Asian J. Exp. Sci., Vol. 19, No. 1, 2005, 131-140

Effect of LASER exposure on scrotal sacs and sperm head morphology of Swiss albino mice, *Mus musculus*

Soma Banerjee[1], C.S. Chakrabarti[1], L.K. Samanta[2]

1. Cytogenetics Section
   Department of Zoology
   University of Burdwan, GolapBag
   Burdwan – 713104
2. LASER Section
   Department of Physics
   University of Burdwan, GolapBag
   Burdwan – 713104

Abstract : LASER is a widely used device in the medical field. In vivo effect of singular and repeated exposure of laser beam on a mammalian model was studied to ascertain any possible effect on mammalian germ cells. Since agents considered to be mutagenic affect sperm head shape, sperm morphology study may be an applicable screen for laser effects on germ cells. When Swiss Albino mice were exposed to laser beam, then significant (at both 1% and 5% levels) morphological changes of sperm heads occurred and increased with repetitive exposure. Also, normal sperm count decreased and scrotal sac lesions increased w.r.t. control. It is suggested that laser may have an adverse effect on male germ cells.

Key Words : Laser exposure, sperm head morphology, scrotal sac, lesions, abnormalities.

Introduction

Studies with Helium-Neon laser (wavelength = 632.8nm) have revealed photo biological and photo damage effects with evidence of interference with cell replication (Cadet *et al*., 1987). However, effects of laser exposure on germ cells have not been characterized on in vivo mammalian systems. As study of sperm head morphology in mammals provides a unique approach to quantiating the effects of environmental agents on germ cells (Koch *et al*., 1989; Pomerantseva *et al*., 1980; Topham *et al.*, 1980), the present study was designed to note the in vivo effects of single and chronic laser exposure on mammalian germ cells.

Materials and Method

Fifteen sets, each comprising of 36 out bred male Swiss albino mice (*Mus musculus*), weighing 28-30 grams, 6 weeks old and maintained on a

standard diet of lentils, gram and gram-flour were subjected to laser exposure for 5, 10 and 15 minutes. The laser source was a 5 mW Helium-Neon Japanese NEO-3M model with a wavelength of 632.8 nm. Laser beam was focused on scrotal sacs.

The treatment protocol and sacrificial day has been summarized in table 1. Three control groups (unexposed and age, sex and weight matched) were maintained for each duration of exposure on similar diet and sacrificed after 7^{th}, 14^{th}, and 21^{st} day.

Mice were killed by cervical dislocation after careful examination of scrotal sacs for any external lesions. Then, the skin of scrotal sacs was removed and examination was done for any internal lesions of skin. Vas deferens and epididymis of testes were dissected out and suspended in 0.87% normal saline, kept at 37^0 C. The contents were teased out, suspended in the saline solution and smeared on clean glass slides. After air drying, the smears were stained in Giemsa : Phosphate Buffer = 1 : 15.

Results

The results have been summarized in tables 2, 3 and 4 and have been evaluated at 1% and 5% level of significance.

Discussion

Only one mouse, sacrificed after 21 days, of the three control sets (3*36 mice) developed an external lesion whereas all the treated sets comprised of different numbers of mice with lesions. So, scrotal sac lesion occurrence is a significant ($p<0.05$) effect of laser exposure. Overall percentage of external lesions was more (28.33%) than internal lesions (23.33%). Thus, the radiation effect is more pronounced superficially. When the lesions spread inwards, sub cutaneous lesions appeared because only 12 out of 540 treated mice developed only inner lesions, whereas, 153 treated mice (total=540) developed lesions first on the skin surface. The skin of scrotal sac is sensitive to laser beam and perhaps to other long amplitude radiations; may be the sub cutaneous reaction occurs after it is initiated on superficial skin. The longer the exposure duration and incubation period, the greater is the percentage of lesions. Also, % lesions increased with repetitive exposure.

Results documented in tables 3 and 4 show that direct irradiation by a laser source led to dosage dependent increase in the fraction of sperms with head shape abnormalities. Even, minimum exposure of 5 minutes and incubation of 7 days showed a significant percentage (11.6 %) of abnormal sperm heads compared to 2.78 % of the control series. Same types of anomalies were recorded in all the treated series, four types of sperm head abnormalities have been shown in the photographs. Effect of incubation periods of 7, 14 and 21 days was also significant for each duration of exposure. With repetition of laser exposure, frequencies of sperm head anomalies increased at 5% level of significance. So, it seems that, chronic exposure is even more harmful. Count of sperms with normal head morphology decreased from 97.22% in untreated mice series to 79.31% in the highest exposure of the C_2 series, *i.e.*, 15 minutes exposure given thrice at 7 days interval. This may adversely affect reproductive capacity of male mice. Evidences from previous mouse studies (Koch *et al.*, 1989; Pomerantseva *et al.*, 1980; Topham *et al.*, 1980; Dobrzynska *et al.*, 2000; Dobrzynsk *et al.*, 1994; Dobrzynsk *et al.*, 2004; Wyrobek *et al.*, 1979), suggest that, in general, sperm head shape is affected by physical and chemical agents, considered to be mutagenic, like X rays, gamma rays etc. These results are documented in other mammalian species, including man (Koch *et al.*, 1989) In the present investigation, the reduction in normal shaped sperms may bear a significance on male fertility of those who experience acute and chronic exposure to laser sources. Also, laser appears to be harmful.

Spermatogenesis is a complex cytomorphological event controlled by various sets of genes and their products, the final shape and size of spermatozoon in a species being determined at the very late phase of spermiogenesis (Ray *et al.*, 1991; Ray *et al.*, 1988). So, change in sperm head morphology may be an applicable screen for radiation exposure effect on germ cells and events of late phase of spermiogenesis. From this discussion, it can be concluded that laser treatment is adversely affecting mammalian germ cell metabolism, which is being manifested by high frequencies of abnormal sperm heads, and its effect on male fertility needs to be determined.

Table - 1

The treatment protocol and sacrifical day has been summarised in table - 1

Duration of Exposure	Sets of mice	Incubation period			
		1st day	7th day	14th day	21st day
5 minutes	a_1	+	s		
	a_2	+		s	
	a_3	+			s
	b	+	+	s	
	c	+	+	+	s
10 minutes	a_4	+	s		
	a_5	+		s	
	a_6	+			s
	b_1	+	+	s	
	c_1	+	+	+	s
15 minutes	a_7	+	s		
	a_8	+		s	
	a_9	+			s
	b_2	+	+	s	
	c_2	+	+	+	s

Key : +' – Laser beam exposure

s – Sacrifice

Table - 2

Internal and External lesions of Scrotal Sacs

Length of laser Exposure	Sets of mice	External lesions		Internal lesions		Both internal & external lesions	
			%		%		%
5 minutes	a_1	4	11.11	3	8.33	1	0.02
	a_2	7	19.40	4	11.11	4	11.11
	a_3	7	19.40	7	19.40	5	13.80
	b	9	25.00	7	19.40	7	19.40
	c	12	33.33	9	25.00	8	22.22
10 minutes	a_4	5	13.80	3	8.33	4	11.11
	a_5	6	16.66	6	16.66	5	13.80
	a_6	8	22.22	7	19.44	6	16.66
	b_1	11	30.55	10	27.77	10	27.77
	c_1	16	44.44	13	36.11	12	33.33
15 minutes	a_7	7	19.44	4	11.11	4	11.11
	a_8	9	25.00	7	19.44	6	16.66
	a_9	11	30.55	11	30.55	11	30.55
	b_2	18	50.00	15	41.66	14	38.88
	c_2	23	63.88	20	55.55	19	52.77

Key : Miceset number are in accordance with table1.Each set comprises of 36 mice Mice showing both types of lesions are overlapping with external and internal lesions (e.g. in a set a_1 ,4+3=7 mice,out of 36,developed either external or internal lesions and 1 out of 7 develop both).

Table - 3

Frequencies of different sperm head shapes of mice in experimental sets a_1 - a_9 (w.r.t. Table1)

Time duration and period of laser exposure	Sets of mice	Types of changes in sperm head morphology scored from 7200 sperms for each dose										
		E	T	Bl	H	R	P	UN	Total aberrations	%	Mean	Standard Error of mean (+\-)
5 Mins												
7 days	a_1	136	116	122	121	115	110	118	838	11.64	119.71	3.11
14 days	a_2	166	138	133	120	128	113	115	913	12.68	130.43	6.88
21 days	a_3	154	144	154	143	137	130	120	982	13.64	140.29	4.69
10 Mins												
7 days	a_4	142	118	124	120	125	128	129	886	12.31	126.57	2.97
14 days	a_5	178	152	138	132	134	131	123	988	13.72	141.14	6.99
21 days	a_6	190	164	166	154	149	141	125	1089	15.13	155.57	7.80
15 Mins												
7 days	a_7	172	127	135	124	163	132	126	979	13.6	139.86	7.34
14 days	a_8	178	160	163	148	174	181	178	1182	16.42	168.86	4.60
21 days	a_9	198	183	187	156	184	152	195	1255	17.43	179.29	6.87

Key : ***E = Elongated head; T = Thread like head; Bl = Balloon-shaped head; H = Hammer-headed; R = Round head; P = Pear-shaped head;UN = Unspecified shapes.***

Table - 4

Frequencies of different sperm head shapes of experimental sets b - b_2, c-c_2 (w.r.t. Table1)

Time duration and period of laser exposure	Sets of mice	Types of changes in sperm head morphology scored from 7200 sperms for each dose										
		E	**T**	**Bl**	**H**	**R**	**P**	**UN**	**Total aberrations**	**%**	**Mean**	**Standard Error of mean (+\-)**
5 Mins												
	b	159	163	151	138	109	163	152	1035	14.38	147.86	7.27
	c	171	197	153	169	131	178	167	1166	16.19	166.57	7.26
10 Mins												
	b_1	166	181	164	186	154	189	181	1221	16.96	174.43	4.95
	c_1	183	198	173	195	162	201	196	1308	18.17	186.86	5.56
15 Mins												
	b_2	203	201	179	207	177	219	208	1394	19.36	199.14	5.87
	c_2	208	213	189	221	209	227	223	1490	20.69	212.86	4.82

Key : ***E = Elongated head; T = Thread like head; Bl = Balloon-shaped head; H = Hammer-headed;***
R = Round head; P = Pear-shaped head;UN = Unspecified shapes.

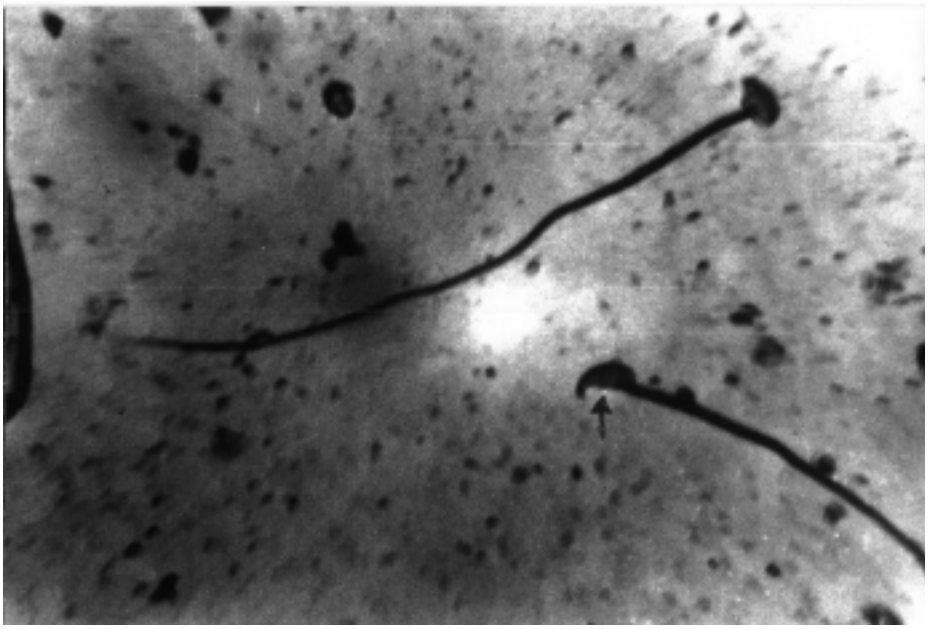

**Photo 1 : Normal head (shown with an arrow),
Hammer shaped head, magnification : 400x**

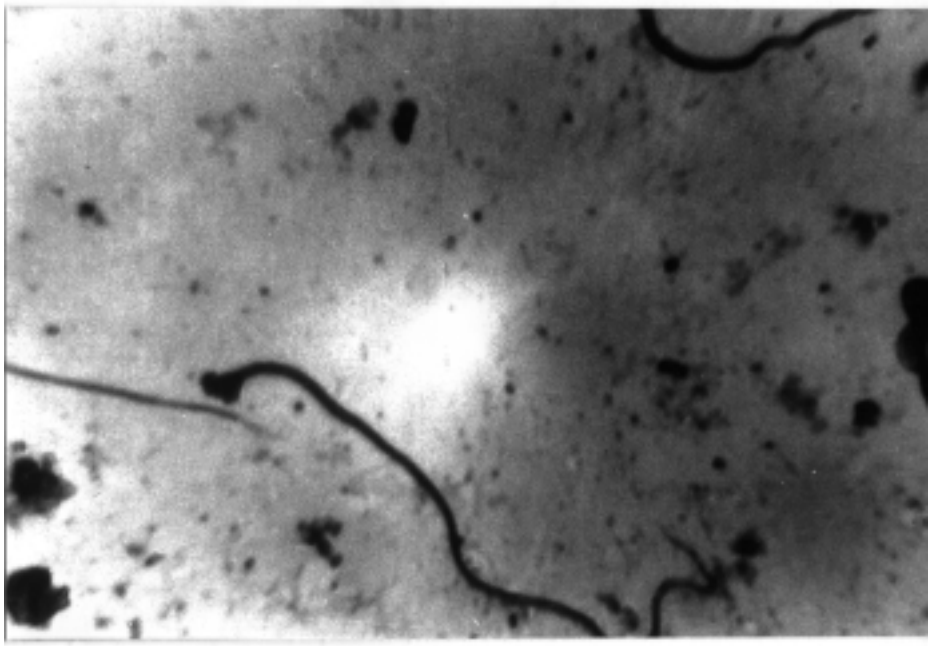

Photo 2 : Pear shaped head, magnification : 400x

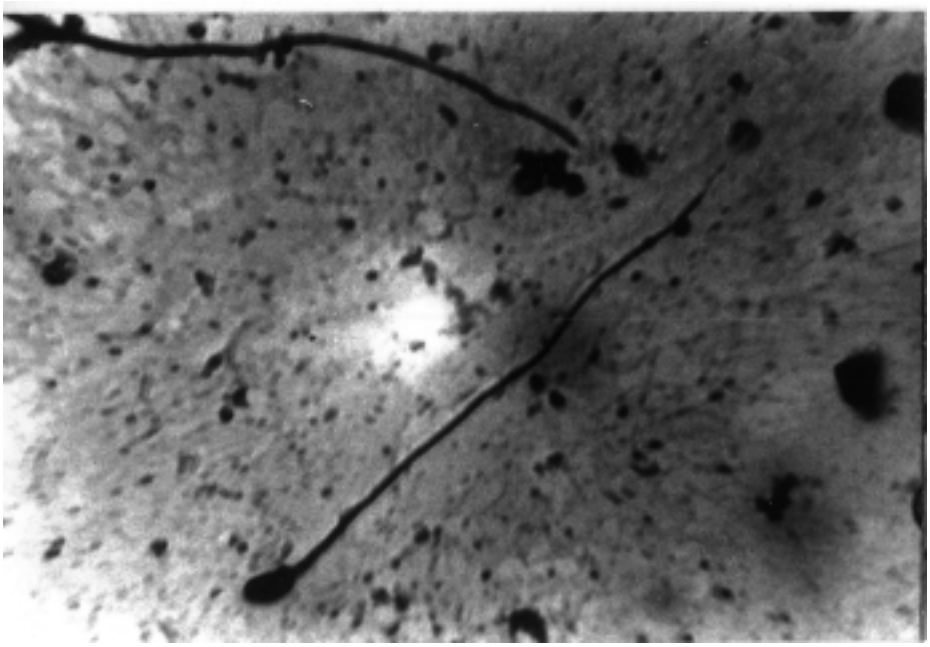

Photo 3 : Balloon shaped head, magnification : 400x

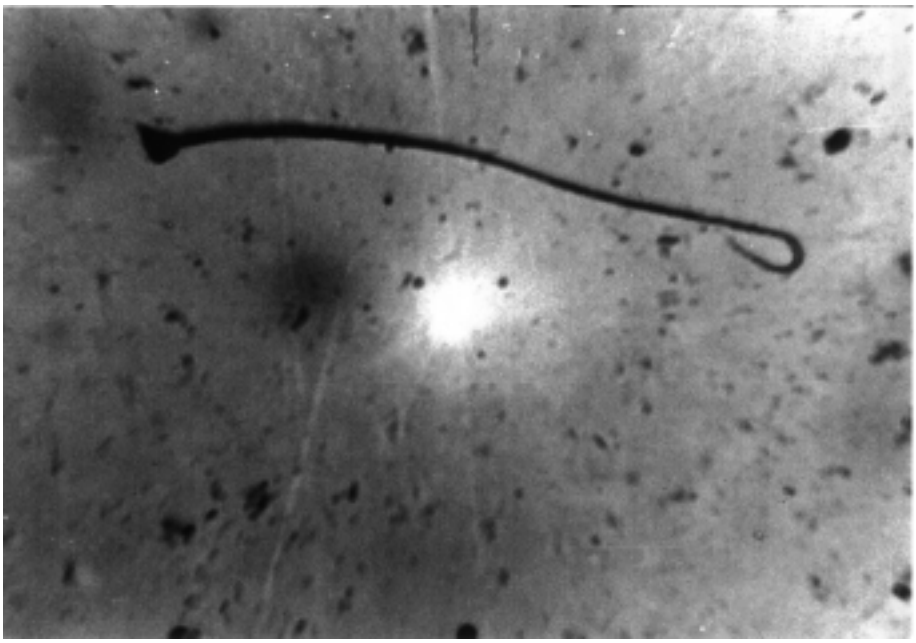

Photo 4 : Unspecified shape or funnel shaped head : 400x

References

Cadet J., Berger M., Decarroz C. *et. al.* (1987) : Ultraviolet photochemistry of nucleic acids using lasers of high intensity ; In : Lasers And Their applications. (ed) A. Spasov (*World Scientific Publishing Co. Pte – Ltd.*), 508.

Dobrzynska M. M. and Gajewski A. K. (2000) : Induction of micronuclei in bone marrow and sperm head abnormalities after combined exposure of mice to low doses of X-rays and acrylamide; *Teratog. Carcinog. Mutagen*., **20(3),** 133 – 40.

Dobrzynska M. M. and Gajewski A. K. (1994) : Mouse dominant lethal and sperm abnormality studies with combined exposure to X-rays and mitomycin-C; *Mutat. Res*., **306(2),** 203 – 9.

Dobrzynska M. M., Slowikowska M. G. and Mikulska U. (2004) : The change in reproductive ability of male mice exposed to vinblastine and X-rays; *Rocz. Panstw. Zakl. Hig*., **55(2),** 147 – 57.

Koch S., Kramer A., Stein J. *et. al*. (1989) : Mutagenicity testing in the sperm head test / mouse and mutagenic potency of 2 disinfectants on he basis of peracetic acid phenols respectively; *Zentralbl. Hyg. Umweltmed*., **188(5),** 391 – 403.

Pomerantseva M. D., Ramaiia L. K. and Vilkina G. A. (1980) : Comparative effectiveness of different tests to determine the mutagenicity of certain factors in mammals. II. Frequency of anomalous sperm head in mice exposed to different factors; *Genetika*, **16(8),** 1397 – 1403.

Ray D., Ghosal S. K. and Maiti C. R. (1991) : Pachytene frequence, RNA synthesis pattern in germ cell line of albino rats treated with ethinyl estradiol and dl – norgestrel; *J. Exptl. Biol*. .

Ray D. and Roy R. (1988) : Meiotic transitions in ethinyl estradiol and dl – norgestrel treated male albino rats; *J. Sci. Res*., **10,** 15 – 19.

Topham J. C. (1980) : Do induced sperm head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens?; *Mutat. Res*., **74(5),** 379 – 87.

Wyrobek A. J. (1979) : Changes in mammalian sperm morphology after X-ray and chemical exposures; *Genetics*, **92 (1 Pt. 1 Suppl.),** 105 – 19.