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Original Contribution

EFFECT OF EXTERNAL GAMMA IRRADIATION ON RABBIT SPERMATOGENESIS

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ABSTRACT

Adult male rabbits were subjected to whole-body external gamma irradiation at 0.5 Gy, 1.5 Gy and 2.5 Gy in order to evaluate its effect on spermatogenesis. The quantitative and qualitative parameters (concentration of spermatozoa and percentage of pathological forms) of semen were determined between post irradiation days 10 and 60. The highest degree of spermatogenesis impairment was observed in rabbits irradiated at 2.5 Gy. Following increase of the challenge dose, a reduction in spermatozoa concentration in semen and increased percentage of pathological spermatozoa were observed. The cytogenetic analysis of irradiated spermatogonia showed a dose-dependent increase in the frequency of radiation-induced reciprocal translocations, manifested primarily as ring and chain configurations.

Key words: gamma irradiation, radiation mutagenesis, reproduction, rabbits

INTRODUCTION

The monitoring and analysis of genotoxic effects, caused by multiple geno- and cytotoxic environmental factors, including ionised radiation, require a proper choice of biological systems that would equally create the potential for extrapolations of data to other species

Mice and rats are the most frequently used species for toxicological studies because they are relatively cheap experimental subjects. In addition, there is a lot of information about their normal development, growth and reproduction. Finally, there is opportunity to use experimental data obtained from them to determine human studies (1, 2, 3).

Aside rats and mice, the peculiarities of development, growth and reproduction are also well studied in rabbits, being both laboratory animals (4) and a principal food source in some countries.

Rabbit semen could be obtained, evaluated and tested for fertilizing qualities of spermatozoa under controlled conditions without artificial insemination (5) that allows a direct comparison with human sperm analysis, a principal component of reproducible and toxicological studies in humans (6).

Ionised radiations, depending on irradiation parameters, influence to an extent the volume, spermatozoa concentration in semen, their motility, morphology, as well as the nuclear chromatin structure of germ cells (7, 8, 9). While radiation-induced death of radiosensitive populations of stem cells results in temporary or permanent sterility, DNA changes, when impossible to be repaired, could be converted in reciprocal translocations, sister chromatid exchange, dominant lethal mutations and abnormalities in spermatozoa that could have a negative impact in next generations (10, 11, 12). These situations thus pose a great danger to reproductive biology.

Therefore, the aim of the present study was to evaluate the quality of semen in male rabbits exposed to various doses of gamma radiation.

MATERIAL AND METHODS

Adult White New Zealand male rabbits, aged 4.5 months, were used in the experiment. All animals had body weight of 4.0 - 4.5 kg and were placed under identical conditions of feeding and housing (individual cages) prior to and during the study.

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The experimental groups (with 6 animals in each) were formed as follows:

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

The rabbits were subjected to whole-body irradiation using a ^{60}Co gamma equipment (*Rokus*) at dose density of 24 cGy/min. The exposure dose was calculated according to the geometrical parameters of the source, its power and the source-object distance (13).

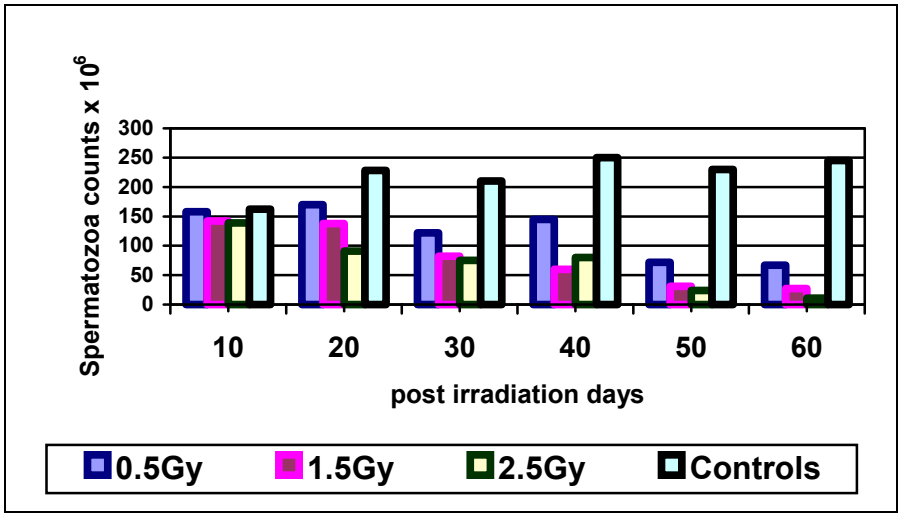
After the procedure, a 10-day period of adaptation of irradiated individuals was allowed.

As controlled parameters, the spermatozoa counts in semen and the percentage of pathological forms were monitored prior to and at 10-day intervals after the irradiation, up to the 60th day. The semen was obtained by the artificial vagina method of Morrel (14) at the respective time intervals. The percentage of pathological forms in 500 cells per rabbit at least was determined after straining with eosin-nigrosin.

Three months after the irradiation, cytogenic analysis was made using spermatocytes in diakinesis Metaphase I. The aim was to determine the frequency of reciprocal translocations that have been induced in the spermatogonia. The method used was according to that of Evans *et al.* (15) but modified for rabbits by the Laboratory of Radiation Genetics at the National Centre of Radiobiology and Radioprotection. The resulting method was a combination of several procedures of protocols described by Evans, et al., and Schleiermacher (16), and the modification for rabbits was only for the initial preparation, i.e. the hypotonic processing of specimens and its duration.

RESULTS AND DISCUSSION

The results of determination of spermatozoa concentration in semen of irradiated rabbits showed a dose-dependent pattern. In all three irradiated groups, spermatozoa counts decreased progressively up to post irradiation day 60 (**Figure 1**).



Treated (*a-p<0.05, b- p<0.01, c- p<0.001) vs the control group.*

***Figure 1**. Changes in the spermatozoa counts in the semen of rabbits, irradiated within the dose range 0.5-2.5Gy*

In the 0.5 Gy group, this parameter ranged from 158±20 – 67±18 x 10^6, within the interval 10th–60th day post irradiation respectively, the differences being significant after the 30th day ($p<0.05$). The most marked reduction in spermatozoa counts at this dose occurred between the 40th and the 50th day, the spermatozoa counts by day 50 being twice lower than that at the 40th day.

The exposure to higher doses, 1.5 Gy and 2.5 Gy resulted in more reduction of spermatozoa counts with time. As early as the 20th day, the counts were significantly lower compared to controls (138±16 x 10^6 – 91±6 x 10^6). Between post irradiation days 40 and 50 the reduction in the 1.5 Gy group was by 50% whereas in the 2.5 Gy group, by 70%. The lowest values of this parameter were determined by day 60 – 27±5 x 10^6 and 11±0.8 x 10^6, for 1.5 Gy and 2.5 Gy groups, respectively.

The analysis of data on the teratogenic effect of acute irradiation showed elevated percentages of pathological spermatozoa in the semen of irradiated rabbits (**Figure 2, 3, 4**).

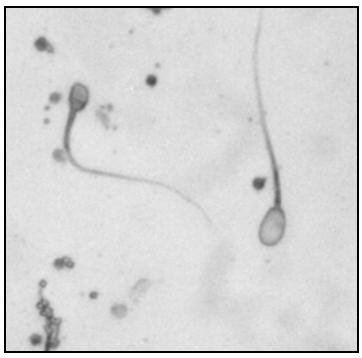


Figure 2. *A primary abnormality – a microhead defect*

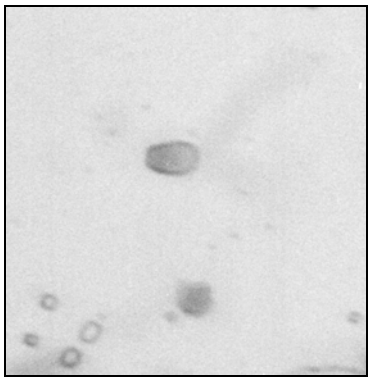


Figure 3*. Secondary abnormality – a detached head defect*

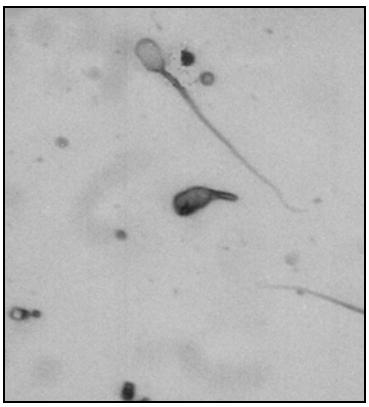


Figure 4. *Primary abnormality – asymmetrically short body, secondary abnormality – detached tail defect.*

The percentage of pathological forms in semen of exposed rabbits correlated positively with increase in applied dose.

While in the 0.5 Gy group, there was no increase in abnormal sperm counts over the physiological range during the entire period of the study, the exposure to 1.5 Gy resulted in significant increase in the percentage of pathological spermatozoa after the 40th day. By post irradiation day 50, the pathological percentage decreased to 22% and thereafter augmented to 26% by day 60.

The changes in this index of semen quality in the 2.5 Gy group followed the tendencies observed in rabbits exposed to 1.5 Gy, but more enhanced. The peak of abnormal sperm cells was again by day 40 – 52%. By day 50, pathological spermatozoa decreased to 28%, with a subsequent elevation to 38% by post irradiation day 60.

The results of the cytogenetic analysis confirmed the data on the negative effect of radiation within the 0.5-2.5 Gy range on spermatogenesis in irradiated rabbits.

In studied metaphase plates, a dose-dependent increase in the incidence of reciprocal translocations induced in spermatogonia and detected at the spermatocyte stage in diakinesis Metaphase I was exhibited.

In rabbits exposed to 0.5 Gy, only RIV configurations were observed.

The irradiation at higher doses: 1.5 and 2.5 Gy resulted in frequency of cells with translocations of 2,33±0,44 and 3,5±0.87, respectively. Both ring and chain quadrivalents were observed. In one of the rabbits irradiated at 2.5 Gy, metaphases involving both RIV and CIV configurations were present (**Figure 5**).

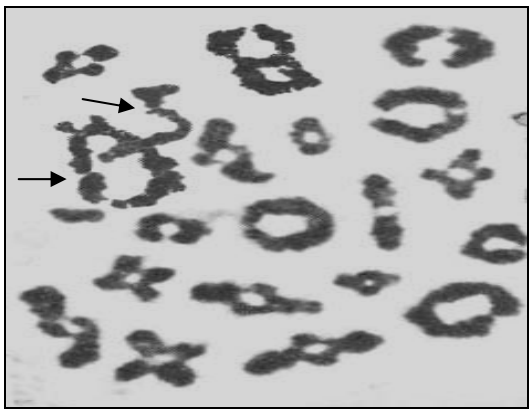


Figure 5. *Metaphase plate with RIV and CIV quadrivalents*

The data of semen analysis of male rabbits, irradiated with the dose range 0,5 Gy – 2,5 Gy showed a dose-dependent aggravation of spermatogenesis defects, manifested by elevated pathological spermatozoa percentages in the ejaculate on one side (17) and reduced spermatozoa counts on the other (18). These processes are, to a significant extent, influenced by the cell stage at the time of radiation exposure.

One the most sensitive visible indicators of the mutagenic effect of ionised radiation is the change in the shape of spermatozoa. The induced morphological alterations were probably due to either damage of responsible genes or their expression. According to Martin et al (19) mutagens cause rather dominant lethal

mutations in genes that determine spermatozoa morphology than chromosome aberrations.

Our results on the genetic radiosensitivity (induction of reciprocal translocations) in spermatogonia, correlate with the results of semen quality evaluation. Literature data (20,21) showed that some translocations provoke a complete or partial discontinuation of spermatogenesis both prior to and after meiosis, and other changes could not be detected at all. This should be considered in the final interpretation of radiation-induced translocations.

CONCLUSION:

Our data showed a dose-dependent alteration in semen quantity and quality. The cytogenetic analysis confirmed the observed relationship in the parameters of irradiated rabbits. The most visible negative effect accompanied by increased incidence of reciprocal translocations, induced in spermatogonia, occurred after exposure to the highest dose – 2.5 Gy, suggesting aggravation of negative changes with increase of the challenge dose.

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