

Damage of the Mouse Testis by Tritiated Water and ^{137}Cs - γ -Rays

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ABSTRACT

Tritiated water at 23.2, 46.3 or 92.5 MBq/animal and ^{137}Cs - γ -rays at 9.5 Gy (equivalent 370 MBq) or lower doses were administered to 6-week old male C3H/HeNCrj and C57BL/6NCrj mice, as well as F₁ Crj: B6C3F1 (C3H \times C57BL) progeny. Each set of six to ten animals were autopsied 30 days after the first irradiation. Testis weights were decreased dose dependently, relative values being highest in the C3H and lowest in the C57BL case, with B6C3F1 intermediate. Vacuolization in seminiferous tubules appeared in the 23.2 MBq group and increased with the dose. Focal pyknosis and karyomegaly were found at 46.3 MBq, while primary and secondary spermatocytes and spermatids disappeared with 92.5 MBq. Only a few spermatogonia and Sertoli cells remained after exposure to 9.5 Gy ^{137}Cs - γ -rays. Sizes of seminiferous tubules were decreased dose dependently, with no strain differences.

When male B6C3F1 mice were irradiated with Cs- γ -rays at 0.119 (equivalent 4.63 MBq tritiated water) or 2.38 Gy (equivalent 92.5 MBq tritiated water), body weights and size of the seminiferous tubules were decreased at both doses, and the larger dose also caused reduction of testis weight and abnormal sperm. However, all changes except for the alteration in weights had disappeared 1 month after the final irradiation. It is considered that the size of seminiferous tubules may be a good parameter for radiation damage in the testis.

Key words: Tritiated water, Cs- γ -rays, Mouse, Testis

Research on nuclear fusion as a new energy source is being actively pursued in technically developed countries, with tritium used as the fuel. Once released into the atmosphere, as has occurred by accident, tritium is rapidly oxidized to tritiated water (HTO) and becomes widely dispersed throughout the environment. The population at large is at risk through inhalation and skin absorption or ingestion of contaminated food or drinking water. Therefore, knowledge of the biological impact of HTO is important and many studies on the somatic, cytogenetic, genetic and carcinogenic effects of HTO in mammals have been conducted^{1,2,4-6,9,14,18,22-26}. Testes are sensitive organs. In the present investigation, safe doses, especially with regard to changes in the testes, were examined in three different strains of mice exposed to HTO and a simulator of tritium irradiation, Cs- γ -rays.

MATERIALS AND METHODS

Animals

Six week-old C3H/HeNCrj and C57BL/6NCrj males and F₁ Crj:B6C3F1 (C3H \times C57BL) progeny

were purchased from Charles River, Japan. Inc., Hino, Japan and housed eight to a polycarbonate cage under constant conditions of temperature ($24 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 10\%$), with a 12 hr light/12 hr dark cycle. The animals were maintained under the guidelines set forth in the Guidelines for the Care and Use of Laboratory Animals established by Hiroshima University. Each set of six to ten animals were used in the present experiment.

Animals treated with HTO were housed in the ^3H -radioactivity facility and those exposed to Cs- γ -rays in a Cs radiation room.

^3H -tritiated water (HTO)

HTO with an activity of 1.75×10^{14} Bq/dm³ purchased from Amersham International Plc, UK, was diluted with saline to various concentrations, and injected i.p. at doses of 23.2, 46.3 or 92.5 MBq/animal.

^{137}Cs - γ -rays

^{137}Cs - γ -rays, generated in our Institute¹⁹) as a simulator of tritium irradiation, were adminis-

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tered by three different radiation sources, 1.11 TBq, 111 GBq and 11.1 GBq, to a mobile animal car on rails, controlled by computer. The dose rate of the ^{137}Cs - γ -rays could be continuously altered by changing the level, speed and distance of the mobile car from 0.7 to 4.5 m from the source. ^{137}Cs - γ -rays were applied at 0.119 (equivalent 4.6 MBq, total dose for 30 days), 2.38 (equivalent 92.5 MBq) and 9.5 Gy/animal (equivalent 370 MBq) to give the same dose rates as for HTO.

Pathological findings

Animals were sacrificed after cumulative irradiation for 30 days. In the case of HTO, the animals were sacrificed in the tritium facility. The main organs were weighed and fixed in 10% neutral formalin. The specimens were routinely processed for embedding in paraffin, sectioned at 3 μm , deparaffinized and stained with hematoxylin and eosin. The smallest diameters of round seminiferous tubules, excluding elongated tubules, were measured under a microscope. After administration of ^{137}Cs - γ -rays, animals were sacrificed, body, testis and epididymides were weighed and testes were fixed in FSA solution (37% formalin 5 ml, 5% sucrose solution 15 ml and acetic acid 0.8 ml) for 5 days, then embedded, sectioned and stained routinely. The sizes of seminiferous tubules were measured. Epididymides irradiated with 0.119 and 2.38 Gy ^{137}Cs - γ -rays were added to 9 volumes of culture medium (MEM) minced, smeared, fixed and stained with Giemsa solution. Then abnormal sperm were counted as described previously^{17,21}.

Briefly the testes were minced in saline and the mince was filtered. Sperm were stained with Giemsa solution and the number of normal sperm and abnormal sperm with one head and two tails were counted.

Statistical analysis

Statistical significance was determined with the Dunnett method for multiple comparisons, the X^2 and the Student's t tests.

RESULTS

Comparison between tritiated water and ^{137}Cs - γ -rays

Tritiated water was injected at 92.5 MBq/animal and ^{137}Cs - γ -rays were irradiated at 2.38 Gy (equivalence 92.5 MBq). All animals were autopsied 30 days after the first irradiation. Body and liver weights in the tritiated water group were significantly decreased as compared to the ^{137}Cs - γ -rays group, and testis weights were greater (Table 1). Relative liver weights in the tritiated water group were decreased as compared to the ^{137}Cs - γ -rays group but testis weights were increased (Table 2). The sizes of the seminiferous tubules in the tritiated water groups were similar in the two groups (Table 3).

Strain differences

Body and organ weights are summarized in Table 1. The values for 0 to 92.5 MBq animals were not compared with those for the 9.5 Gy

Table 1. Body and organ weights

	Number of animals	BW (g)	Liver (g)	Kidney (g)	Spleen (g)	Prostate (g)	Testis (g)	Preputial gland (g)
C57BL								
Non-irradiation	10	21.7 \pm 1.6	1.196 \pm 0.119	0.294 \pm 0.047	0.065 \pm 0.006	0.251 \pm 0.059	0.165 \pm 0.012	0.078 \pm 0.017
23.1 MBq	10	20.5 \pm 1.1**	0.969 \pm 0.131**	0.275 \pm 0.022*	0.062 \pm 0.007	0.251 \pm 0.045**	0.135 \pm 0.010**	0.065 \pm 0.029
46.3 MBq	10	18.6 \pm 1.3**	0.889 \pm 0.073**	0.266 \pm 0.014*	0.072 \pm 0.006	0.158 \pm 0.027**	0.116 \pm 0.011**	0.044 \pm 0.006**
92.5 MBq	10	20.1 \pm 0.7**	0.881 \pm 0.082**	0.271 \pm 0.013*	0.062 \pm 0.004	0.236 \pm 0.031**	0.092 \pm 0.017**	0.057 \pm 0.011*
9.5 Gy	10	22.4 \pm 0.8	1.106 \pm 0.072	0.280 \pm 0.016	0.059 \pm 0.007	0.213 \pm 0.052**	0.049 \pm 0.041**	0.088 \pm 0.017
B6C3F1								
Non-irradiation	10	27.5 \pm 0.4	1.536 \pm 0.084	0.455 \pm 0.024	0.106 \pm 0.023	0.377 \pm 0.088	0.211 \pm 0.018	0.141 \pm 0.043
23.1 MBq	10	24.7 \pm 1.1**	1.010 \pm 0.110**	0.406 \pm 0.023	0.059 \pm 0.006**	0.345 \pm 0.035	0.187 \pm 0.027	0.116 \pm 0.039
46.3 MBq	10	25.4 \pm 1.6**	1.145 \pm 0.185**	0.422 \pm 0.035	0.083 \pm 0.032**	0.313 \pm 0.041	0.179 \pm 0.013	0.127 \pm 0.034
92.5 MBq	10	24.0 \pm 1.5**	1.072 \pm 0.157**	0.404 \pm 0.035	0.065 \pm 0.014**	0.323 \pm 0.065	0.103 \pm 0.017**	0.112 \pm 0.022
2.38 Gy	10	25.8 \pm 1.7 ^a	1.286 \pm 0.142 ^b	0.401 \pm 0.032	0.087 \pm 0.012 ^b		0.093 \pm 0.005	0.122 \pm 0.021
9.5 Gy	10	25.4 \pm 1.4**	1.198 \pm 0.110**	0.415 \pm 0.032	0.065 \pm 0.013**	0.280 \pm 0.081*	0.064 \pm 0.004**	0.111 \pm 0.022*
C3H								
Non-irradiation	10	27.3 \pm 0.8	1.452 \pm 0.039	0.454 \pm 0.041	0.112 \pm 0.010	0.270 \pm 0.076	0.159 \pm 0.009	0.107 \pm 0.025
23.1 MBq	10	24.8 \pm 1.0**	1.117 \pm 0.072**	0.450 \pm 0.027**	0.080 \pm 0.012**	0.280 \pm 0.029**	0.143 \pm 0.017**	0.101 \pm 0.023
46.3 MBq	10	24.4 \pm 0.6**	1.054 \pm 0.059**	0.462 \pm 0.023*	0.082 \pm 0.007**	0.299 \pm 0.024**	0.138 \pm 0.015**	0.099 \pm 0.015
92.5 MBq	10	25.6 \pm 1.2**	1.207 \pm 0.080	0.501 \pm 0.037	0.086 \pm 0.011**	0.298 \pm 0.024**	0.109 \pm 0.011**	0.104 \pm 0.016
9.5 Gy	10	27.6 \pm 1.0	1.320 \pm 0.091	0.433 \pm 0.046**	0.094 \pm 0.007**	0.235 \pm 0.070**	0.053 \pm 0.005**	0.092 \pm 0.028*

(mean \pm SD)

*: Significantly different from Non-irradiation ($p < 0.05$); **: Significantly different from Non-irradiation ($p < 0.01$);

^a: Significantly different from 92.5 MBq in B6C3F1 ($p < 0.05$); ^b: Significantly different from 92.5 MBq in B6C3F1 ($p < 0.01$)

Table 2. Relative organ weights (Organ weight/Body weight × 1000)

	Liver/BW	Kidney/BW	Spleen/BW	Prostate /BW	Testis/BW	Preputial gland/BW
C57BL						
Non-irradiation	49.84 ± 5.50	14.51 ± 6.93	2.91 ± 0.17	13.95 ± 2.22	7.18 ± 0.40	3.73 ± 0.77
23.1 MBq	47.02 ± 4.05	13.37 ± 0.88	3.01 ± 0.27	12.16 ± 1.76	6.57 ± 0.45	3.14 ± 1.21
46.3 MBq	47.71 ± 1.97	14.29 ± 0.78	3.90 ± 0.52**	8.47 ± 1.13**	6.27 ± 0.75*	2.36 ± 0.29**
92.5 MBq	43.80 ± 3.28**	13.48 ± 0.49	3.09 ± 0.17	11.79 ± 1.82*	4.60 ± 0.85**	2.81 ± 0.50
9.5 Gy	49.45 ± 2.21	12.51 ± 0.64	2.62 ± 0.31	9.52 ± 2.17**	2.20 ± 0.22**	3.96 ± 0.77
B6C3F1						
Non-irradiation	55.90 ± 2.76	16.56 ± 0.76	3.85 ± 0.82	13.73 ± 3.14	7.67 ± 0.61	5.14 ± 1.55
23.1 MBq	41.00 ± 4.42**	16.47 ± 0.90	2.40 ± 0.24**	14.01 ± 1.42**	7.58 ± 1.15	4.70 ± 1.59
46.3 MBq	44.93 ± 4.68**	16.63 ± 0.51	3.23 ± 1.04**	12.37 ± 1.82	7.08 ± 0.62	4.99 ± 1.10
92.5 MBq	44.47 ± 4.33**	16.81 ± 0.62	2.68 ± 0.43**	13.38 ± 2.20	4.27 ± 0.66**	4.66 ± 0.82
2.38 Gy	49.70 ± 2.92 ^b	15.53 ± 0.87 ^b	3.36 ± 0.43		3.60 ± 0.25 ^b	4.32 ± 0.60
9.5 Gy	47.07 ± 3.25**	16.32 ± 0.99	2.53 ± 0.39**	11.10 ± 3.35*	2.54 ± 0.13**	4.34 ± 0.77
C3H						
Non-irradiation	53.29 ± 0.83	16.66 ± 1.55	4.12 ± 0.32	12.09 ± 3.58	5.83 ± 0.31	3.94 ± 0.87
23.1 MBq	45.05 ± 1.66	18.17 ± 0.85	3.21 ± 0.45**	11.28 ± 1.04*	5.75 ± 0.65	4.05 ± 0.85
46.3 MBq	43.13 ± 1.98**	18.92 ± 0.57	3.35 ± 0.24**	12.23 ± 0.86	5.65 ± 0.54	4.07 ± 0.59
92.5 MBq	47.11 ± 2.33	19.54 ± 0.87	3.35 ± 0.35**	11.61 ± 0.68*	4.24 ± 0.43**	4.06 ± 0.57
9.5 Gy	47.75 ± 2.02	15.67 ± 1.25**	3.40 ± 0.23**	8.52 ± 2.55**	1.94 ± 0.16**	3.34 ± 1.05*

(mean ± SD)

*: Significantly different from Non-irradiation(p<0.05); **: Significantly different from Non-irradiation (p<0.01)

^a: Significantly different from 92.5 MBq inB6C3F1 (p<0.01);

Table 3. Sizes of seminiferous tubules and percentage of spermless seminiferous tubules in irradiated mice

Strain	Sizes (μm)						Percentages of spermless	
	0	23.1 MBq	46.3 MBq	92.5 MBq	3.28 Gy	9.5 Gy (370 MBq)	0	9.5 Gy (370 MBq)
C3H	195.7 ± 19.7	160.6 ± 19.0**	151.8 ± 14.0** ^a	139.1 ± 18.5** ^{a,b}		114.6 ± 13.5** ^{a,b,c}	3.6 ± 3.1	38.5 ± 12.1**
B6C3F1	200.5 ± 24.3	163.2 ± 15.9**	155.4 ± 13.8** ^{a,d}	134.3 ± 17.8** ^{a,b}	127.4 ± 14.9	113.5 ± 14.7** ^{a,b,c}	3.6 ± 2.9	22.0 ± 4.6**
C57BL	200.3 ± 19.4	153.0 ± 17.6**	146.9 ± 15.3**	131.8 ± 13.4** ^{a,b}		114.7 ± 14.8** ^{a,b,c}	4.1 ± 2.3	70.5 ± 22.0**

(mean ± SD)

** : Significantly different from the 0 MBq value (p<0.01)

^a: Significantly different from the 23.1 MBq value (p<0.01) ^c: Significantly different from the 92.5 MBq value (p<0.01)

^b: Significantly different from the 46.3 MBq value (p<0.01) ^d: Significantly different from the 23.1 MBq value (p<0.05)

group, because of differences in conditions. Body weights were decreased by doses up to 92.5 MBq in all strains, generally along with liver and kidney weights in the C57BL and C3H cases. Spleen weights were reduced in the B6C3F1 and C3H and prostate weights in all but the B6C3F1 cases. Testis weights were significantly and dose-dependently decreased. The relative weights (organ weight/body weight × 1000) are shown in Table 2. Testis weights were significantly decreased and results for the other organs were in line with those for actual weights. The 0 MBq relative weight was set at 100% and applied to estimate change.

Histological Findings

Pathologically there were few changes except in the testes (Fig. 1–1), where seminiferous tubule vacuolization (Fig. 1–2) appeared in the 23.2 MBq group and increased with the dose (data was not shown). Focal pyknosis and karyomegaly were found, and primary and secondary spermatocytes and spermatids disappeared with 46.3 MBq to

92.5 MBq. Only a few spermatogonia and Sertoli cells remained in the 9.5 Gy groups (Fig. 1–3). Spermless seminiferous tubules were most frequent in the C57BL, next the C3H and then the B6C3F1 strain (Table 3). In all irradiated groups diffuse interstitial cell (Leydig cell) hyperplasia was observed. Sizes of seminiferous tubules are given in Table 5, a dose-dependent decrease being observed in all strains.

¹³⁷Cs-γ-rays irradiation

After 0.119 Gy irradiation, data for testes and epididymides were not significantly different from non-irradiation values in B6C3F1 mice. Body weights and sizes of seminiferous tubules were decreased. However, abnormal sperm were not increased, but rather demonstrated a significant reduction (Table 4). After 2.38Gy ¹³⁷Cs-γ-rays irradiation, body, testis and epididymides weights were significantly decreased, and this extended to relative weights of testes. The size of seminiferous tubules were decreased and abnormal sperm were

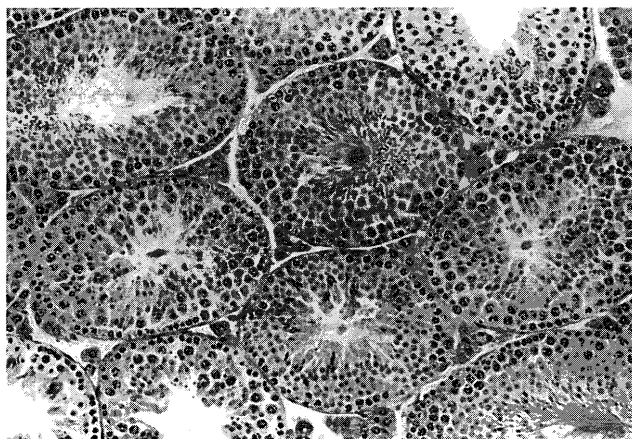


Fig. 1-1

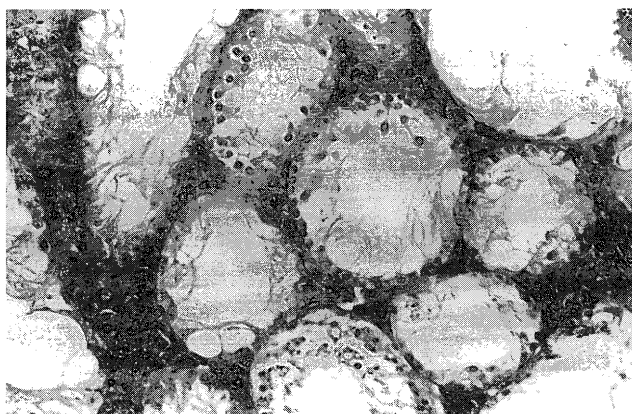


Fig. 1-2

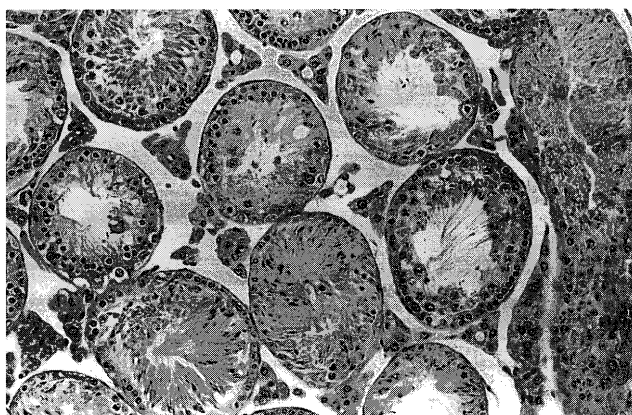


Fig. 1-3

Fig. 1-1. Normal testis, $\times 200$, HE staining

Fig. 1-2. Testis after 2.38 Gy ^{137}Cs - γ -rays irradiation. Note focal pyknosis and karyomegaly in primary and secondary spermatocytes and disappearance of spermatids, $\times 200$, HE staining

Fig. 1-3. Testis after 9.5 Gy ^{137}Cs - γ -rays irradiation. Note the few spermatocytes and Sertoli cells, $\times 200$, HE staining

increased. One month after the end of the irradiation period, body and testis weights were still significantly decreased but relative weights and other parameters were not significantly different from non-irradiated control values (Table 5).

Table 4. Body, testis, epididymis weights and abnormal sperm after 0.119 Gy ^{137}Cs - γ -rays

	0.119 Gy	0 Gy
Number of animals	6	6
Body weight (g)	27.4 \pm 0.8**	28.8 \pm 0.5
Testis (g)	0.193 \pm 0.080	0.199 \pm 0.009
Epididymis (g)	0.082 \pm 0.005	0.081 \pm 0.006
Testis/BW	7.06 \pm 0.43	6.92 \pm 0.37
Epididymis/BW	2.98 \pm 0.22	2.81 \pm 0.21
Seminiferous tubules (μm)	164.4 \pm 11.7**	174.6 \pm 13.3
Abnormal sperm (%)	0.92 \pm 0.39*	1.52 \pm 0.59

(mean \pm SD)

** Significantly different from the 0 Gy value ($p < 0.01$)

* Significantly different from the 0 Gy value ($p < 0.05$)

Table 5. Body, testis, epididymus weights and abnormal sperm after 2.38 Gy ^{137}Cs - γ -rays irradiation

Group	0 Gy	2.38 Gy
0 month		
Number of animals	10	10
Body weight (g)	27.5 \pm 1.2	25.7 \pm 0.7**
Testis (g)	0.192 \pm 0.012	0.096 \pm 0.006**
Epididymid (g)	0.070 \pm 0.003	0.066 \pm 0.004*
Testis/BW	7.00 \pm 0.62	3.72 \pm 0.24**
Epididymis/BW	2.54 \pm 0.17	2.56 \pm 0.17
Seminiferous tubules (μm)	174.8 \pm 13.5	127.4 \pm 14.9**
Abnormal sperm (%)	1.4 \pm 0.7	4.3 \pm 1.1**

1 month after end of exposure		
Number of animals	10	10
Body weight(g)	30.5 \pm 1.5	28.5 \pm 1.5**
Testis (g)	0.213 \pm 0.017	0.191 \pm 0.010**
Epididymid (g)	0.090 \pm 0.004	0.087 \pm 0.005
Testis/BW	6.92 \pm 0.58	6.70 \pm 0.57
Epididymis/BW	2.94 \pm 0.19	3.04 \pm 0.19
Seminiferous tubules (μm)	179.9 \pm 14.8	178.9 \pm 14.6
Abnormal sperm (%)	1.7 \pm 0.7	1.4 \pm 0.7

(mean \pm SD)

*: $p < 0.05$ compared with 0 Gy

** : $p < 0.01$ compared with 0 Gy

DISCUSSION

In the present experiment, the biological effects of HTO and ^{137}Cs - γ -rays at the same dose rate varied with some organs, but there were no differences in the size of seminiferous tubules between the two sources of irradiation. It can thus be considered that the biological effects of irradiation might be similar, independent of the source. Since use of high dose HTO is circumscribed by regulations, a simulator of tritium irradiation can be a good tool for determination of HTO damage or safety doses. In the present experiment pathological changes were found in the testes, and weights were decreased in a dose dependent manner, especially in C57BL mice. Spermless seminiferous tubules were most prevalent in this strain. The

testis is known to be one of the most radiosensitive organs^{8,11-13,20} with strain differences^{3,11}. Moreover, van der Meer et al¹⁰ reported that spermatogonial stem cells were found to be most sensitive to X-rays during quiescence and most resistant during active proliferation. However, staging is very difficult. In this experiment, all strains demonstrated a dose-dependent decrease in the size of seminiferous tubules between 23.2 MBq and 92.5 MBq ($y = -0.62x + 186$, $r^2 = 0.89$, $p < 0.001$) and between 0 MBq and 9.5 Gy ($y = -0.17x + 169$, $r^2 = -0.80$, $p < 0.001$). This may therefore be considered a good parameter for determination of radiation damage in the testis. In the case of 0.119 Gy ¹³⁷Cs- γ -rays irradiation, body weights and size of seminiferous tubules were still decreased, but other parameters were not altered as compared to non-irradiated controls. The threshold dose for ¹³⁷Cs- γ -rays for testis damage in young adults may thus be near to 0.119 Gy. After 2.38 Gy ¹³⁷Cs- γ -rays irradiation, body, testis weights and size of seminiferous tubules were decreased, but spermatogonia remained and spermatogenesis had recovered almost completely after 1 month. Carr and Nolan⁴ reported that, except at the lowest injected amount of HTO (10 μ Ci/g body mass), the testis mass decreased to a minimum by 4–5 weeks, followed by a somewhat irregular recovery towards the control value, but which was complete in all the HTO injected groups by 16 weeks. However, it remains to be determined whether this may also be true after 9.5 Gy irradiation. High radiosensitivity during fetal life in germ cells has been reported^{13,20}, with a decrease after birth^{7,15,16}. In the adult, differentiating spermatogonia remain the most radiosensitive cells of the testis. Thus, further experiments are required to assess the effects of HTO *in utero*.

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