin structure of germinative cells is different (Sailer *et al.*, 1995; Hasegawa *et al.*,

1997; Haines *et al.*, 1998; Russell *et al.*, 1998). The processes of injury are influenced by factors such as the physiological status, the age, the species affiliation and the individual radiosensitivity.

The available literature data showed a considerable heterogeneity in the sensitivity of the various types of cells included in spermatogenesis. While the spermatogonial cell population is especially sensitive to the induction of cellular death (Searle and Beechey, 1974), the differentiating germinative cells are injured at DNA level and the resulting defects could be repaired or transformed into mutations. The mutations induced in germ-cells apart the risk of reducing the reproductive potential of irradiated individuals, are a treat for future generations under the form of late genetic effects (Friedler, 1996; Hoyes *et al.*, 1994; Wakeford and Town, 2000; Brinkworth, 2003).

The numerous studies in this field gave proofs of species-related peculiarities of the observed effects on spermatogenesis from one part and the ability for reproduction and quality of offsprings from the other.

The lack of explicit data about the effect of irradation at various doses on semen quality and the effect of radiation on the progeny in rabbits motivated the present study.

## MATERIALS AND METHODS

The experiment was performed with 84 sexually mature (4.5–month old) male and female New Zealand rabbits. All animals were with an equal body weight (4.0–4.5 kg) and housed in individual cages under uniform conditions prior to and during the experiment. Male rabbits (n=24) were alloted to 4 groups with 6 animals in each as followed:

- Group I: male rabbits irradiated at 0.5 Gy;
- Group II: male rabbits irradiated at 1.5 Gy;
- Group III: male rabbits irradiated at 2.5 Gy;
- Group IV: non-irradiated male rabbits (controls).

The total irradiation of male rabbits was performed with a $^{60}Co$ gamma equipment (Rokus) with a dose density of 24 cGy/min. The procedure was followed by a 10-day period of adaptation.

The concentration of spermatozoa in semen and the percentage of abnormal spermatozoa were determined prior to the irradiation and until post irradiation day 60 at 10-day intervals. The semen was collected using an artificial vagina. The percentage of abnormal spermatozoa was determined after staining with eosin-nigrosin on a minimum of 100 cells per rabbit.

In 60 female rabbits, 5 groups of intact animals were formed (groups V–IX) with 12 rabbits in each. Four groups were used for mating with 2.5 Gy males at post irradiation days 10, 20, 30 and 40, respectively in order to obtain offsprings from males whose germinative cells were irradiated at different stages of spermatogenesis (according to the 40–44-day duration of spermatogenesis in the rabbit; Desjardins, 1972; Robert, 1998). One of the female groups was mated with intact males and served as control.

The pregnancy in females was diagnosed by palpation 8 days after mating.

Radiation-induced dominant lethal mutations (DLM) were determined on the basis of the increased prenatal loss in $F_1$ per female mated with irradiated male in comparison with spontaneous prenatal loss in $F_1$ per female mated with non-irradiated (control) male. The prenatal

loss in $F_1$ for each group was defined using the respective mean number of live newborns (LN) per female. The calculation was performed according to the equation (Ehling, 1971):

$$DLM = 1 - \frac{\text{LN / ♀ in } F_1 \text{ of irradiated male parent}}{\text{LN / ♀ in } F_1 \text{ of non-irradiated male parent}} \times 100$$

The data were statistically processed using the Student's t-criterion. Differences were considered statistically significant at $p \leq 0.05$.

## RESULTS AND DISCUSSION

The results of determinations of spermatozoa concentration and the percentage of abnormal spermatozoa in semen of irradiated males within the dose range 0.5–2.5 Gy are presented on Fig. 1, 2 and 3. A dose-dependent decrease in sperm counts between post irradiation days 10 and 60 was observed. In the 0.5 Gy group (Fig. 1), the studied parameter varied between 158±20 $\times 10^6$/mL and 67±18 $\times 10^6$/mL at post irradiation days 10 and 60, respectively. The dynamics of those changes was variable in the various post irradiation periods. While during the first 20 days after the treatment there were no significant differences vs non-irradiated controls, thereafter a significant lower sperm counts occurred up to day 60 ($p<0.05$, $p<0.01$) compared to controls. At post irradiation days 50 and 60, sperm conts were lower vs the previous intervals in irradiated individuals themselves.

The irradiation within the dose range 1.5–2.5 Gy intensified the reduction of spermatozoa in semen with time. In the 1.5 Gy group (Fig. 2), sperm counts were statistically lower vs controls as early as

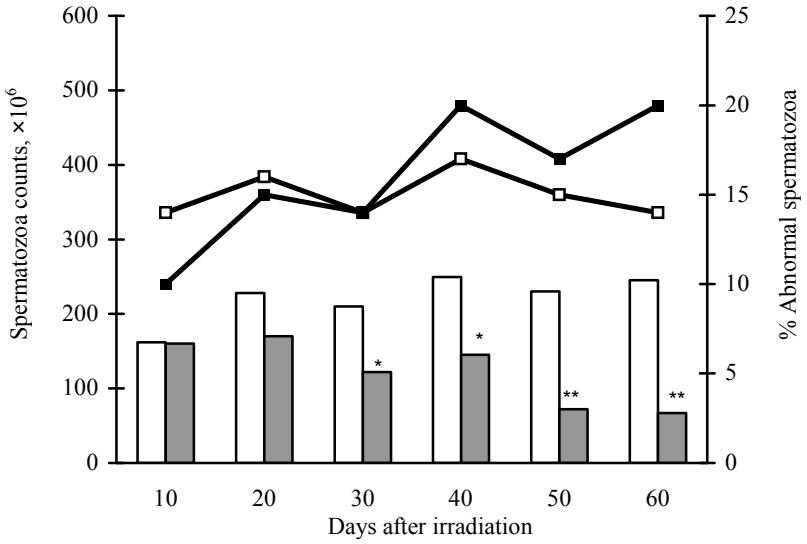


**Fig. 1.** Changes in spermatozoa counts in male rabbits from the control group (white bars) and rabbits, irradiated at 0.5 Gy (grey bars) and abnormal spermatozoa percentages in control (–□–) and irradiated at 0.5 Gy rabbits (–■–); significant differences – *$p<0.05$, **$p<0.001$ vs control (non–irradiated) group.

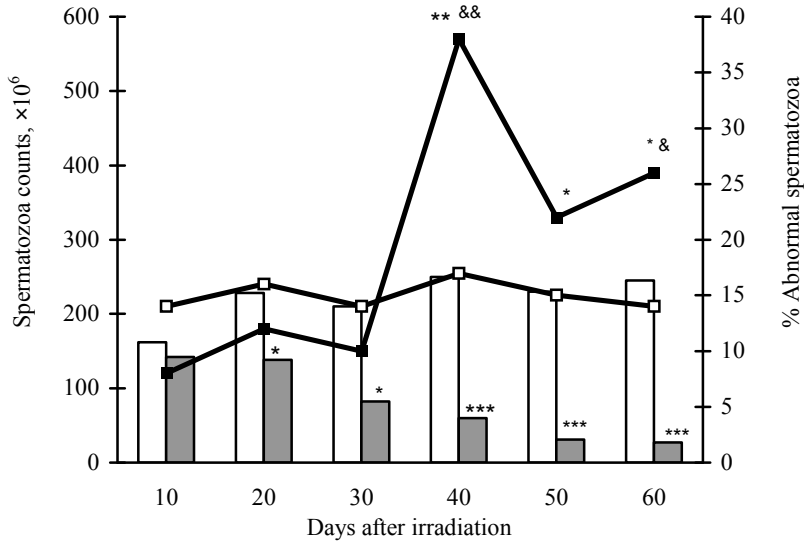


**Fig. 2.** Changes in spermatozoa counts in male rabbits from the control group (white bars) and rabbits, irradiated at 1.5 Gy (grey bars) and abnormal spermatozoa percentages in control (–□–) and irradiated at 1.5 Gy rabbits (–■–); significant differences – *p<0.05, **p<0.01; ***p<0.001 vs control (non–irradiated) group; $^{\&}$ p<0.05, $^{\&\&}$ p<0.01 vs the preceding period of time.

the post irradiation day 20. The rate of spermatozoa concentration diminution was considerably higher and by post irradiation day 60, sperm counts reached 27±5 $\times 10^6$/mL

In the 2.5 Gy group (Fig. 3), sperm counts by day 20 was significantly lower vs controls (91±6 $\times 10^6$/mL; p<0.01) as well as vs day 10. Despite the slower rate of decrease in sperm counts by days 20–40, there was a collapse by days 50 and 60. The counts by day 60 were 11±0.8 $\times 10^6$/mL – a value that was significantly lower than the respective values of controls (p<0.001) and the 0.5 Gy group (p< 0.01).

The analysis of data about the teratogenic effect of radiation showed that during the first 30 post irradiation days no significant increase in the percentage of abnormal spermatozoa occurred and it was between 8–18%.

After the irradiation at 0.5 Gy (Fig. 1), the teratogenic effect was the lowest (no significant differences vs controls) despite the trend towards increased abnormal spermatozoa percentages after the 30$^{th}$ day.

The irradiation at 1.5 Gy (Fig. 2) resulted in statistically significant teratogenic effect by 38% on post irradation day 40 vs both controls and the preceeding term (p<0.01).

The most obvious teratogenic effect was observed after the irradiation at 2.5 Gy (Fig. 3). The percentage of abnormal spermatozoa by post irradiation day 40 (52%) demonstrated that this term was critical with regard to the morphological and functional status of sperm. This percentage was statistically significantly higher vs both controls (p<0.001) and the preceeding time interval (p<0.01). Regardless of the relative decrease in ab-

normal spermatozoa in subsequent terms (days 50 and 60), their percentage at the end of the experiment was still considerably higher vs controls ($p<0.01$) and vs day 50 ($p<0.05$).

The parameters characterizing the DLM are presented in Table 1.

A prominent finding was that when the dose of 2.5 Gy was used for irradiation of male rabbits, the length of post irradiation intervals had a particular influence on percentages of pregnant females. They varied from 66.6% to 70 % in animals fertilized 10, 20 and 30 days after irradiation but at day 40, pregnant females were 41.6% whereas in group IX (control) – 83%. The same phenomenon was observed in the total and average number of live newbrorns. The reduction of the progeny was especially indicative, being 20% and 46% following fertilization at post irradiation days 30 and 40, respectively.

The decrease in the average number of live newborn rabbits is used as an indice for induced DLM (Searle and Beechey, 1974; Haines *et al.*, 2002). The greatest reduction in the percentage of offsprings in our studies occurred between 30$^{th}$ and the 40$^{th}$ post irradiation days and was observed by other authors. This corresponded to the observations of Sankaranarayanan (1991) and Russel *et al.* (1998) in mice and rats and the phenomenon was attributed to spermatid irradiation. Furthermore, the lowest percentage of pregnant female was observed within this time interval, most probaly due to the worsened quality of semen and the lower fertilization capability of spermatozoa. The num-

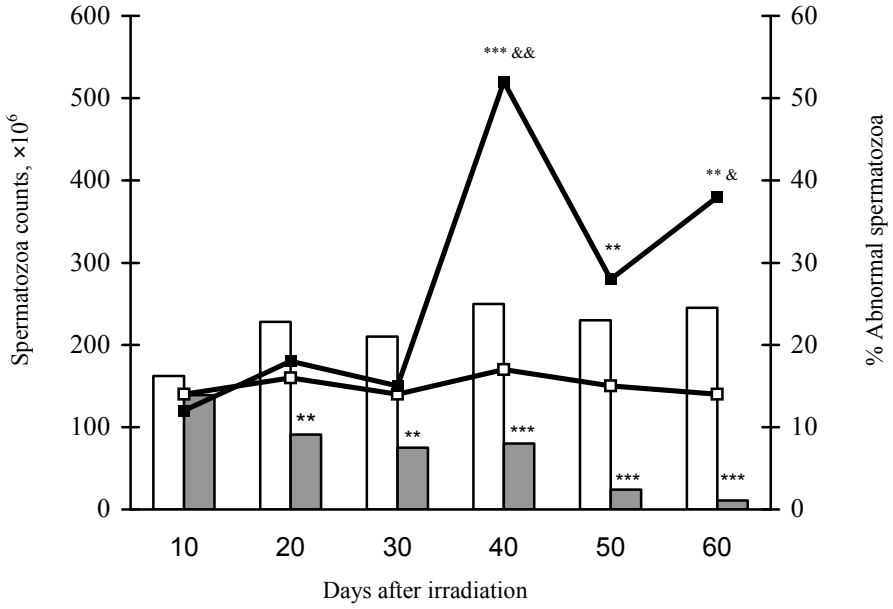


**Fig. 3.** Changes in spermatozoa counts in male rabbits from the control group (white bars) and rabbits, irradiated at 2.5 Gy (grey bars) and abnormal spermatozoa percentages in control (–□–) and irradiated at 2.5 Gy rabbits (–■–); significant differences – **p<0.01, ***p<0.001 vs control (non–irradiated) group; $^{\&}$ p<0.05, $^{\&\&}$ p<0.01 vs the preceding period of time.

**Table 1**. Induced DLM following mating of non-irradiated females with males, irradiated at 2.5 Gy

| Parameters | Groups | | | | |
|---|---|---|---|---|---|
| | V | VI | VII | VIII | IX (control) |
| Number of inseminated females | 12 | 12 | 12 | 12 | 12 |
| Number of pregnant females | 8 | 9 | 8 | 5 | 10 |
| Percentage of pregnant females | 66.6 | 75 | 66.6 | 41.6 | 83 |
| Total of live newborns | 56 | 63 | 48 | 24 | 75 |
| Average number of live newborns per female | 7.0 | 7.0 | 6.0 | 4.8 | 7.5 |
| Percentage of reduction of the progeny (DLM) | 6.7 | 6.7 | 20.0 | 46 | |

Legend: Group V: fertilized by males at post irradiation day 10; Group VI: fertilized by males at post irradiation day 20; Group VII: fertilized by males at post irradiation day 30; Group VIII: fertilized by males at post irradiation day 40; Group IX: fertilized by non-irradiated males.

ber of live newborn per one female in the previous terms was not significantly different from that in controls.

It has to be stated that the data calculated according to the used equation are an indirect indication at the appearance of a genetical damage such as DLM because of the impossibility to make a differentiation between radiation-induced preimplantation loss and loss of non-fertilized ova.

The analysis of semen of irradaited male rabbits showed that within the dose range 0.5–2.5 Gy, a dose-dependent impairment of spermatogenesis occurred, manifested by increased abnormal spermatozoa percentages from one hand (Lion, 1981) and decreased sperm counts from the other (Hasegawa *et al.*, 1998). The dynamics of those processes were also significantly influenced by the cellular stage at the moment of radiation challenge (Lion, 1981).

One of the most sensitive detectable indices for the mutagenic potential of ionized radiation is the change in spermatozoa's shape. The induced morphological abnormalities were probably due either to damage of coding genes or to their expression. According to Martin and Rademarker (1988), mutagens are causing DLM of genes that determine sperm morphology rather than chromosomic abberations.

Our data about the genetic sensitivity to radiation (DLM induction) of differentiating germinative cells correlate with the data for semen evaluation and correspond to those observed by Russell *et al.* (1998); Lion (1981) etc.

Undoubtedly, spermatids are the most sensitive stage of spermatogenesis, when genetic defects resulting in DLM appearance are induced. Moreover, spermatogonial cells, being precursors of differentia-

ting and mature germ-cells are sensitive to apoptosis as well as to mutations induction (Russell and Rinchik, 1993).

## CONCLUSION

Our data evidenced that the entire mechanism of spermatogenesis was impaired following irradiation in the used dose range. The lesions were manifested in changes in both quantitative and qualitative parameters of semen and DLM induction and resulted in decreased reproductive potential of irradiated individuals (lower fertility, and lower number and vitality of offsprings) and in potential lesions that could affect the next generations (late genetic consequences).

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