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The Radioprotective and Radiomitigative Effects of Resveratrol Supplementation at Different Time After the Start of Irradiation on the Sperm Count and Quality of Male Mice

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Abstract

Background: Agents with free radical-scavenging features may act as radiation modifiers, protectors, or mitigators. Methods: We investigated if supplementation of resveratrol (RSV) in mice, since different time after the start of irradiation may influence the sperm count and quality during the irradiation and recovery. Results: Irradiation significantly reduced the sperm count. RSV supplemented with 1 Gy since 24 h increased sperm count. Combination of low doses increased, whereas of high doses reduced DNA damage. Coadministration of two high doses since 8th day significantly increased of DNA damage and slightly sperm count. The supplementation of RSV during recovery was toxic to irradiated males. The sperm parameters were a little better in the absence of RSV. The level of DNA damage of germ cells was significantly lower in groups combined with 1 Gy. Conclusions: Resveratrol counteracted the killing of gem cells by ionizing radiation and is very useful to improve the sperm count. RSV may work both as radioprotector and radiomitigator of lethal effects in male gametes. Combination of high doses of irradiation with RSV since 24 h mitigated of DNA damage. Contrarily, the supplementation during the recovery is not recommended since it may works toxic during long-lasting irradiation.

Keywords: irradiation; resveratrol; sperm count; sperm quality; radiomitigation and radioprotection; recovery; DNA damage

1. Introduction

Human activities involving the use of radiation and radioactive substances, which cause man-made radiation exposure in addition to natural exposure from cosmic rays and naturally occurring radioactive substances. The use of radioactive materials in industry, agriculture and research is expending, and people may be subjected to adverse effects by mishandled radiation source [1]. Moreover, there is known that 60 % of cancer patients received radiotherapy, so interesting and useful seems to be to identify possibly protectors of normal tissues inevitably or accidentally exposed to ionizing radiation during such procedure [2].

Spermatogenesis is particularly important process, which takes place within seminiferous tubules of the testes. This process leads to development of the male gametes from spermatogonial stem cells through spermatocytes and spermatids to spermatozoa, ready to fertilize eggs. Previous studies showed that genetic damage after radiation or chemical exposure might be transmitted to the offspring leading to the male-mediated developmental toxicity [3]. Both male and female contribute half of the genetic information of the genome of developing offspring, so the transmission of congenital malformations via the sperm is incredibly significant [4].

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Ionizing radiation is well-known mutagenic and carcinogenic agent. Radionuclide present in soil, water and air, and cosmic radiation are classified as natural sources. However, as the greatest source of human exposure is considered radiodiagnosis and radiotherapy, used for health care. The worldwide average background exposure for a human is estimated about 2.4 mSv per year, whereas the average from medical diagnosis ranges from 0.043 to 2 mSv per year [1,5]. Ionizing radiation has an adverse effect on male gametes. The most radiosensitive organ in the body are testes together with germ cells. According to the International Commission on Radiological Protection [6] report the weighting factor for the gonads is 0.08. In workers occupationally exposed to ionising radiation several authors observed such changes like diminished quantity, motility, increased sperm abnormalities, sperm fragmentation and global hypermethylation in human [7–10].

Resveratrol (trans-3,4,5-trihydroxystilbene, RSV) is a natural, non-flavonoid polyphenol from a group of stilbenes, the compounds, which characterize significant biological activities. It is structurally similar to diethylstilbestrol and estradiol, due to this is called phytoestrogen. RSV is present in fruits such as grapes, peanuts, strawberry, blueberry, cranberry, mulberry, lingberry, sparkleberry, bilberry, and in wine, especially in red [11,12]. Its dietary intake is estimated to be about 100 μg daily [13]. RSV is also produced by several plants in the response of injury, stress, bacteria and fungi infections, UV radiation and exposure to ozone [14,15]. RSV shows many pro-health properties, such as antioxidant, anticarcinogenic, anti-inflammatory, neuroprotective, antidiabetic, analgesic, antiviral, cardioprotective action [16–22]. RSV is widely present in Mediterranean diet, which is known for beneficial properties for health, mainly for prevention of cardiovascular diseases despite to high fat diet [23,24].

RSV is also considered as a modulator or protector of ionizing radiation induced damage [15–28]. Contrary. there are also reports showed that RSV enhances the radiosensitivity of cancer cells [29–31].

The aim of the study was investigation if supplementation of RSV since different time after the start of irradiation may influence the sperm count and quality during the irradiation and during recovery.

2. Materials and Methods

2.1. Animals and Exposure

Seven-week-old male Swiss outbreed laboratory mice obtained from the "Kołacz" Animal Breeding Laboratory (Warsaw, Poland), were housed in standard rodent cages in a room with controlled temperature, humidity, and light cycles (12 h dark, 12 h light). Tap water and rodent diet were available *ad libitum*. The mice were randomly assigned to either control or exposed groups, one week after acclimatization. Eight-week old male mice were exposed to RSV dissolved in a small amount of ethanol and diluted in drinking water to obtain the desired dose (7 mg/kg body weight (bw) or 28 mg/kg bw, daily), irradiated with X-rays (0.5 Gy or 1 Gy daily), or exposed to a combination of both agents (0.5 Gy + 7 mg/kg bw RSV, 0.5 Gy + 28 mg/kg bw RSV, 1 Gy + 7 mg/kg bw RSV, 1 Gy + 28 mg/kg bw RSV daily). Animals were irradiated 5 times per week (working days), whereas RSV was continuously supplied in drinking water starting from 24 hours or 1 week following the initiation of daily irradiation. Body weight of the animals was checked weekly, and the volumes of control water and RSV-water solution were checked daily. The solution of RSV in water was prepared twice a week. A therapeutic Roentgen unit Medicor type THX-250 (Hungary) was used for irradiation, and operated with the following parameters: 155 kV, 18 mA, added filtration 0.25 mm Cu and HVL 2 mm Al. Mice were subjected to whole-body irradiation at the dose rate of 0.20 Gy/min. Control animals were sham irradiated and unexposed to RSV, or were exposed only to RSV. The groups of animals were sacrificed 24 hours after the last irradiation. Other groups of animals were observed during recovery process for additional 2-weeks. During the recovery there were two different groups of mice, with RSV (recovery in the presence of RSV - RPR) or without RSV (recovery in the absence of RSV - RAR).

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Eight to ten mice were used for each of the doses and time periods.

The study was conducted in accordance with the relevant institutional and national guidelines and standards and approved by the IV Local Ethical Commission for Animal Experiments.

2.2. Sperm Count and Quality.

Both testes and epididymides were removed and weighed from each male. One epididymidis was macerated in 0.2 ml of 1 % solution of trisodium citrate for 5-8 min and minced. Then the solution was made up to 2 ml and mixed for about 1 min. The sperm suspension was diluted 1:1 in 10 % buffered formalin. The spermatozoa were counted using an improved Neubauer haemocytometer [32–34].

The content of the second epididymides were placed into 0.2 ml of warm (37°C) physiological saline. An aliquot was placed on warm (37°C) microscope slide and covered with a cover slip. 200 cells per animal were evaluated for motility within 5 min after killing the animal [34].

The remaining sperm was distributed evenly in the saline. The study of frequency of morphologically abnormal spermatozoa was performed according to the procedure described by Wyrobek and Bruce [35]. Smears were prepared on microscope slides, air-dried overnight and stained with eosin Y. Then one thousand spermatozoa per mouse were analyzed using a light microscope, and abnormal spermatozoa morphology (e.g. lacking hook, amorphous, banana-shaped head, two tails) were recorded.

2.3. Comet Assay

For the Comet assay the method of tissue preparation described previously was used [36]. One testis from each animal was decapsulated and placed in the RMPI 1640 medium and minced with scissors. Before using the cells, tubes were swirled so that single cells remained in the suspension. 5 µl of cell suspension were mixed in an eppendorf tube with 75 µl low melting point agarose (LMA) for embedding on slides. The slides were immersed in alkaline lysis (2.5 m sodium chloride (NaCl), 100 mM ethylene diamine tetra-acetic acid, sodium salt Na_2 (EDTA), 10 mM Tris, 1% sodium sarcosinate, pH 10) overnight at 4°C. Then they were drained and placed in gel electrophoresis tank, and left in the solution for 20 min. The electrophoresis was conducted at 4°C for 20 min using 19V and 300 mA. After neutralization slides were stained with EtBr and examined using a fluorescence microscope (Nikon, Japan). Images of one hundred randomly selected cells from each animal were recorded and analyzed using CASP image-analysis software [37]. The Comet tail moment and % DNA in the Comet tail was chosen to determine induction of DNA breaks.

2.4. Statistical Analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) with post hoc Fisher's test. The significance level was established at $P<0.05$.

3. Results

The results of irradiation mice supplemented with RSV since 24 h after the first exposure to X-rays are shown in Table 1. The body weights of males significantly decreased in all groups except of 28 mg/kg bw of RSV and 0.5 Gy compared to control. In groups 0.5 Gy + 7 mg/kg bw RSV and 0.5 Gy + 28 mg/kg bw of RSV the body weight was also reduced as compared to 0.5 Gy alone. The mean testes weight significantly decreased in all irradiated and combined groups as compared to control and additionally in groups 1 Gy + 7 mg/kg bw RSV and 1 Gy + 28 mg/kg bw of RSV also as compared to 1 Gy. The mean epididymis weight was reduced in irradiated groups and in combined groups exceptof 0.5 Gy + 7 mg/kg bw of RSV and 0.5 Gy + 28 mg/kg bw of RSV compared to control and was increased in both mentioned groups as compared to 0.5 Gy alone. The mean sperm count was significantly reduced after irradiation with 0.5 Gy or 1 Gy daily as compared to control and increased in combined with 1 Gy groups as compared to irradiation alone. The sperm motility was decreased

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in all groups except of 7 mg/kg bw of RSV. The percentage of abnormal spermatozoa and the percentage of DNA in the Comet Tail were significantly increased in the group of 0.5 Gy + 7 mg/kg bw RSV. DNA damage in this group was significantly higher as compared to 0.05 Gy alone, and lower in 1 Gy + 28 mg/kg bw group as compared to 1 Gy.

The results after supplementation of RSV since 8th day after the start of irradiation are presented in Table 2. The mean body weight was significantly decreased after irradiation with 1 Gy and in combined groups, except of 0.5 Gy + 7 mg/kg bw RSV. The mean testis weight was reduced in all groups except of 7 mg/kg bw and 28 mg/kg bw RSV groups. The mean epididymes weight significantly decreased in both irradiated groups and in the group of 0.5 Gy + 28 mg/kg bw RSV compared to control and increased in the group of 1 Gy + 7 mg/kg bw RSV compared to 1 Gy alone. The sperm count significantly reduced after irradiation to 0.5 Gy or to 1 Gy daily and in combined with 1 Gy groups. The motilities of spermatozoa were diminished in all experimental groups, except of 28 mg/kg bw RSV. There were no effects on the sperm abnormality nor on the DNA damage of male gametes.

The effects of 2-weeks recovery after supplementation of RSV since 24 h the start of irradiation are shown in Table 3. In groups of 1 Gy + 7 mg/kg bw RSV and 1 Gy + 28 mg/kg bw RSV almost all (7 of 9 and 6 of 8) animals have died during the recovery period, whereas in the group of 1 Gy alone, only 1 male of 9 has died. The mean

Table 1. The effects of resveratrol supplementation since 24 h after the start of irradiation on the sperm count and quality.

Daily dose	Mean body weight (g) ± SD	Mean testes weight (mg) ± SD	Mean epididymis weight (g) ± SD	Sperm count x10⁶ /ml±SD	Percent of motile spermatozoa ±SD	Percent of abnormal spermatozoa ±SD	Comet tail moment ±SD	Percent of DNA in Comet Tail ±SD
Control	37.79±2.93	258.90±33.58	244.00±37.32	6.23±0.99	35.93±17.80	17.36±4.49	3.60±3.41	8.17±5.20
0.5 Gy	34.83±4.17	192.70±31.87*	159.80±34.34*	4.05±1.33*	14.95±7.35*	19.63±6.23	3.87±2.75	8.06±4.34
1 Gy	30.59±5.17*	208.00±46.10*	178.30±54.28*	2.88±1.47*	12.08±7.21*	22.08±6.88	4.01±2.42	8.47±3.94
7 mg/kg RSV	32.96±4.36*	235.33±48.29	198.33±51.10	5.42±4.40	24.82±17.92	19.51±6,96	4.97±2.95	10.41±4.70
28 mg/kg RSV	34.03±2.41	215.13±59.86	217.50±22.97	4.71±1.61	16.36±7.18*	22.54±8.06	5.79±2.66	11.82±4.02
0.5 Gy+7 mg/kg RSV	28.65±2.54*[a]	172.39±28.15*	231.88±75.90[a]	3.98±1.79	9.94±16.26*	23.69±7.99*	7.58±5.10[a]	12.32±4.27*[a]
0.5 Gy+28 mg/kg RSV	29.76±3.73*[a]	173.75±25.09*	227.00±60.74[a]	4.47±1.75	16.44±13.43*	19.69±5.80	3.33±2.85	6.03±2.75
1 Gy+7mg/kg RSV	29.01±3.73*	170.57±13.54*[b]	188.57±36.13*	4.66±2.08[b]	19.21±11.18*	19.27±3.64	2.07±1.37	5.07±1.50
1 Gy+28mg/kg RSV	27.14±3.66*	162.57±20.31*[b]	191.57±64.21*	4.62±2.15[b]	13.71±7.71*	19.59±7.30	1.80±1.59[b]	4.36±2.81[b]

* p<0.05 compared to control; [a] p< 0.05 compared to 0.5 Gy alone; [b] p< 0.05 compared to 1 Gy alone by post hoc Fisher's test.

Table 2. The effects of resveratrol supplementation since 8th day after the start of irradiation on the sperm count and quality.

Daily dose	Mean body weight (g) ± SD	Mean testes weight (mg) ± SD	Mean epididymis weight (g) ± SD	Sperm count x10⁶/ml ±SD	Percent of motile spermatozoa ±SD	Percent of abnormal spermatozoa ±SD	Comet tail moment ±SD	Percent of DNA in Comet Tail ±SD
Control	37.79±2.93	260.50±31.52	248.33±36.82	6.22±1.00	35.93±17.80	20.18±3.29	3.89±2.53	8.27±3.66

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0.5 Gy	34.83±4.17	192.70±31.87*	159.80±34.34*	4.05±1.33*	14.95±7.35*	22.40±6.11	3.87±2.75	8.06±4.34
1 Gy	30.59±5.17*	204.10±38.47*	183.40±63.66*	3.01±1.68*	12.08±7.21*	21.93±6.66	4.01±2.42	8.47±3.94
7 mg/kg RSV	32.90±3.80	218.60±30.12	226.40±71.90	5.98±2.05	9.30±7.45*	17.38±4.03	5.71±3.00	11.26±4.33
28 mg/kg RSV	34.86±2.36	245.80±34.12	197.20±38.28	5.83±1.56	8.30±7.80	19.64±5.31	5.72±1.30	11.16±1.36
0.5 Gy + 7 mg/kg RSV	33.22±2.32	213.00±42.22*	253.33±41.01	4.49±2.07	10.59±7.85*	20.80±8.90	3.85±2.08	7.96±3.70
0.5 Gy + 28 mg/kg RSV	33.20±2.80*	218.33±13.22*	191.33±30.53*	6.25±1.91	16.94±11.32	16.18±4.25	3.91±1.68	7.91±2.94
1 Gy + 7 mg/kg LYC	27.23±2.62*	183.86±27.69*	255.57±44.38[b]	3.63±1.64*	8.72±5.94*	18.08±6.58	4.25±1.66	8.40±2.44
1 Gy + 28 mg/kg LYC	28.60±3.04*	186.29±49.88*	227.71±44.66	3.96±2.30*	12.71±12.55*	23.60±7.45	6.03±4.78[b\]	11.26±7.31

[*] p<0.05 compared to control; [a] p< 0.05 compared to 0.5 Gy alone; [b] p< 0.05 compared to 1 Gy alone by post hoc Fisher's test.

Table 3. The effects of resveratrol supplementation since 24 h after the start of irradiation on the sperm count and quality – effects after reconvalescence.

Daily dose	Mean body weight (g) ± SD	Mean testes weight (mg) ± SD	Mean epididymis weight (g) ± SD	Sperm count x10^6 /ml±SD	Percent of motile spermatozoa ±SD	Percent of abnormal spermatozoa ±SD	Comet tail moment ±SD	Percent of DNA in Comet Tail ±SD
Control	36.72±3.94	246.06±91.44	145.60±91.66	6.39±2.31	18.09±16.73	21.98±4.66	2.60±0.99	6.24±1.91
0.5 Gy	39.06±4.17	158.00±83.02*	223.11±64.49	3.09±2.07*	20.17±15.61	25.66±14.15	2.26±0.60	5.73±1.44
1 Gy	33.97±7.55	216.00±128.92	245.70±107.4*	3.14±2.04*	34.94±17.83*	26.44±11.40	2.32±0.66	5.96±1.41
7 mg/kg RSV	39.24±3.47	236.38±36.98	212.44±123.45	6.04±2.10	20.13±17.52	20.21±5,65	2.85±1.93	6.59±3.65
28 mg/kg RSV	36.47±2.41	221.89±574.29	192.10±34.38	6.14±2.18	16.46±7.56	19.20±2.70	3.32±2.06	7.05±4.11
0.5 Gy+7 mg/kg RSV RPR	32.50±3.30*[a]	128.50±40.37*[a]	147.00±38.68	2.45±1.19*	8.34±8.34[a]	34.91±19.94*	2.38±1.18	5.74±2.18
0.5 Gy+28 mg/kg RSV RPR	33.77±3.70*[a]	91.80±37.97*[a]	213.20±57.36	2.58±1.12*	14.23±12.60	36.35±15.32[a]	2.70±1.76	5.65±2.74
0.5 Gy+7 mg/kg RSV RAR	27.49±6.57*[a]	160.96±33.56*	184.00±42.61	3.60±1.43	9.71±10.94	29.98±10.85	2.58±0.83	4.36±1.99
0.5 Gy+28 mg/kg RSV RAR	29.49±2.86*[a]	179.27±18.41*	238.36±46.91*	3.34±2.72	17.86±11.47	26.22±16.55	2.83±1.75	5.21±1.18
1 Gy+7 mg/kg RSV RAR	28.60±6.47*[b]	176.87±21.55*	177.00±66.73	2.42±0.93*	15.75±9.29	40.20±35.20*	1.86±1.60	4.03±3.18
1 Gy+28 mg/kg RSV RAR	26.40±4.06*	170.75±16.48*	224.33±49.35	2.83±0.48*	14.58±11.29	46.70±15.14*[b]	3.54±0.85	6.77±1.38

[*] p<0.05 compared to control; [a] p< 0.05 compared to 0.5 Gy alone; [b] p< 0.05 compared to 1 Gy alone by post hoc Fisher's test.

animal weights were significantly lower compared to control in all combined groups. Additionally, results of all combined groups except of 1 Gy + 28 mg/kg RSV (RAR) were significantly different compared to appropriate dose of irradiation alone. The mean testes weights were significantly decreased in 0.5 Gy and in combined groups as compared to control, and in combined with 0.5 Gy RPR groups also as compared to 0.5 Gy alone. Epididymis weight was significantly higher in 1 Gy and in 0.5 Gy + 28 mg/kg bw RSV (RAR) groups. Sperm count was significantly reduced in irradiated, and in combined with 1 Gy RAR groups and in combined with 0.5 Gy RPR groups. Percent of motile spermatozoa was significantly different compared to control in 1 Gy group.

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The percent of abnormal spermatozoa was increased in 0.5 Gy + 7 mg/kg bw RSV (RPR), and in both combined with 1 Gy (RAR) groups compared to control and in 0.5 Gy + 28 mg/kg bw RSV RPR group compared to 0.5 Gy alone, and in 1 Gy + 28 mg/kg bw RSV RAR as compared to 1 Gy alone. There were not significant changes in the level of DNA damage.

The effects of 2-weeks recovery after supplementation of RSV since 8th day after the start of irradiation are shown in Table 4. Almost all (8 of 10) males from 1 Gy + 7 mg/kg bw RSV RPR have died during the recovery, whereas among males exposed to 1 Gy only 2 of 10 have died. The mean body weight was significantly different in all combined RPR and RAR groups. Mean testes weight was significantly decreased in irradiated and in all combined RPR and in 0.5 Gy + 7 mg/kg bw RSV (RAR) groups compared to control and additionally all RPR groups and 0.5 Gy + 7 mg/kg bw RSV (RAR) group were significantly different compared to irradiation alone. The epididymes weights significantly increased in all combined RAR groups except of 0.5 Gy + 7 mg/kg RSV. This parameter was also significantly lower in the group of 1 Gy + 28 mg/kg bw RSV RPR group as compared to 1 Gy alone. Sperm count significantly decreased after exposure to 0.5 Gy and in all combined groups. Additionally results of 0.5 Gy + 28 mg/kg bw RSV RAR, 1 Gy + 28 mg/kg bw RSV (RPR), 1 Gy + 7 mg/kg bw RSV (RPR) and in 1 Gy + 28 mg/kg bw RSV (RPR) were significantly different as compared to irradiation alone. Percent of motile spermatozoa was not significantly different from control, except of 1 Gy + 7 mg/kg bw RSV (RAR). Additionally, result of 1 Gy + 28 mg/kg bw RAR groups was significantly different from 1 Gy. Percent of abnormal spermatozoa was significantly higher in 1 Gy and all combined groups compared to control. Additionally, the result of 1 Gy + 28 mg/kg bw RSV (RPR) was significantly higher compared to 1 Gy alone. The level of DNA damage of germ cells was significantly lower in groups combined with 1 Gy (RAR).

Table 4. The effects of resveratrol supplementation since 8th day after the start of irradiation on the sperm count and quality – after reconvalescence.

Daily dose	Mean body weight (g) ± SD	Mean testes weight (mg) ± SD	Mean epididymis weight (g) ± SD	Sperm count x10⁶/ml ±SD	Percent of motile spermatozoa ±SD	Percent of abnormal spermatozoa ±SD	Comet tail moment ±SD	Percent of DNA in Comet Tail ±SD
Control	37.14±2.72	263.42±60.42	139.60±35.08	6.19±1.09	16.72±9.80	18.56±4.26	3.34±1.05	7.48±1.61
0.5 Gy	38.72±4.32	176.00±90.95*	189.50-±41.44	3.35±2.49*	24.70±11.62	29.13±9.13	2.11±2.75	4.64±1.87
1 Gy	33.07±4.10	186.50±115.18*	185.57±96.55	4.97±2.65	9.48±5.22	33.88±23.57*	2.92±2.16	6.60±3.26
7 mg/kg RSV	35.14±2.62	240.63±85.54	120.71±49.42	5.36±2.38	9.46±8.49	16.63±3.05	4.60±2.09	9.77±3.92
28 mg/kg RSV	35.36±2.72	234.71±107.54	120.29±58.22	5.96±2.50	13.55±8.19	18.89±5.06	3.64±1.53	7.61±2.05
0.5 Gy + 7 mg/kg RSV RPR	32.21±4.89*	87.67±11.81*,a	155.67±58.07	3.67±1.77*	18.11±10.77	34.52±13.15*	3.28±1.63	5.94±3.10
0.5 Gy + 28 mg/kg RSV RPR	33.24±2.80*	99.75±27.26*,a	187.17-±58.07	2.81±0.95*,a	19.32±8.85	35.57±8.72*	3.14±2.30	6.75±4.21
1 Gy + 28 mg/kg RSV RPR	32.33±3.26*	82.75±15.78*,b	100.50±57.24b	1.67±1.24*,b	10.81±3.9	46.95±16.55*,b	3.50±2.23	8.81±6.38
0.5 Gy + 7 mg/kg RSV RAR	31.60±2.26*,a	79.33±12.50*,a	165.33±68.41	2.60±2.66*	13.75±13.28	37.82±16.41*	1.76±1.78	7.21±3.21
0.5 Gy + 28 mg/kg RSV RAR	33.20±3.80*,a	218.57±19.71	229.14±52.55*	2.34±1.33*	15.07±11.26	37.3±12.23*	2.71±3.29	4.37±2.88
1 Gy + 7 mg/kg RSV RAR	28.87±2.12*	212.40±15.57	237.2±39.19*	2.45±1.57*,b	8.57±7.87*	40.9±6.21*	2.74±0.83	2.17±0.39*
1 Gy + 28 mg/kg RSV RAR	28.20±3.46*	203.0±23.07	257.33±38.73*	1.77±1.55*,b	24.13±16.40b	32.04±13.87*	3.02±0.96	2.27±1.84*

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* p<0.05 compared to control; [a] p< 0.05 compared to 0.5 Gy alone; [b] p< 0.05 compared to 1 Gy alone by post hoc Fisher's test.

4. Discussion

Approximately, 15 % of couples after 12 months of regular unprotected cohabitation cannot get pregnant and for at least 50% of infertility cases the responsibility of the male factor is considered [38]. The World Health Organization (WHO) characterized the male factor infertility as alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyses between 1 and 4 weeks apart [39]. The most important seems to be oligozoospermia i.e. low sperm count and quality, which is responsible for 90 % of male infertility [40,41].

Male infertility is caused mainly by oxidative stress. The fertilization capacities of spermatozoa relate to the presence of controlled level of free radicals, however the excess of oxidants or reactive oxygen species in the comparison of not delivered antioxidant defense mechanism may be harmful for germ cells [42]. Cell membrane of spermatozoa is rich in polyunsaturated fatty acids, which consist of unconjugated double bonds containing electrons, which may be donated to reactive oxygen species (ROS), leading to the generation of lipid peroxides. As a result, the germ cell membrane is disrupted and in the consequence the sperm viability and motility is decreased [43–45]. The decreased sperm viability take place by the modification of membrane proteins and an abnormal acrosome reaction that resulted in a reduction of the ability spermatozoa to fertilize oocytes. The reason of diminished sperm motility is a reduction in axonemal protein phosphorylation. ROS may also induce DNA damage in spermatozoa by direct attack on the bases or the phosphodiester backbones. In turn, DNA damage may lead to incorrect fertilisation, reduced implantation, and poor embryo development, and in consequence to induction of increased mortality and risk of developing cancer. The next consequence may be apoptosis of highly damaged gametes leading to reduced sperm count [43–48]. Sperm morphology is a result of complex cellular modifications occurring during spermatogenesis [49]. Sperm abnormalities induced by high ROS level, may lead to teratozoospermia and decreased fertility or even to temporally or permanent infertility [50,51].

Agents which show free radical-scavenging features may act as radiation modifiers or protectors, if added before or shortly after irradiation, or mitigators, which work if administered after radiation [52].

Plants are naturally protected against radiation. For example, phytophenols present in many plants are structurally adapted in order to be activated by electron donating substituents, which inhibit energy transfer mechanism, supressing oxidative stress and stabilising redox processing cells [53] .

RSV activates antioxidant enzymes, i.e. catalase and superoxide dismutase, which are involved in lipid damage of spermatozoa [54]. This compound is able to activate anti- and proapoptotic mediators and this way protect cells from DNA damage and apoptosis [18]. Moreover, it induces cell cycle arrest, apoptosis, differentiation and inhibits cancer cell proliferation [55].

RSV is well tolerated, harmless and not affects the reproductive ability of both sexes and is also non-toxic in embryo of rodents [56]. It is relatively safe natural medication, rapidly absorbed, metabolised, transported and distributed, however long-term administration is unknown [57].

Due to its abilities, resveratrol is considered to be promising compound for the treatment of male infertility. RSV, however, structurally like oestrogens, does not show estrogenic properties and does not affect reproductive organs [58,59].

Increase in the relative weights of the testes and epididymides and normal features of testis, as well as enhanced epididymal sperm motility and testicular sperm count were observed in ICR mice given 50 mg/kg of resveratrol daily for 28 days [60]. Contrary, other studies show that RSV may cause reduction in testicular weights impairs seminiferous tubules morphology and spermatogenesis [61–63]. Other authors showed that RSV increased the relative testes weight, improved apoptotic index and testosterone levels in mice [64]. In the current study the mean body, testes and epididymis

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weights as well as sperm count, and quality were not significantly different in RSV exposed groups compared to control.

There is postulated that RSV modulates the oestrogen-response system, acting as a regulator of male reproductive function [59]. Moreover, it has capacity to pass through the blood–testis barrier, imparting its protective effects in the testis [65]. RSV directly stimulates the hypothalamic–pituitary–gonadal axis, with no adverse effects on testes and promotes spermatogenesis by ameliorating the effect induced by 2,5-hexanedione [59].

In human, RSV caused improvement of sperm concentration and count (Illiano et al. 2020), and decreases of DNA damage in fertile and infertile patients [66–69]. RSV also enhances sperm production and motility in laboratory animals, as well as enhances blood testosterone levels, testicular sperm count and sperm motility in rabbits [60]. Lower doses of RSV act against lipid peroxidation, preserving sperm chromatin and plasma membranes [59]. It is a potent inhibitor of the oxidation of polyunsaturated fatty acids found in lipoprotein [70]. Therefore, RSV could be active by decreasing the level of ROS and proinflammatory factors in seminiferous tubules, thus increasing sperm and androgen production [59]. As earlier papers showed, RSV at low doses improves cell survival, but at high doses is cytotoxic [71,72]. This may be useful in cancer therapy. Moreover, RSV is able to preserve sperm chromatin texture, but not acrosome [68]. Low concentration has a positive effect on the sperm motility, but higher concentrations showed opposite results [71,73–76].

According to our knowledge there are no published reports regarding the effects of RSV on the irradiated male germ cells. The current study reported effects on the germ cells in irradiated male mice co-administered with RSV since different time after the start of irradiation and compared the effects during convalescence in presence or absence of RSV.

The 2 weeks irradiation significantly diminished the sperm count and quality similarly to earlier results [77–80]. The current study showed that irradiation leads to a death of approximately 35 to 54 % of germ cells and confirmed that ionizing radiation diminish sperm count and quality. The reduced testes and epididymis weights correlated with the diminished sperm count. The reduced sperm count after irradiation is mainly attributed to apoptosis of germ cells with genomic abnormalities initiated likely by Trp53 protein [7].

In the mice the spermatogenesis cycle continues 8 weeks, so during 2 weeks of experiment all stages of male germ cells present in the animal body were irradiated simultaneously. After 2 weeks of exposure the effects of irradiation on the spermatozoa and late spermatids were shown, whereas after additional 2 weeks of convalescence on the early spermatids and late spermatocytes.

As earlier papers showed, the male reproductive organs and cells are susceptible to ionizing radiation. Highly sensitive are spermatogonial stem cells and differentiating spermatogonia. Spermatocytes are less sensitive, while spermatids are rather radioresistant [81–83]. The current study did not confirm the above finding, since spermatids, spermatocytes and spermatozoa showed high sensitivity to irradiation.

Our study showed that co-administration of RSV since 24 h after the start of 1 Gy irradiation daily significantly increased the sperm count and additionally combination with high dose of RSV decreased the level of DNA damage. Contrary, combination of 7 mg/kg bw RSV with 0.5 Gy of irradiation leads to increased DNA damage and percent of abnormal spermatozoa.

In the case of coadministration of 1 Gy + 28 mg/kg of RSV since 8th day after the first irradiation, the significant increase of DNA damage and slight increase of sperm count was noted. In turn, the sperm count was markedly improved after combined exposure to 0.5 Gy of irradiation and RSV. This correlated with increase of the testes weight. The best result was seen after exposure to 0.5 Gy + 28 mg/kg bw RSV, where the level of sperm count reached control value. The above results confirmed the earlier results of RSV alone, i.e. increased the level of sperm count and concentration [59,71,72,84]. Reduced DNA damage may reflect stimulation of DNA repair-DNA damage-control biosystem by low dose radiation [85]. RSV reduces germ cells apoptosis in rodents and protects the male reproductive tract [86,87]. According to results of other study, RSV enhance sperm production in rats by stimulating the hypothalamic-pituitary-gonadal axis without inducting adverse effects [59].

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Similarly to our results in the study of Juan et al. [59], sperm count was significantly (about 1.7 times) higher in the resveratrol treated male rats compared to control group, however the sperm quality did not differ. The increase in the sperm production observed following RSV administration may be also caused by an overall increase in the size of spermatogenic tissue (decrease in the mean diameter of the seminiferous tubules with increase in the testicular tubules density) observed when RSV co-administered with isoflurane [88]. The current results for germ cells confirmed our earlier results for somatic cells, where RSV reduced the frequency of micronuclei induced by irradiation in reticulocytes [27].

Results of convalescence showed that longer exposure to RSV (4 weeks) is harmful to male mice irradiated before. In the experiment regarding to mice supplemented with RSV since 24 h after the first irradiation almost all males have died when RSV was supplemented for additional 2 weeks, whereas after irradiation to 1 Gy alone only 1 has died. Surpassingly, the germ cells parameters were better after the recovery of absence of RSV. In the case of supplementation of RSV since 8th day after the first irradiation (3 weeks of RSV exposure) majority of males exposed to 1 Gy + 7 mg/kg bw RSV have died. This may confirm earlier results regarding to toxic effects of RSV after longer administration i.e. after higher doses [71,72].

Results after convalescence i.e. when cells were exposed as late spermatids and early spermatocytes, showed that RSV at each dose given within 2 weeks after the end of irradiation decreased the sperm count and increased the percent of abnormal spermatozoa compared to the results of 0.5 Gy alone. The response was slightly improved in the absence of RSV. In groups of 1 Gy irradiation in the absence of RSV the sperm count was slightly lower, and the percent of abnormal spermatozoa was significantly higher compared to results of 1 Gy alone. The germ cells parameters during the recovery in the presence of RSV were a little better, especially after irradiation with 0.5 Gy.

Results of convalescence showed that mice exhausted by 2 weeks irradiation respond poorly to further supplementation of RSV and the effect of such administration is adverse and may reflect the toxic long-lasting effect of RSV, which was so far not exactly recognized.

In the case of convalescence if supplementation of RSV was started since 8th day after the first irradiation, the presence of resveratrol, especially after combination of higher doses, increased the percent of abnormal spermatozoa. Additionally decreased the epididymis weight and the sperm count if RSV was combined with 1 Gy of irradiation and after 0.5 Gy + 7 mg/kg bw RSV. In the absence of RSV, the sperm count was reduced compared to results of irradiation alone and the percent of abnormal spermatozoa was markedly increased except of combination 1 Gy + 8 mg/kg bw RSV, where the percent of abnormal spermatozoa was similar. The only advantage is that combination of 1 Gy with each dose of RSV reduces the level of DNA damage. The present study regarding to male germ cells confirmed the earlier results for somatic cells where supplementation of RSV after the termination of irradiation delayed the recovery [27].

Results obtained are very important and promising in the point of view of public and reproductive health. They are consistent with the “One Health” concept.

5. Conclusions

Based on the results, we concluded that resveratrol is very useful compound to improve the sperm count diminished by irradiation. RSV counteracted the killing of gem cells by ionizing radiation both after the start of RSV administration since 24 h as well as since 8th day following the irradiation. In the case of combination of high doses of irradiation with RSV since 24 h the mitigation of DNA damage was also observed. RSV may work both as radioprotector and radiomitigator of lethal effects in male gametes. Contrarily, the supplementation during the recovery after irradiation is not recommended since it may works toxic during long-lasting exposure, worsening the condition of the body and gametes.

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