THE ASSESSMENT OF DNA DAMAGE IN POULTRY SPERMATOZOA AFTER EXPOSURE TO LOW DOSES OF IONISING RADIATION

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INTRODUCTION

Male exposure to ionising radiation can cause dramatic effects such as decrease in fertility, lower sperm production, increased frequency of germ line mutations that can all predispose future generations to dominant lethal mutations or to cancer [1,2,3]. In this kind of exposure, sperm DNA integrity represents an important parameter in the evaluation of semen functionality, particularly regarding dose-related induction of DNA strand breaks in spermatozoa following *in vitro* exposure to ionising radiation in humans and mouse samples [4]. Maintaining of normal sperm becomes even more important when it is known that DNA in semen samples is already fragmented in certain amount in human and turkey semen [5]. It is also worth mentioning that sperm lacks the DNA repair mechanisms, and that makes sperm DNA damage irreversible [6,7], showing the importance of studies on sperm DNA damage.

One of the methods for detecting primary DNA damage at the individual cell level is comet assay with different variants (alkaline, neutral, enzyme modified) [8]. The technique is well established in DNA damage evaluation for mammalian semen samples [9] but less for poultry semen [6,7,10]. Since there are differences in DNA sensitivity between different

poultry species, there are different protocols used, but the approach for avian samples is similar considering the use of proteinase K and dithiotreitol (DTT, for cross-linked proteins-protamines removal).

The aim of this paper was to provide an insight in the amount of DNA damage detected in chicken spermatozoa after radiation with small doses of ionising radiation and to address the question of the potential ecological consequences of the damage.

MATERIALS AND METHODS

For this study 5 cocks of heavy line (45 weeks old) were used. The cocks were trained to give regular semen samples for 30 days before experiment. Ejaculates from each male were collected by dorso-abdominal massage method. Immediately after collection, samples were diluted 1:1 with modified Ringer solution, divided into 5 plastic tubes and irradiated with doses of 0.3, 0.5, 1 and 2 Gy gamma radiation from panoramic ^{60}Co source (activity about 3 PBq) at Ruđer Bošković Institute, Zagreb [11].

The dose rate was about 0.0117 Gy/s. For measuring DNA damage we used method described by Gliozzi *et al.* [7] and Sakkas *et al.* [10] with slight modifications. All samples were made in duplicate and on each slide we measured 50 randomly captured comets avoiding the edges of the slide. Images were captured with black and white camera and software from Perceptive Instrument Comet Assay IV. Scored parameters included tail intensity or tail DNA% (TI), tail length (μm; TL) and tail moment (TM; (tail mean × head mean) × (TI/100)).

One-way ANOVA was used to compare differences in DNA damage among different doses. Data are expressed as mean ± S.E. (standard error). For normalisation of results distribution, all the values were logarithmed with the function $\log_{10}(v1+1)$.

RESULTS AND DISCUSSION

It has been known that the sensitivity of comet assay can be seen after exposure of the cells to 0.25 Gy doses, but the significant differences in DNA damage values can be seen only after 0.5 Gy [12]. Our results have shown that comet assay can detect significant DNA damage even after exposure to 0.3 Gy doses and that this modified protocol is equally sensitive like the other techniques used to measure sperm DNA damage [12]. On the other side, after irradiation with 0.5, 1 and 2 Gy doses, no dose-related responses were detected in any of the observed parameters (Table 1, Figure 1). We have also shown dose related increase in the number of

detected apoptosis. Since there are no articles for comparison of the results for TI, TL and TM, we can just confirm that our TI and TM values were lower than the values for fresh chicken semen samples described by Gliozzi *et al.* [7]. Although it is possible that adaptive response is involved in DNA damage response after exposure to doses higher than 0.3 Gy, since it is known that poultry semen does not have DNA repair mechanisms, we must be careful in conclusions. Namely, when we compare the distribution of DNA damaged cells (Figure 2), although the mean values for DNA damage are lower for doses higher than 0.3 Gy, the proportion of damaged DNA is more widely spread after exposure to 0.5, 1 and 2 Gy doses. That actually means that higher number of cells is more damaged, but that the damage does not have high values in the individuals cells like in the lower doses and that can contribute to explanation of adaptive response.

Table 1. Effects of ionising radiation on comet parameters of DNA damaged spermatozoa, values are represented as mean ± standard error.

	Tail length (μm)	**Tail moment**
Control samples	23.11 ± 0.35$^{\#}$	1.27 ± 0.06$^{\#}$
0.3 Gy	27.87 ± 0.36*^{z}	2.50 ± 0.11*
0.5 Gy	22.09 ± 0.35$^{\#}$	1.43 ± 0.07$^{\#}$
1 Gy	20.97 ± 0.29*$^{\#}$	1.15 ± 0.08$^{\# z}$
2 Gy	26.85 ± 0.35*^{z}	1.76 ± 0.12$^{\#}$

*significant difference P<0.05 from the control samples; $^{\#}$significant difference P<0.05 from 0.3 Gy,^{z} significant difference P<0.05 from 0.5 Gy

CONCLUSION

In conclusion, we could only speculate that exposure to low doses and higher doses of ionising radiation can have different effects considering the amount of DNA damage and a number of damaged cells and different mechanisms involved in creation of the damage (such as adaptive response) Since there are no articles on this topic, further larger studies are needed to answer that question.

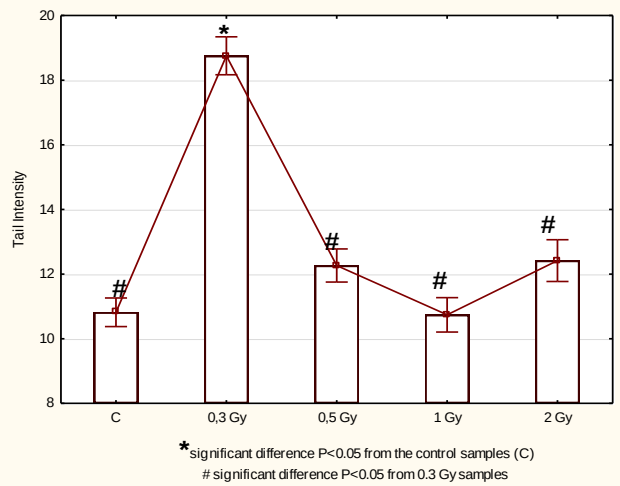
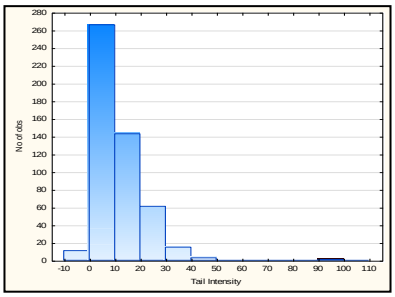
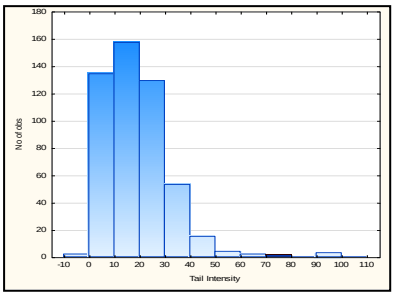


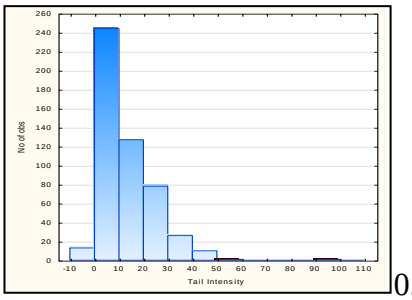
Figure 1. Values (Mean ± S.E.) for tail intensity in chicken sperm samples before radiation (C), and after radiation with 0.3, 0.5, 1 and 2 Gy doses



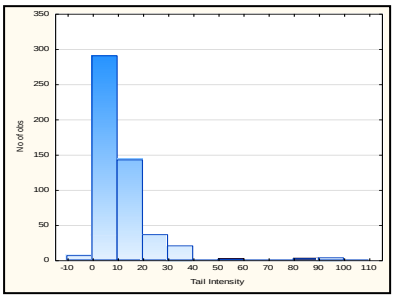
C



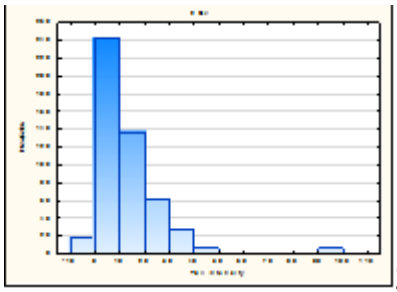
0.3 Gy



0.5 Gy



1 Gy



2 Gy

Figure 2. Distribution of damaged DNA considering tail intensity in chicken sperm samples before radiation (C), and after radiation with 0.3, 0.5, 1 and 2 Gy doses

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The existence of dose-related induction of DNA strand breaks in spermatozoa following *in vitro* exposure to ionising radiation represents sperm DNA integrity as an important parameter in the evaluation of semen functionality. Maintaining of normal sperm becomes even more important when it is known that DNA in semen samples is already fragmentated in certain amount in human and turkey semen and that it lacks DNA repair mechanisms making DNA damage irreversible. The aim of this paper was to provide an insight in the amount of DNA damage detected in chicken spermatozoa (5 cocks, 45 weeks old) of heavy line after radiation with doses of 0.3, 0.5, 1 and 2 Gy gamma radiation and to address the question of the potential ecological consequences of the damage that was measured with comet assay. Scored parameters included tail intensity, tail length and tail moment. Results showed sensitivity of comet assay technique that detected significant DNA damage even after exposure to 0.3 Gy, but also showed no dose-related responses after 0.5, 1 and 2 Gy. Distribution of damaged cells was widely spread for the higher doses, showing the influence of possible adaptive response, but for further conclusions, larger studies are needed to answer that question.