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Electron Beam Irradiation – a Potential Inducer of Sperm Abnormalities in Swiss Albino Mouse, *Mus Musculus* Exposed to Median Lethal Dose

KEYWORDS: Electron beam; Sperm shape abnormalities; Sperm count; Median lethal dose

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ABSTRACT *Electron beam (E-beam), an ionizing radiation consists of very tiny electrically charged particles generated by a particle accelerator. There are no reports documenting E-beam induced toxicity with special reference to male germinal cells. A pilot study was carried to evaluate E-beam induced effects on morphology and density of sperms of mice that survived median-lethal dose (10-11 Gy) after five weeks following whole-body irradiation. The present study demonstrates that E-beam at median lethal dose level induces radical damaging effects on sperm morphology and density. It suggests that E-beam affects the fertility status of the survived mice and may also have an impact on the next generation if bred. Studies further in details are warranted to understand the impact of E-beam radiations on the health of the reproductive system, in particular the gamete cells.*

The present data demonstrates that the survived animals following exposure to median lethal dose of electron -beam exhibited severe germinal toxicity as indicated by altered sperm morphology and reduced sperm count. It suggests that E -radiations may affect the fertility status of the irradiated mouse or of the next generation if breed successfully The radical damaging effects observed at median lethal dose indicate that E-beam would induce testicular toxicity, may be at low magnitude at sub-lethal exposure, which may cause impairment in reproductive functions and other testes associated health problems.. Further studies, however are warranted to evaluate differential effects of E-beam radiations on male reproductive system at sub-lethal / therapeutic doses to evaluate the safe dose particularly in clinical applications.

Introduction:
E-beam, a corpuscular radiation with definite mass and travel as a stream of particles very different from those of electromagnetic radiations. E-beam irradiation is a special type of radiotherapy that consists of very tiny electrically charged particles generated in a machine called a linear accelerator. As a radiotherapy, it is useful in the treatment of various disorders, including skin cancer, mycosis, fungoids,melanoma, ymphoma, for mastectomy and lumpectomy (Loevinger et al, 1961;Klein, 2008) .Therefore, it necessitates toxicity evaluations for its safer mode of usage to avoid the health risks. The responses of mammalian testicular cells involved in spermatogenesis process to electromagnetic ionizing radiations (gamma and X-rays) are well-known (Oakberg, 1955, 1956 and Meistrich,1986). However, there are only few reports documenting E-beam induced toxicity on experimental models. Earlier study reports a dose-dependent induction of micronuclei in mouse (Suchetha and Madhu., 2011). However, there is no report on mouse with special reference to testicular toxicity. The present pilot study was carried out to assess effect of E- beam radiations on reproductive status in particular the alterations in sperm shape abnormalities and the sperm density in survived animals (Swiss albino mice) following exposure to median lethal dose (10-11 Gy) of E-beam as assessment of abnormalities in shapes and density of sperms is a potential indicator of infertility and testicular cancer (Lambert and Fisch, 2007).

2. Materials and Methods:
2.1. Experimental animals
Swiss albino male mice (*Mus musculus*) aged 6 -8 weeks, weighing 25+5 g maintained under controlled conditions of light (light: 10 h; dark: 14 hour) and temperature (26-28°C) were housed in a polypropylene cage containing sterile paddy husk as the bedding material and were provided with standard mouse feed and water ad libitum.

2.2. Irradiation:
The experimental animals were randomly divided into three groups Viz: Group 1. served as control while 2nd and 3rd group subjected to whole-body irradiation using E-beam at a dose of 10 Gy/ min and 11 Gy /min respectively. The mice were irradiated at a distance of 30 cm from the beam exit point of the Microtron accelerator at Microtron centre, Mangalore University, Mangalore, Karnataka, India.

2.3. Sperm abnormality Assay:
Sperm abnormality assay was performed as described earlier (Wyrobek and Bruce, 1983). The control and experimental animals were sacrificed on 35th day following exposure to radiations.The cauda epididymis were excised, minced using fine scissors in buffered physiological saline (pH 7.2), filtered and smears were made on clean, grease-free slides following staining of sperm cells with 1% aqueous eosin (9 :1) for 30 min. The slides were air-dried. A total of 2000 sperms (including normal and abnormal) were scored using the microscope at were photographed. Sperm density was determined using method of Vega et al. (1988). The experimental data was subjected to statistical analysis using Students t test.

3. Results and Discussion :
E-beam irradiation has versatile applications in food industry and in particular as a therapy agent On the other hand electron beam ionising radiations have been reported to induce damaging effects on reproductive system in particular on sperm cells in mammals (Dobson and Felton,1983; Matalka et al, 1994; Georgieva et al, 2005; Bonde, 2010)

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Exposure of tissues to ionizing radiations cause excitation of some of the atoms and ejection of orbital electrons resulting in ionization and alteration in the chemical structure of molecules of biological importance leading to damaging effects. The median lethal dose (LD-50) of electron beam irradiated Swiss albino mouse lies between 10 Gy-12 Gy /min as recorded in the present study and 10 Gy as documented in earlier study (Suchetha and Madhu, 2011). The survived irradiated mice following exposure to E-beam radiations (10 and 12Gy /min) exhibited significant reduction in sperm counts and abnormalities in shape (hook-less, banana shaped, amorphous, double tailed, double-headed and folded) compared to control (Table 1 & Figs.1 to 3).The data suggest a statistically significant (P<0.01) dose dependent effect compared to control group.

Table 1: Percent frequency[A] of different types of abnormal sperms in mouse, Mus musculus induced by E-beam (10 and 11 Gy/min) after 35 days of exposure.

Treatment/ Dose	Normal	Abnormal						Total aberrations % ± SD	Sperm count (x10^6)
		Hookless	Banana	Amor-phous	Double Tailed	Double eaded	Folded		
Control	98.1	0.3	0.2	0.7	0.2	-	0.5	1.9±0.32	17.96±1.03
E-beam 10 Gy	87.7	2.1	1.4	4.8	0.8	0.5	2.7	12.3±0.98*	4.85±0.76*
E-beam 11 Gy	84.2	2.9	1.9	5.2	1.2	0.9	3.8	[a]15.8±1.22*	[a]2.3 ±0.47*

[A] From 2,000 sperms per animal; 5 animals/group., *P<0.001; (Control vs Treated).

[a]P<0.01 (10 Gy Vs 11 Gy).,. P < 0.05 considered as significance difference.

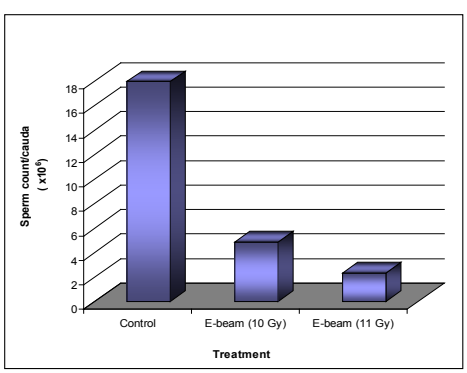

Fig. 1: Effect of E- beam irradiation (10 - 11 Gy) on sperm count of mouse , Mus musculus exposed to E beam radiation at a dose of (10 -11 Gy) compared to control

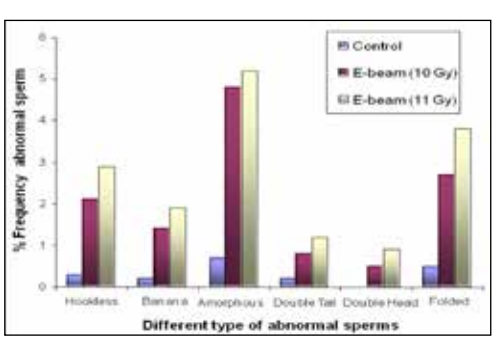

Fig 2: Percent (%) frequency of various types (Hookless, Banana, Amorphous , Double tail, Double head, and Folded) of abnormal sperms in mouse, Mus musculus exposed to whole body irradiation following exposure to median lethal dose (10 -11 Gy) of electron beam compared to control

Defects in sperm morphology recorded in irradiated mouse may be a consequence of genetic damage to spermatogenic cells (Wyrobek & Bruce, 1978). It has been recorded that the frequency of abnormal sperms increase markedly following treatment with mutagen agents, including radiations (Hugenholtz and Bruce, 1983;Aubele et al.,1990).According to Kranowska (1974) and Ferlin et al. (2005), Y-linked genes contribute to the overall percentage of abnormal sperms while, autosomal factors control relative type of abnormalities. Yamauchi et al. (2010) has proposed that deficiency in mouse Y chromosome long arm gene complement is associated with sperm DNA damage leading to sperm abnormalities. Thus, electron beam irradiation either directly or through generation of reactive oxygen species (ROS) or by both the mechanisms induces DNA damage, which may be the probable reason for induction of the abnormal sperms. Using Comet assay, E-beam irradiation has been reported to induce DNA damage in cultured human lymphocytes (Joseph et al., 2011) in larvae of chestnut weevil (Setsuko et al., 2006), in bone marrow cells and lymphocytes of mice (Madhu et al., 2012).

A significant dose-dependent reduction in sperm count indicates that E-beam induces a depressive effect on spermatogenesis in mammals due to inhibition of cell multiplication and interference in sperm differentiation process (D'Souza, 2003) affecting in particular the spermatogonial cell population on 35th day following exposure to radiations (Wyrobek and Bruce, 1983).

The present data demonstrates that the animals survived following exposure to Median lethal dose of E-beam exhibit severe germinal toxicity as demonstrated by altered sperm morphology and reduced sperm count. It is suggested that E-beam radiations would affect the fertility status of the irradiated mouse or the next generation if breed successfully. The radical damaging effects observed at median lethal dose indicate that E-beam at sub-lethal exposure would induce the testicular toxicity, may be at low magnitude, which may cause impairment in reproductive functioning and other testes associated health problems. Hence, it warrants further studies to evaluate differential effect of E beams on male reproductive system at sub-lethal / therapeutic dose to evaluate safe dose particularly in clinical applications.

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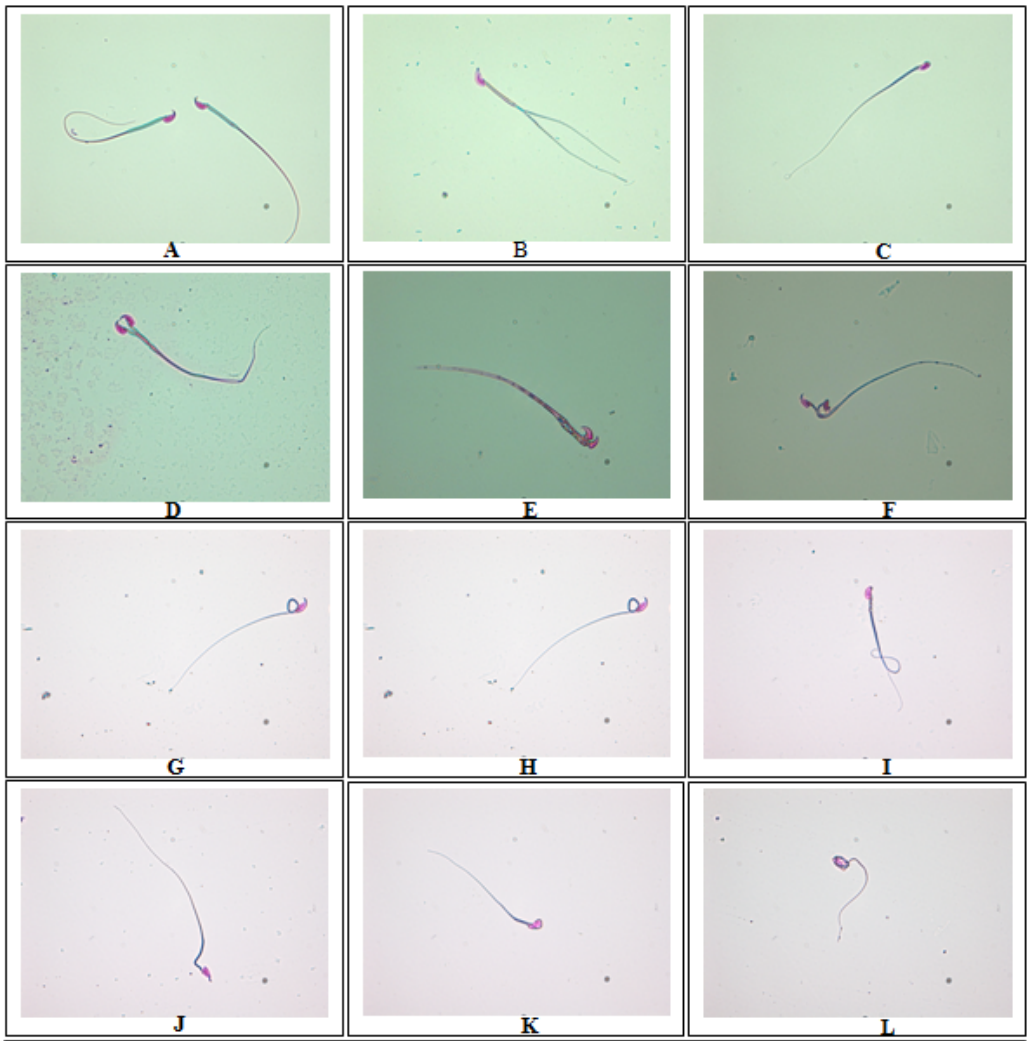

Fig-1 : Showing normal (A) and abnormal sperms (B) - Double tailed ., (C)- Hookless., (D ,E and F) Double headed ., (G and H)- folded neck (I)- Folded tail ., (J) - Banana ., (K and L) - amorphous in mouse, *Mus musculus* following whole body exposure to a medianlethal dose (11-12 Gy) of E beam radiations

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