Radiation exposure exerts its adverse effects on sperm maturation through estrogen-induced hypothalamohypophyseal axis inhibition in rats

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Irradiation has adverse effects on reproductive aspects such as spermatogenic cell population and cell malformation, leading to reduced sperm count and non-viable spermatozoa. This has overshadowed possible effects of radiation exposure on biochemical environment throughout the epididymis and the viability of spermatozoa that appeared morphologically normal. The effects of radiation exposure on sperm quality were evaluated through mating trials and assessment of the cauda epididymal sperm motility. Sprague Dawley rats with body mass of 300–400 g were selected at random. Two experimental groups received acute ^{60}Co γ-radiation doses of 3.5 and 6.0 Gy, respectively. Data were collected 2, 7, 14, 21, 28 and 35 days post-irradiation. Each male was housed with a non-irradiated super-ovulated female during mating trials. Cauda epididymal sperm motility was assessed with the CASMA. Hormone analyses were carried through chemiluminescence diagnostic tests to determine the endocrine status. Results suggest that irradiation causes an overproduction of estrogens, which suppresses the hypothalamic–pituitary axis and inhibits LH and FSH secretions. Both LH and FSH deficiencies have negative effects on the testicular index and local reproductive hormones. Elevated estrogen levels influenced the epididymal internal milieu negatively, resulting in rigid, flagella bending sperm tail, impaired progressive movement of the spermatozoa and hence infertility.

Key words: radiation exposure, spermiogenesis, estrogen, sperm motility.

INTRODUCTION

Mammalian spermatozoa leaving the testis have to undergo distinct morphological and biochemical changes during the epididymal transit before they acquire motion and the ability to fertilize the oocytes (Van der Horst *et al.* 1999). A suitable microenvironment should be created along the epididymal ducts for successful sperm maturation to occur. Sertoli cell and principal cell secretions provide a suitable chemical composition of the epididymal internal milieu (More & Akhondi 1996). A specialized barrier between the interstitial blood compartment and the luminal compartment of the seminiferous tubules also plays a vital role by ensuring controlled movement through tight junctions between adjacent Sertoli cell and endothelial cells (Brooks 1983).

The composition of this epididymal internal milieu has been reported to influence the functioning of the sperm cells. Forward motility protein (FMP) and calcium ions have been implicated in the calcium-dependent mechanism, which involves transformation from an irregular and circular type movement of caput epididymal spermatozoa into a progressive movement of cauda epididymal spermatozoa (Serres & Kann 1984) resulting in acquisition of mobility and ability to fertilize the oocytes (Kohane *et al.* 1980).

The mammalian sperm tail presents a complex organization in which a number of additional structures, such as outer dense fibers (ODF) and fibrous sheath (FS), are involved in the regulation of flagella motility (Yu *et al.* 2002). The ODF normally form the disulphide bound in the presence of zinc ions causing a rigid flagella motion at the earlier spermatid development within the seminiferous tubules initial parts of the epididymis. The fibrous sheath (FS) is a unique cytoskeletal structure and is believed to influence the degree of flexibility, plane of flagella motion, and the shape of the flagella beat (Eddy *et al.* 2003).

Research on the effects of radiation exposure on the mammalian reproductive tract dates back to the early 20th century when Bergonie and Tribondeaus exposed rabbit testes to X-rays in 1906. It was then discovered that proliferating cells are more radiosensitive (Dowd 1994). Many

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studies have since been conducted (Rowley *et al.* 1974; Meistrich 1986; Pinon-Lataillade *et al.* 1991). It has been determined that the dominant lethal inductions observed *in vivo* and *in vitro* (Matsuda *et al.* 1985) were mainly on the cells involved in the mitotic and meiotic divisions by the time of radiation exposure. The effect of irradiation on testicular parameters has, to a great extent, obscured the possible effects on maturing spermatozoa in their transit through the epididymis. The current study investigated the effects of gamma radiation on the fertilizing ability of Sprague Dawley rat spermatozoa that survived radiation exposure. This was carried out through mating trials, assessment of epididymal sperm motion parameters using the Computer Aided Sperm Motility Analyser (CASMA) and the evaluation of post-irradiation endocrinological status.

METHODS

Anaesthesia and radiation exposure

Sprague Dawley rats with the body mass of 300–400 g, selected at random, were obtained from the Wits Central Animal Service. These rats were maintained under controlled temperature (20–22°C), humidity (40–50%), and on light schedule of 12L:12D. They were allowed a balanced diet of commercial food in the form of dry pellets (Rat cubes; Epol, South Africa) and tap water *ad libitum.* The conditions and facilities under which the rats were treated were approved by the Animal Ethics Committee (University of Pretoria, Onderstepoort).

Prior to radiation exposure, male rats were divided into control and two experimental groups of 36 rats each. Each group was further subdivided into six post-irradiation sub-groups, each consisting of six rats. The experimental groups were classified as Group A and B according to the irradiation doses they received. Both control and experimental rats were then anaesthetized with a mixture of 20 mg/ml xylazine and 100 mg/ml ketamine in HCI at a ratio of 1:4 injected intraperitoneally at a dose of 0.1 ml/100 g body mass. All invasive, surgical and radiation procedures were performed under the supervision of a registered veterinary nurse and a veterinarian at University of the Witwatersrand Medical School.

During irradiation, a protective lead shield was used to cover the entire body of the animal. The scrotal region of each experimental rat was exposed to ^{60}Co-gamma rays through an opening made in the lead cloth. A single dose of 3.5 Gy and 6.0 Gy was directly aimed on the scrotal region of both groups A and B animals, respectively, at a dose rate of 0.48 Gy/min. Each animal then received 0.25 ml of 20% Temgesic analgesia in sterile water (0.3 mg/ml Buprenorphine) intra-peritoneally and allowed 12 hours recovery. Control animals received similar treatments including treatment in the radiation room, except that they were not exposed to gamma rays.

During mating trials, each male was housed with a non-irradiated super-ovulated female in the breeding cage. Females were super-ovulated by an intraperitoneal injection with pregnant mare's serum gonadotropin (PMSG) and human chorionic gonadotropin (HCG) as described by Matsuda *et al.* (1985). Successful copulation was confirmed by the presence of a copulation plug. Once the copulation was confirmed, the females were then housed in separate cages (one animal per cage) for the entire duration of gestation. Litter size was recorded immediately at birth.

Sperm sampling

Flourothane ($C_2HBrCLF_3$, SIGMA) was used to anaesthetize the male rats in an enclosed chamber. The male rats were sacrificed after 2, 7, 14, 21, 28 and 35 days post-irradiation. Only males that had inseminated females were sacrificed. Blood was collected by the cardiac puncture method and centrifuged. Plasma collected was stored at –20°C for hormone analysis.

Testes and epididymides were pushed with a slight force through the incisions made on the scrotal sac. The two organs were separated under a stereomicroscope and testis weight recorded for the testis and body weight ratio (testicular index) determination.

Once separated, epididymides were rinsed in a diluting medium (0.12 g/100 ml $NaHCO_3$; 9.8 g/l SIGMA Ham's H_2O; Cat. No. N6013). Epididymal tubules were held under slight pressure and punched in the late proximal to mid cauda epididymis to release the luminal contents in 4 ml of the diluting medium incubated at 36°C.

Video-recording of sperm preparation

Two hundred μl of the sperm suspension was delivered by a displacement pipette onto a clean slide pre-warmed on a heated platform maintained at 36°C. The sperm suspension was covered with a 21 × 26 mm^2 cover slip to create a 40 μm

deep swimming chamber recommended for sperm motion analysis. The microscope heating stage was equilibrated and maintained at the same temperature as a heated platform during the entire analyses. A phase contrast Olympus CH2 microscope with negative phase optics, set at ×20 objective, was used to study and record sperm motion.

Video recording was made at 50 frame/sec using a photo-ocular camera and video recorder interfaced with a digital timer. The sperm activities were video-recorded for an overall time of approximately 2–5 minutes and 10 seconds at a given field. Any incidence of abnormally shaped spermatozoa was noted and any field with abnormal/normal spermatozoa ratio of 1:25 or less was interpreted as normal, although there were very few of such fields. The Sperm Motion Quantifier (SMQ; Wirsam Scientific & Precision Equipment, Johannesburg), a computer aided sperm analyser (CASMA) was used to analyse video recordings of sperm images.

Motion parameters measured

For each field analysed, the positions of motile sperm on the monitor were marked and head movement from one video frame to the next was manually tracked. A minimum of 100 motile spermatozoa was analysed per animal and a total of six motility parameters were measured using a computerized image analysis system. Sperm motion parameters presented in this study, which are: curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), mean amplitude of lateral displacement of the centroid (mnALH), straightness (STR) and beat/cross frequency (BCF), were abbreviated according to Boyers *et al.* (1989) and Van der Horst *et al.* (1999)

Chemiluminescent determination of estradiol

Chemiluminescent diagnostic tests were conducted on fibrin-free plasma using the Automated Chemiluminescent System (ACS: 180 Plus, VI instruments). Plasma samples in 50 μl aliquots were placed in plastic sample cuvettes together with 50 μl of antibody reagent (1.5 μg rabbit anti-estradiol antibody and 125 ng/vial steroid releasing agent in 2.5 ml of 1% sodium azide buffer) incubated for 5.5 minutes at 37°C, 50 μl lite reagent (5 ng estradiol derivate labelled with acridinium ester in 2.5 ml of 1% sodium azide) and 250 μl of solid phase (625 μg mouse anti-rabbit antibody coupled with paramagnetic particles in 2.5 ml of 1% sodium azide). Both lite reagent and solid phase were incubated at 38°C for 5 min. Three hundred μl of both Reagent 1 and 2, respectively, were added to initiate the chemiluminescence reactions. The rest of the procedure was performed as recommended and illustrated in the system's operation manual.

Statistical analysis

All data were analysed with the Sigma-Stat version 2.0. Both ANOVA on Ranks and Student's *t*-tests were used when comparing multiple and two variants, respectively. The correlation coefficient (R) was calculated as stated by Matson (1981) to determine the relationship between these parameters and the ability of the spermatozoa to fertilize the oocyte during oestrus. Since the correlation coefficient was calculated from the data drawn from the small sample size ($n = 6$) in this study, the statistical test employed to make any interference concerning the presence of a correlation should contain the variable r both in the numerator and denominator. To test the null hypothesis (H_0: $P = 0$), t-statistics were calculated using the equation below:

$$t_{\text{experimental}} = \frac{r\sqrt{(n-2)}}{\sqrt{(1-r^2)}}. \qquad (1)$$

To test the correlation between the litter size and sperm motion parameters, litter size and testicular index as well as litter size and estrogen levels, the $t_{\text{experimental}}$ values were calculated from the r-values, using Equation (1). At 0.05 level of significance with the application a one-tailed test and four degrees of freedom (d.f.), the value of $t_{0.05,4}$ was found to be 2.132, as was statistical tabulated by Federighi (1959). By comparing the $t_{0.05,4}$ value with $t_{\text{experimental}}$ values, according to Edward (1984), it was shown that there is a significant correlation between the litter size and testicular index ($P < 0.0025$), litter size and sperm motion parameters (VSL, $P < 0.005$; VCL, $P < 0.005$; STR, $P < 0.05$; VAP, $P < 0.01$; mnALH, $P < 0.005$; and BCF, $P < 0.001$) and a negative correlation between litter size and estrogen level ($P < 0.01$). The correlation between litter size and the parameters mentioned above were also determined in group A animals. It was also found that there was a positive correlation between litter size and testicular index, litter size and sperm motion parameters and a negative correlation between litter size and estrogen levels at the significance level of $P < 0.05$.

RESULTS

Radiation exposure to the scrotal region of male rats has no significant effects on body growth as indicated by a slight gain ($P > 0.05$) in body weights (Table 1). By contrast, testicular weights showed a gradual decrease.

Table 2 shows sperm motion parameters as observed after manual tracking of the sperm heads by computer aided sperm motion analysis (CASMA). The control sperm motion parameters did not differ statistically ($P > 0.05$) over time. Both groups A and B sperm motion parameters showed significant decrease over time ($P < 0.05$) compared to the time-matched control from post-irradiation day 14 and day 7, respectively. The decrease in VAP was more pronounced in animals exposed to high irradiation dose ($P > 0.05$).

The results presented in this study also showed a decrease in fertility rate at any time-matched control as indicated by a decrease in litter size (Fig. 1). Group A litter sizes fell within normal litter size range of 6–12 pups/litter at any time-matched control, except day 35 ($P < 0.001$). Group B values were below the normal range, indicating the severity of high irradiation dose. A decrease in regression lines indicates that other factors such as stress (Almeida *et al.* 2000), prolonged caging (Nass *et al.* 1982) and ageing (Tain *et al.* 2000) might have influenced the decline in litter size.

The incidences of abnormally shaped spermatozoa (e.g. double-headed) and spermatozoa with branched or bent tails, were low, with a ratio of less than 1:25. There was no incidence of offspring malformation. This might be due to the fact that radiation-induced sperm morphological changes are expected to be more evident in the F_1 generation than in the F_0 paternal group. The fact that the fertility rate decreased in experimental groups to the point that group B failed to produce offspring, 35 days after irradiation, showed that most of the spermatozoa that appeared morphologically normal had sustained internal injuries.

From the data collected in the current study, the plasma estrogen level was affected by radiation exposure. Table 2 shows a fluctuation in estrogen levels initially, but then a progressive increase starting from day 21 and 14 in group A and B animals, respectively, compared to the time-matched control. The difference in plasma estrogen level of group A showed a statistically insignificant increase ($P > 0.05$) compared to the time-matched group B until day 21. Chemiluminescent tests indicated that both LH and FSH concentrations were below the detectable levels of 0.07 mIU/ml and 0.3 mIU/ml, respectively, and hence no data are shown.

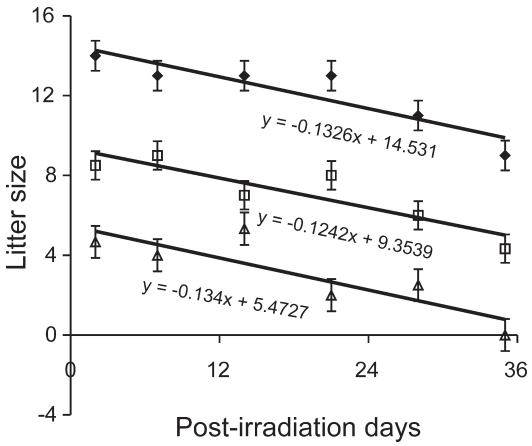

Fig. 1. Comparison of the control and experimental Sprague Dawley rats' litter sizes over a period of 35 days. Each point on the graph (♦ control; □ Group A; Δ Group B) represents the mean ± S.E.M. values ($n = 6$).

DISCUSSION

Radiation exposure had no effects on body weight, which increased following local exposure of the rat testes to an acute exposure of gamma radiation. By contrast, testicular weight declined as observed previously by Pinon-Lataillade *et al.* (1991) who considered this to result from the destruction of spermatogonia and preleptotene spermatocytes. The destruction of spermatogonia and preleptotene spermatocytes were reported to coincide with the time they undergo DNA replication (Pineau *et al.* 1989; Meistrich, 1993) and the extent of damage increased with post-treatment interval and increasing dose (Bansal *et al.* 1990). Pinon-Lataillade *et al.* (1991) observed a 41% decrease after 35 days in the testicular weight of Sprague Dawley rats exposed to irradiation doses of 9.0 Gy. This was attributed to the difference in the rate of irradiation. In the present study an irradiation dose of 6.0 Gy was delivered at the dose rate of 0.48 Gy/min for 12.5 minutes. This exposure time is far more than the three minutes exposure time used to deliver a total dose of 9.0 Gy (Pinon-Lataillade *et al.*1991). This suggests that a prolonged low-dose radiation exposure has a more devastating effect on seminiferous epithelial cells than a short-term high-dose exposure as was reported by Pinon-Lataillade *et al.* (1991).

The scientific evidence has shown that low and

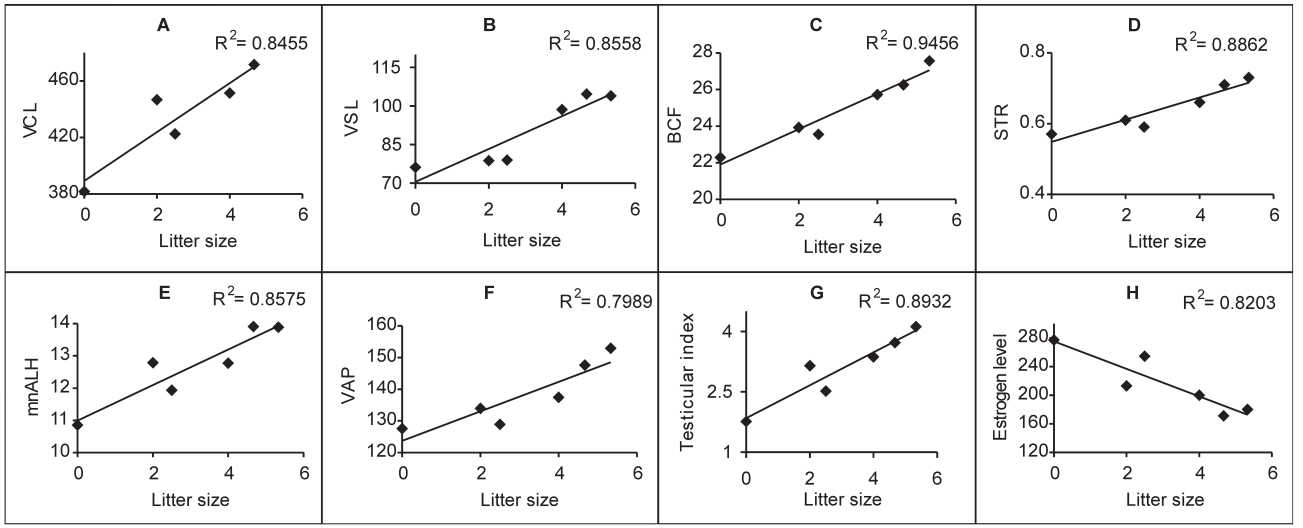

Fig. 2. The correlation coefficient (r^2) of group B animals that best described the relationship with the litter size. **A–F** show the sperm motion parameters (i.e. VSL, VCL, BCF, STR, mnALH and VAP); **G** shows testis body mass ratio (testicular index) and **H** shows endocrine parameter (estrogen level).

Table 1. Comparison of the effects of an acute ^{60}Co γ-irradiation on body weight, testicular weight and the testicular index. Each value represent the mean ± S.E.M. ($n = 6$)

Post irradiation period (days):		2	7	14	21	28	35
Control	Body weight	381.74 ± 20.04	422.23 ± 16.82	431.3 ± 20.14	435.18 ± 18.69	482.22 ± 23.09	491.20 ± 27.47
	Testis weight	1.75 ± 0.07	1.92 ± 0.08	2.00 ± 0.13	1.93 ± 0.07	2.08 ± 0.13	2.03 ±0.05
	Testis/body ratio × 10^{-3}	4.58 ± 0.22	4.54 ± 0.10	4.63 ± 0.09	4.45 ± 0.08	4.33 ± 0.12	4.22 ± 0.01
Group A	Body weight	377.85 ± 20.34	396.33 ± 15.40	418.93 ± 16.14	419.87 ± 20.51	443.50 ± 21.73	531.50 ± 26.97
	Testis weight	1.83 ± 0.08	1.73 ± 0.05	1.55 ± 0.03	1.38 ± 0.02	1.17 ± 0.06	1.19 ± 0.60
	Testis/body ratio × 10^{-3}	4.50 ± 0.12	4.17 ± 0.13	4.03 ± 0.15	3.37 ± 0.19	2.65 ± 0.07	2.27 ± 0.15
Group B	Body weight	390.83 ± 16.71	417.62 ± 13.58	446.33 ± 17.73	448.76 ± 17.64	465.87 ± 18.09	490.20 ± 15.67
	Testis weight	1.60 ± 0.07	1.55 ± 0.07	1.50 ± 0.06	1.45 ± 0.03	1.12 ± 0.08	0.86 ± 0.05
	Testis/body ratio × 10^{-3}	4.12 ± 0.13	3.72 ± 0.09	3.37 ± 0.11	3.15 ± 0.12	2.52 ± 0.19	1.76 ± 0.11

Table 2. Comparison of the sperm motion parameters obtained by manual tracking of the sperm heads using CASMA, and plasma estrogen level. Each value represents the mean ± S.E.M. ($n = 6$).

Post irradiation days	Parameters	VCL (μm/s)	VSL (μm/s)	BCF (Hz)	STR (μm/s)	MnALH (μm)	VAP (μm/s)	Estrogen level (pmol/ml)
2	Control	480.80 ± 8.79	120.36 ± 5.66	28.43 ± 0.34	0.77 ± 0.01	13.71 ± 0.46	157.90 ± 4.34	143.25 ± 31.23
7		481.34 ± 17.46	122.45 ±1.13	27.91 ± 0.78	0.73 ± 0.06	13.73 ± 0.58	165.71 ± 1.68	148.33 ± 37.28
14		499.20 ± 6.05	121.14 ± 4.61	29.58 ± 0.91	0.77 ± 0.02	14.14 ± 0.26	158.07 ± 6.51	188.67 ± 26.30
21		492.20 ± 4.52	118.50 ± 3.98	27.57 ± 0.75	0.77 ± 0.03	13.97 ± 0.23	157.62 ± 1.79	128.01 ± 30.84
28		500.18 ± 10.58	121.40 ± 1.15	27.03 ± 0.56	0.76± 0.04	14.20 ± 0.35	161.86 ± 3.62	137.67 ± 32.49
35		490.67 ± 4.01	120.38 ± 7.50	26.91 ± 0.37	0.78 ± 0.02	14.08 ± 0.13	159.84 ± 2.78	150.25 ± 32.93
2	Group A	482.87 ± 2.9	113.85 ± 3.90	28.08 ± 0.26	0.72 ± 0.04	13.89 ± 0.85	165.27 ± 4.26	156.60 ± 31.68
7		504.63 ± 15.9	109.33 ± 7.50	28.67 ± 0.89	0.74 ± 0.01	13.91 ± 0.73	154.13 ± 7.26	156.54 ± 51.24
14		449.36 ± 7.83	99.74 ± 2.5	26.26 ± 0.97	0.68 ± 0.02	12.78 ± 0.62	151.81 ± 4.21	183.69 ± 16.81
21		452.45 ± 9.76	89.69 ± 1.9	25.62 ± 0.74	0.67 ± 0.03	12.98 ± 0.32	150.60 ± 6.64	167.60 ± 23.35
28		427.76 ± 12.05	84.21 ± 3.4	24.82 ± 1.24	0.63 ± 0.04	12.93 ± 0.53	146.09 ± 3.93	195.69 ± 36.57
35		399.93 ± 5.83	80.61 ± 4.9	22.92 ± 1.70	0.64 ± 0.03	11.43 ± 0.31	136.47 ± 5.58	207.02 ± 11.53
2	Group B	474.78 ± 12.74	103.99 ± 1.5	27.57 ± 1.74	0.73 ± 0.04	13.57 ± 0.67	152.95 ± 7.01	180.83 ± 46.50
7		471.53 ± 15.69	104.68 ± 4.02	26.25 ± 0.97	0.71 ± 0.02	13.25 ± 0.83	147.58 ± 4.81	171.01 ± 48.21
14		451.35 ± 17.60	98.61 ±1.78	25.72 ± 0.75	0.66 ± 0.04	12.51± 0.55	137.44 ± 6.95	205.21± 25.52
21		446.69 ± 16.61	78.75 ±1.29	23.93 ± 0.54	0.57 ± 0.03	12.79 ± 0.45	133.97 ± 7.31	211.33 ± 21.83
28		422.49 ± 12.71	79.04 ±3.91	23.55 ± 1.32	0.59 ± 0.04	11.94 ± 0.66	137.54 ± 5.99	254.54 ± 25.25
35		381.78 ± 6.89	76.14 ± 3.63	22.29 ± 1.52	0.61 ± 0.02	10.85 ± 0.34	128.88 ± 7.66	277.03 ± 18.14

controlled testicular concentration of ROS has beneficial effect on sperm functioning. Although ROS play an important role in sperm physiology (Oehninger *et al.* 1995), such as capacitation, acrosome reaction and fusion with the oocyte membrane (Griveau & Le Lannou), they have been reported to cause ATP depletion leading to peroxidative damage to the sperm plasma membrane if allowed to escalate (Aitken & Fisher 1994). Malondialdehyde is the end product of lipid peroxidation, the process through which ROS causes destruction of the antioxidative capacity of spermatozoa and peroxidative damage to the sperm plasma membrane. Since acute ^{60}Co-gamma irradiation has been reported to increase the formation of malondialdehyde (Ronai & Benko 1984), it can be suggested radiation exposure breaks the fine balance between beneficial and detrimental effects of ROS, leading to loss of sperm motility (Alvarez & Storey 1989), loss of viability (de Lamirande *et al.* 1997), ineffective spermatozoonoocyte fusion (Koksal *et al.* 2003) and hence infertility.

During the formation of the outer dense fiber (ODF) between steps 8–19 of spermatogenic development in the seminiferous tubules, zinc binds to the sulphydryl group of cysteine and prevents the formation of disulphide bridges. This leads to rigid sperm flagellum motion and immotile spermatids, which is very essential at this stage of spermatogenic development. The spermatozoa normally acquire a progressive motility as they transit through the caput, corpus, and cauda regions of the epididymis (Srivastava *et al.* 1982). During this transit, the zinc content of the outer dense fiber is normally reduced by about 54–60%, leading to an increase in the diameters and stabilization of ODF proteins as a result of oxidation of the sulphydryl group (Kaminska *et al.* 1997). Flagellum bending, which influences the sperm motion pattern, is highly dependent on the diameter of ODF and its stiffness

(Henkel *et al.* 1999). An elevation in the estrogen level, which was observed in the current study, has previously been reported to inhibit the formation of disulphide bridges by preventing the reduction of spermazoal zinc levels during epididymal transit (Srivastava *et al.* 1983) hence retaining their rigid flagellum motion and poor fertilizing capacity (Samanta & Pal 1986).

The radiation-induced estrogen level escalation observed can also be associated with hyperactivity of testicular aromatase ($P450_{arom.}$), an enzyme reported to cause male infertility in Sprague Dawley rats (Akiyama 1997). Reabsorption of epididymal fluids influences the luminal elementary composition of the caput epididymis, a site where sperm maturation is initiated (Jenkin *et al.* 1983; Hess 1998). Increased estrogen level was reported to reduce caput calcium content (Jenkin *et al.* 1983). Lubart *et al.* (1997) also reported that Ca^{2+} ion uptake by the spermatozoal mitochondria could be inhibited by exposure of mammalian spermatozoa to laser irradiation, another type of ionizing radiation. Radiation-induced plasma estrogen production suggests the reduction in calcium intake by the spermatozoa suggesting inhibition of calcium-dependent mechanism that is involved in transformation from an irregular and circular type movement of caput epididymal spermatozoa into a progressive movement of cauda epididymal spermatozoa. This also contributes to the reduction in fertilizing capability (Cohen *et al.* 1998).

Mature spermatozoa with full fertilizing capacity are characterized by high average path velocity (VAP), straight-line velocity and drastic improvement in straightness (STR) as a result of more flexible mid-piece and symmetrically beating tail with regular rotation (Dyson & Orgabin-Crist 1973). In this study the reduction in VSL, VCL, STR, VAP mnALH and BCF, which correlated positively with animal mating trials was shown. This reduction in the sperm motion parameters is considered to be responsible for the infertility as reported previously that the spermatozoa with low fertility abilities have characteristic low VSL and VAP accompanied by a low swim path (STR) both *in vivo* and *in vitro* (Moore & Akhondi 1996; Kaneto *et al.* 1999). The reduction in sperm motion parameters observed also correlate positively with the decrease in litter size. This suggests that the sperm motion analysis or CASMA could be considered as a useful tool for the detection of cytotoxic effects caused by acute irradiation on the epididymal spermatozoa.

From the results obtained in the current study it can be suggested that radiation-induced infertility causes the inhibition of the hypothalamo-pituitary axis as the results of elevated estrogens levels, which in turn suppresses the secretion of both LH and FSH. Deficiency of LH and FSH and the resultant inhibition in testosterone biosynthesis (Vanage *et al.* 1997) is thought to impede the normal Sertoli cell functioning resulting in poor maintenance of spermatogenesis as indicated by a decrease in the testicular index and supported by Smith *et al.* (1982).

Since LH and FSH are required to allow the pachytene spermatocytes to progress efficiently to stage 7 spermatids (Vernon *et al.* 1975), LH and FSH deficiency will block the progression of these cells into spermatids leading to spermatogenic arrest and infertility. Excess estrogen level has been reported to depress *in vivo* ABP synthesis and androgens transportation through its inhibitory effects on testosterone biosynthesis (Smith *et al.*1982). Since epididymal functioning and secretion are regulated by androgens of Leydig cell origin, a lack of ABP production by the Sertoli cell can be associated with the interference of the normal maturation process. It has been found that estrogens cause infertility by accelerating the epididymal transit time resulting in the appearance of immature spermatozoa in the ejaculate (Meistrich *et al.* 1975). Since there was no incidence of abnormal spermatozoa in the current study a decrease in litter size may also be related to the presence of immature spermatozoa in the ejaculate.

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