

Restoration of fertility in male rats exposed to lead by testosterone

Reshma Anjum M¹, Hari Priya P¹, Madhu P¹, Pratap Reddy K¹, Sreenivasula Reddy P^{2*}

1. Department of Biotechnology, S.V. University, Tirupati-517502, India

2. Department of Zoology, S.V. University, Tirupati-517502, India

Abstract

Lead or lead together with testosterone was given to adult rats in order to assess the protective effect of testosterone supplementation on lead-induced suppressed fertility in male rats. Male Wistar rats were exposed to lead acetate at dose level of 0.05 and 0.15 % for 55 days through drinking water and injected intraperitoneally with either testoviron depot at a dose of 4.16 mg/Kg body weight or vehicle alone on day 1, 7 and 14. To assess the fertility, control and experimental males were cohabited with sexually mature 100 day old females for 8 days. Significant decrease in mating index and fertility index were observed with an increase in conception time with male rats exposed to lead. Significant decrease was also observed in fertility rate, measured by counting live fetuses in the uterus of females mated with males exposed to lead. Pre- and post- implantation losses were increased significantly in females mated with lead exposed males. Testosterone supplementation restored the suppressed fertility in Pb-exposed rats. These data demonstrate that fertilization capacity of adult male rats exposed to lead decreased significantly and testosterone supplementation mitigated lead-induced suppressed male fertility.

Keywords: Lead acetate, Rat, Testosterone, Fertility, Implantation

1. Introduction

Now-a-days, a major concern is towards the protection of male reproductive health, since; a range of endocrine disruptors (EDs) target the machinery of male reproduction which is supported by hormones of diverse nature and origin (1). Metal contaminants are unique EDs and interfere with endocrine regulated processes in animal systems (2). The preponderance of these pollutants is mainly due to anthropogenic life style and rapid industrialization (3, 4). Lead (Pb) is one of the metal pollutants which is a ubiquitous toxic heavy metal and widely distributed environmental contaminant (5). Pb exposure targets many vital processes including reproduction (6).

Reproductive consequences of Pb poisoning are severe and almost all compartments of reproductive system are vulnerable targets to Pb stress (7). Studies of Bonde et al. (8) and Naha et al. (9) suggested that men working in lead based factories showed reduced sperm production and poor sperm quality. It has also been reported that workers exposed to Pb suffered with oligospermia (10) and asthenozoospermia with altered sperm morphology (11). In addition to Pb

induced spermatotoxic effects, studies of Biswas and Ghosh (6) demonstrated that Pb administration reduces the activity levels of testicular steroidogenic enzymes in rats. Thus it appears that, lead burden disturbs hormonal-mediated spermatogenesis and steroidogenesis of male reproduction (12-13).

Lead is an endocrine disruptor which can modify hormonal metabolism by altering synthesis and/or breakdown of testosterone, FSH, LH, or other hormones (14). Testosterone plays a vital role in spermatogenesis. Earlier it has been reported that exogenous administration of testosterone improves the spermatogenesis (15). Mc Lachlan et al. (15) also suggested normalization of maturation from round to elongated spermatids was a predominant action of testosterone in the restoration of spermatogenesis in rats. Further, high doses of testosterone substantially promote spermatogenesis by maintaining or restoring testicular testosterone to 10-40% of normal levels (16).

Hormone replacement therapy is one of the commonly used approaches to treat male reproductive disorders. Since, hypothalamus-pituitary-testicular axis is a vulnerable target to a range of pollutants including lead (17) and exogenous supplementation of hormones such as gonadotropin releasing hormone (GnRH) and

***Corresponding Address:** Prof. P. Sreenivasula Reddy, Department of Zoology, S.V. University, Tirupati – 517502, Andhra Pradesh, India. Email: psreddy1955@gmail.com

testosterone are generally used to support male fertility. It has long been known that testosterone replacement therapy restores partially or completely many of the adverse pathophysiological events which occur during androgen deficiency, including restoration of libido and erectile function, increase in muscle mass, increase in bone mineral density, improvement in mood, cognition and general sense of well-being (18).

The purpose of the present set of experiments was two-fold. First, we studied the effect of lead acetate on fertility output of adult male rats. This study was conducted in an effort to complement our earlier studies, which employed sperm parameters and levels of circulatory reproductive hormones as reproductive endpoints (19). Second, we established whether supplementation of testosterone reduces the risk of lead on suppressed male fertility in adult rats.

2. Materials and methods

2.1. Chemicals

Lead acetate of AR grade was procured from E-Merck and testoviron depot which is available in the form of oily solution (Manufactured by German Remedies, India) was purchased from local drug store, Tirupati. All other chemicals used in study were of the highest purity available and obtained from local commercial sources.

2.2. Animals

Wistar strain albino rats were used for the present study. Adult male (n=60) (body weight 220 ± 10 g) and virgin female (n=60) (body weight 200 ± 10 g) rats were purchased from authorized dealer (M/S Raghavendra Enterprises, Bengaluru, India). Rats were housed in polypropylene cages (18" x 10" x 8") lined with sterilized paddy husk, and provided filtered tap water *ad libitum* and standard rat feed (purchased from Sai Durga Agencies, Bengaluru, India). They were maintained in a well controlled environment (temperature $25 \pm 2^\circ\text{C}$; 12-hour light and 12-hour dark cycle, humidity $50 \pm 10\%$). The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (20) and approved by the Institutional Animal Ethical Committee (Regd. No. 438/01/a/CPCSEA/ dt. 17-07-2001) in its resolution No. 10/(i)/a/CPCSEA/ IACE/ SVU/PSR-MRA).

2.3. Experimental design

Male rats were randomly divided into six groups. The rats in group I served as control and was allowed *ad libitum* access to tap water containing 0.15%

sodium acetate for 55 days. The animals in group II were maintained in a similar way and received intraperitoneal injection of testosterone at a dose of 4.16 mg/Kg body weight on days 1, 7 and 14 respectively. The animals in groups III and IV were allowed *ad libitum* access to tap water containing either 0.05 or 0.15% lead acetate solution (equivalent to 273 or 819 mg/L lead respectively) for 55 days. The rats in groups V and VI were maintained in a similar way as rats in groups III and IV but received testosterone also as in group II. The desired amount of lead acetate was dissolved in distilled water and used immediately. The body weight of rats was recorded at the time of initiation and completion of the experiment. Rats were also observed daily for clinical signs of toxicity (salivation, rhinorrhea, lacrimation, ptosis, squinted eyes, convulsions, stupor, tremors), postural changes (erection of fur, exophthalmia) and nonsexual behavior (such as cleaning of face, self grooming, climbing in the cage) from the first day of treatment.

2.4. Fertility studies

After completion of treatment, each male from the control and experimental group was transferred to a mating cage and cohabited with untreated female of proven fertility on a 1:1 basis in the home cage of the male. The maximum duration of pairing was 8 days. The following morning, females were checked for the presence of vaginal plugs and vaginal smears were examined for the presence of spermatozoa to determine whether copulation had occurred; this day was defined as gestation day 0 (21). All confirmed pregnant females were moved into separate cages and housed individually. The conception time, the interval between the first day of cohabitation and the day of vaginal plug/or sperm in vaginal smear, was recorded for each female. The number of pregnant rats with each experimental or the control group was recorded for determination of fertility index. All confirmed pregnant females were caged individually. On the 6th day of pregnancy, laparotomies were performed using 6 pregnant rats from each group, numbers of corpora lutea (representing the numbers of ovulated oocytes) and numbers of implantations were counted. Cesarean sections were also performed on day 19 of gestation in the remaining pregnant rats from each group; the uteri were dissected and the numbers of live fetuses and resorption sites were recorded. Mating index, fertility index, pre- and post-implantation loss were calculated using the following formula:

Mating index (%) = [(No. of females kept for mating – No. of mated females)/No. of females kept

for mating] X 100

Fertility index (%) = [(No. of females mated – No. of pregnant females)/No. of females mated] X 100

Pre-implantation loss = [(No. of corpora lutea – No. of implantations)/No. of corpora lutea] X 100

Post-implantation loss = [(No. of implantations – No. of live fetuses)/No. of implantations] X 100

2.5. Statistical analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P < 0.05$ was considered significant. The data were presented as mean $\pm$ S.D. All statistical tests were performed using Statistical Package for Social Sciences (SPSS) version 16.0.

3. Results

The animals exposed to lead acetate at 0.05 and 0.15 % for 55 days did not show any clinical signs of toxicity or none of the rats were excluded from the experiment. No deaths occurred in any of the groups. Treatment of rats with testosterone or lead did not induce significant changes in the body weight at any of the test doses (data not shown).

Generally, rats in all six groups, exhibited signs of sexual motivation as soon as the female was introduced, including licking the female genitalia, grooming, chasing the female, and passing under

each other's body. Though visual observations on male sexual behavior are not quantified, they made several attempts to mount the female. The mean conception time by the control male was 1.21 ± 0.92 to impregnate the female, whereas the mean conception time was 4.5 ± 0.96 and 6.54 ± 1.03 for 0.05% and 0.15% groups, respectively (Table 1).

The fertility index of Pb-exposed male rats was calculated by their ability to impregnate unexposed female rat. Although all females mated with males in the control, 0.05 % or 0.15% groups had copulatory plugs and produced pups (fertility index 100%), the mean number of fetuses per rat was 12.16 ± 0.75 , 7.1 ± 2.12 and 6.5 ± 0.55 in the control, 0.05% and 0.15% Pb-exposed rats, respectively. The mean number of corpora lutea in females mated with males from different groups was comparable among the six groups (Table 1). The mean pre-implantation loss was 7.23 % for the control group, in contrast to 30.98% and 49.24% for 0.05% and 0.15% groups, respectively. The mean post-implantation loss for the control, 0.05% and 0.15% groups was 5.22%, 27.55% and 11.08% respectively (Table 1). Rats exposed to lead and received testosterone took less conception time to impregnate females when compared to lead alone exposed males. Marginal increase in number of implantations and decrease in pre- and post-implantation loss in females mated with

Table 1: Effect of testosterone (T) on reproductive performance in adult male rats exposed to lead acetate (Pb)

Parameter	Control (C)		Lead (Pb) exposed rats			
			0.05%		0.15%	
	C	C + T	Pb	Pb + T	Pb	Pb + T
Conception time* (days)	$1.21^a \pm 0.92$	$1.31^a \pm 0.60$ (8.26)	$4.5^b \pm 0.96$ (271.90)	$2.3^a \pm 0.44$ (-48.89)	$6.54^c \pm 1.03$ (440.50)	$3.6^b \pm 0.65$ (-44.95)
Mating index	100(20/20)	100(20/20)	75(15/20)	90(18/20)	40(8/20)	60(12/20)
Fertility index	100(20/20)	100(20/20)	100(15/15)	100(18/18)	100(8/8)	100(12/12)
No. of corpora lutea/rat	$13.83^a \pm 1.16$	$14.16^a \pm 0.75$ (2.38)	$14.2^a \pm 1.3$ (2.67)	$13.25^a \pm 0.95$ (-6.69)	$14.4^a \pm 1.09$ (4.12)	$13.1^a \pm 1.15$ (-9.03)
No. of implantations/rat	$12.83^a \pm 0.51$	$13.16^a \pm 0.51$ (2.53)	$9.8^b \pm 1.64$ (-23.61)	$12.75^a \pm 1.5$ (30.10)	$7.31^b \pm 0.83$ (-43.03)	$10.2^c \pm 1.41$ (39.54)
Pre-implantation loss/rat (%)	7.23	7.06	30.98	3.77	49.24	22.14
No. of live fetuses/rat	$12.16^a \pm 0.75$	$12.9^a \pm 0.53$ (6.08)	$7.1^b \pm 2.12$ (-41.61)	$9.6^c \pm 0.51$ (35.21)	$6.5^c \pm 0.55$ (-46.54)	$9.3^c \pm 0.81$ (43.08)
Post-implantation loss/rat (%)	5.22	1.98	27.55	24.71	11.08	8.82

* Values are given as mean $\pm$ S.D of ten individuals

Mean values that do not share same superscript differ significantly from each other at $p < 0.05$

Values in the parentheses are percent change from that of control. For calculation of % change and evaluation of 'p' for C+T group, Pb groups untreated rats served as controls; for Pb+T groups respective Pb groups served as controls.

rats co-administered with lead and testosterone. No significant change in fertility parameters was observed in normal rats injected with testosterone when compared with control rats (Table 1).

4. Discussion

In this study, we evaluated the effect of subchronic exposure to lead on fertility output in a rat model. Our main objective was to assess whether the effects of lead on these parameters were reversible. Rats were exposed to Pb for 55 days in order to evaluate its effect through a complete spermatogenic cycle, which takes approximately 55 days in Wistar rats and the length of spermatogenic cycle is considered as biological constant (22).

There was no change in body weight gain of lead-exposed animals indicating that the general metabolic condition of the animals was within normal range after exposure to low levels of lead acetate. The fertility output that was tested was the male's ability to sire offspring in a fixed time period. Male rats exposed to lead were able to impregnate the unexposed female and the rate of pregnancy was observed to be comparatively lower as compared to unexposed male rats. This clearly suggests that exposure to lead for 55 days was effective in reducing male fertility significantly. Whether the reduced fertility observed in lead exposed rats resulted from lower sperm numbers, decreased viable and motile sperms, altered sperm membrane integrity or depressed sexual desire cannot be determined from the present data. Though there was delay in conception time, all the lead exposed males were able to copulate and fertilize the females indicating depressed sexual desire is not a causative factor in affecting fertility in lead exposed male rats.

Females mated with control males had more implantations. The fetal loss may occur both before and after implantation. The observed pre-implantation loss may represent unfertilized, ovulated oocytes or the death of early embryos prior to implantation. The cause of this manifestation is probably due to fertilization of oocytes with damaged spermatozoa. We have reported earlier a significant reduction in daily sperm production, epididymal sperm numbers with deterioration in spermatozoa quality in rats exposed to lead (23). The reduced fertilization capacity of lead-exposed male is probably due to decrease in sperm quality, such as motility, viability and sperm membrane integrity. This is well supported by the observations of Meistrich (24) who reported that infertility occurs when the sperm count falls significantly ($p < 0.05$)

below normal. Our earlier studies also showed reduction in fertility output in lead exposed male rats (25). Additional in-depth fertility studies are needed involving artificial insemination using a fixed number of sperm from the epididymis of control and treated animals and compare fertility between the groups because it will allow assessment of sperm fertility.

The present study was exclusively carried out to know the protective role of testosterone on fertility output in lead exposed rats. It was well established that bioavailability of testosterone is not only important for the maintenance of structural integrity of testis, accessory sex organs and maintenance of spermatogenesis (23) but also essential for expression of secondary sex characters (24). Significant decrease in circulatory testosterone levels was observed in rats after exposure to lead (23). It was also reported that rats exposed to lead exhibited decreased spermatid production and spermatozoon concentration in cauda epididymis (23). The administration of testosterone mitigated lead-induced spermatotoxic effects and restored sperm concentration in lead-exposed rats (26) suggesting testosterone's reproductive protective effect in lead-exposed rats. The reversal effects observed in lead-induced reproductive toxic effects in testosterone supplemented rats suggest that testosterone levels might be maintained in lead-exposed rats after supplementation.

In conclusion, our results show that the reproductive toxic effects of lead in rats can be attributed to lead-induced reduction in steroidogenesis. This burden can be greatly reduced when supplemented with testosterone. Extrapolation of rat data to humans is always difficult. However, it should be noted that the concentrations of lead used in the present study (0.05 and 0.15 %) did not differ markedly from that often found in drinking water in several lead-polluted areas (27). Our observations, if translated to the human situation, emphasize that spermatozoa produced by males working in lead based factories or living in lead contaminated areas may increase risk for abnormal progeny outcome.

Authors' Contribution Statement

PSR conceived the idea, participated in its design, supervised the work, provided the grants for the study, evaluated the data, and coordinated the study. MRA, PHP, PM and KPR participated in designing the study, carried out the treatment of animals, and performed fertility studies. All authors drafted the manuscript for publication, read, and approved the final manuscript.

Acknowledgements

The authors are thankful to the Head, Department of Zoology, S.V. University, Tirupati for providing facilities. The authors thank UGC for financial support in the form of research grant to PSR. We also thank Prof. K.V.S. Sarma for the statistical analysis of data and Mr. S. Umasankar for maintaining rat colony. The authors declare that the experiments conducted during these studies comply with the current laws of their country. PM and KPR are highly thankful to University Grants Commission, New Delhi for financial support in the form of UGC–BSR (RFSMS) fellowships.

Conflict of interest statement

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References

1. Sharpe RM. Pathways of endocrine disruption during male sexual differentiation and masculinisation. *Best Pract Res Clin Endocrinol Metabol.* 2006; 20: 91-110.
2. Castellini C, Mourvaki E, Sartini B, et al. In vitro toxic effects of metal compounds on kinetic traits and ultrastructure of rabbit spermatozoa. *Rep Toxicol.* 2009; 27: 46-54.
3. Fisher IJ, Pain DJ, Thomas VG. A review of lead poisoning from ammunition sources in terrestrial birds. *Biol Conser.* 2006; 131: 421-432.
4. Monsefi M, Alaei S, Moradshahi A, Rohani L. Cadmium-induced infertility in male mice. *Environmental Toxicology.* 2010; 25:94-102.
5. Herman DS, Geraldine M, Venkatesh T. Case report: Evaluation, diagnosis, and treatment of lead poisoning in a patient with occupational lead exposure: a case presentation. *J Occup Med Toxicol.* 2007; 2: 7-10.
6. Biswas NM, Ghosh P. Effects of lead on gonadal activity in albino rats. *Kathmandu Univ Med J.* 2004; 2: 43-46.
7. Marchlewicz M, Wiszniewska B, Gonet B, et al. Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of rats chronically exposed to lead. *BioMetals.* 2007; 20: 13-19.
8. Bonde JP, Joffe M, Apostoli P, et al. Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occup Environ Med.* 2002; 59: 234-242.
9. Naha N, Chowdhury AR. Toxic effect of lead on human spermatozoa: a study among pigment factory workers. *Internat J Occup Env Med.* 2005; 9: 118-123.
10. Gennart JP, Bernard A, Lauwerys R. Assessment of thyroid, testes, kidney and autonomic nervous system function in lead exposed workers. *Internat Arch Occup Env Health.* 1992; 64: 49-57.
11. Chowdhury AR. Recent advances in heavy metals induced effect on male reproductive function-A retrospective. *Al Ameen J Med Sci.* 2009; 2: 37-42.
12. Hsu P, Liu M, Hsu C. et al. Lead exposure causes generation of reactive oxygen species and functional impairment in the rat sperm. *Toxicology.* 1997; 122: 133-143.
13. Singh J, Oneill C, Handelsman DJ. Induction of spermatogenesis by androgens in gonadotropin-deficient (Hpg) mice. *Endocrinology.* 1995; 136: 5311-5321.
14. Queiroz EK, Waissmann W. Occupational exposure and effects on the male reproductive system. *Cader Saude Publica.* 2006; 22: 485-493.
15. McLachlan RI, Wreford NG, Meachem SJ, et al. Effects of testosterone on spermatogenic cell populations in the adult rat. *Biol Reprod.* 1994; 51: 945-955.
16. Zirkin BR, Santulli R, Awoniyi CA, et al. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. *Endocrinology.* 1989; 124: 3043-3049.
17. Sant Ana MG, Spinosa HS, Florio JC, et al. Role of early GnRH administration in sexual behavior disorders of rat pups perinatally exposed to lead. *Neurotoxicol Teratol J.* 2001; 23: 203-212.
18. Bain, J. Testosterone and the aging male: To treat or not to treat. *Maturitas.* 2010; 66: 16-22.
19. Kasperczyk A, Kasperczyk S, Horak S, et al. Assessment of semen function and lipid peroxidation among lead exposed men. *Toxicol Appl Pharmacol.* 2008; 228: 378-384.
20. CPCSEA. CPCSEA guidelines for laboratory animal facility. *Indian J Pharmacol.* 2003; 35: 257-274.
21. Dunnick JK, Gupta BN, Harris MW, et al. Reproductive toxicity of dimethyl methyl phosphonate in male Fischer 344 rat. *Toxicol Appl Pharmacol.* 1984; 73: 480-490.
22. Clermont Y. Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycle and spermatogonial renewal. *Physiol Rev.* 1972; 52: 198-265.
23. Reshma Anjum M, Sainath SB, Suneetha Y, et al. Lead acetate induced reproductive and paternal

- mediated developmental toxicity in rats. *Ecotoxicol Environ Saf.* 2011; 74: 973-978.
24. Meistrich ML. Evaluation of reproductive toxicity by testicular sperm head counts. *J Am College Toxicol.* 1989; 8: 551-567.
25. Hari Priya P, Girish BP, Reddy PS. Restraint stress and lead-induced reduction in progeny output by male rats. *J Infertil Rep Biol.* 2013; 1(2): 26-30.
26. Reddy PS, Reshma Anjum M. Lead-Induced Reproductive Toxicity in Rat: Recovery of suppressed reproduction by testosterone. 2012; Lambert Academy Publication, Germany. pp 196.
27. Troesken W. The Great Lead Water Pipe Disaster. 2006; MIT Press, Hayward street, Cambridge. pp 318.