



TESTICULAR VOLUME; INDICATOR FOR MALE FERTILITY IN ALBINO RATS

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ABSTRACT... Objective: To determinate that male fertility influenced by testicular volume in albino rats. **Study Design:** Experimental. **Place and Duration of study:** Institute of Basic Medical Sciences, Dow University of Health Sciences, Karachi, 10 months (November 2009 to August 2010). **Methodology:** Sixty four adult albino rats were obtained from animal house Jinnah Postgraduate Medical Centre for the study and divided into 2 groups. Group A received injection normal saline 1 cc intraperitoneally (IP) daily for 8 weeks. Group B received lead chloride in a dose of 10 mg/kg body weight IP daily. On the day of completion of treatment the animals were sacrificed testes along with epididymis removed and place in Petri dish. The length, breath and width of testes were measured with help of vernier caliper. The spermatozoa were obtained from cauda epididymis. **Results:** The mean \pm SEM of volume testes in group A and B after eight week of treatment were $0.77142 \pm 0.04778 \text{ cm}^3$ & $0.11768 \pm 0.01673 \text{ cm}^3$ respectively. The volume of testes of group B was significantly decreased as compare to group A ($P = 0.000$). Mean \pm SEM number of sperm cells million / ml in groups A and B after eight week of treatment was 7.65 ± 186706.553 & 1.84 ± 132792.770 respectively. Number of sperms in group B were significantly decreased as compared to group A ($P = 0.000$). **Conclusion:** There was relationship between volume of testes and male fertility.

Key word: Testicular Volume, Male Fertility, Lead Chloride, Sperms, Albino Rats, Cauda Epididymis

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INTRODUCTION

Assessment of testicular volume provides useful information about the spermatogenesis.¹ The testicular volume reflects the spermatogenesis, as about 70 to 80 % of testicular mass generated by the seminiferous tubules. Testicular volume is indicating the male fertility and helpful for semen profiles in infertile men, and it has been useful in estimating spermatogenesis. The different methods to measure the testicular volume are vernier calipers, orchidometers and ultrasonography (US). Orchidometry is a conventional method and has been used for about 40 years; currently ultrasonography is the most popular and accurate technique to calculate the testicular volumes and can be used for actual testicular volume.²

Sexual maturation and differentiation is a continuous process starting early at the time of fertilization and during the 7th & 12th after fertilization. At

time of puberty the process of sexual maturation is indicated by rapid and accelerated somatic development and increased secretion of male sex hormones. At time of puberty the earliest secondary sex characteristic which in male observed in male are increase in the size of testis, scrotum, and later an increase in the size of penis.³

There is evidence that sperm competition might have been important in the evolutionary lineage leading to humans. In primates, testis size is related to the mating system, the species having single male partner (monogamous species) they have smaller testes as compare to their body size as compare to species having multiple male partners (promiscuous).⁴ Those species have relatively larger testes, the motility of sperm motility is more⁵, and this leads to rapid evolution of genes which are involved in productions of sperm and seminal fluid.⁶ The size of testes in human is mod-

erate according to their body size, midway between those of the socially monogamous (having one male partner) gorilla, and the promiscuous (having multiple male partner) chimpanzee.

The adult male workers who are engaged in different occupations, their blood lead level (BLL) ranging 40 to 70pg /dl. At this blood lead level different biological changes may occur in human body and these changes produce disturbance in the heme biosynthesis pathway and some of the neuropsychological changes and reduce conduction in peripheral nerves. The functions of other body organs may also alter like thyroid, testes, kidney and autonomic nervous system which are exposing to different BLL for long-term.⁷

Precise measurement of testicular volume is important for evaluating testicular function because testicular volume represents the quality of spermatogenesis⁸. Some investigators also demonstrated the relationship of testicular volume and semen quality in infertile patients.⁹⁻¹⁰

Prader's orchidometer is the most popular method for the measurement of testicular volume¹¹. The other methods are ordinary ruler, vernier caliper and ultrasonography used for measurement of volume of testis however; its inaccuracy is a problem. The volume of testes measured in milliliter. The most accurate and popular orchidometer is Lambert's equation (L (length) x W (width) x H (height) x 0.71).¹²

At present the most important and widely distributed environmental toxicant considers to be is the lead. The exposure to lead influences multiple organ systems and also it has ill effects on the male reproductive system in both humans and rodents. Exposure to lead decreases organ weight this is an important index to provide general health status of animals and used as one of parameter to access the adverse effects on reproductive system. Lead toxicity on testes may occur due direct effect on the hypothalamic-pituitary-testicular axis or the high doses of lead crosses the blood-testis barrier and disturbs the activities within the seminiferous tubules. As the lead toxicity is reducing

the appetite which results in loss of body weight and testicular weight both the absolute weights and /or relative weights. Assessment of absolute weight of organ is very important as compare to relative weight of organ as a reduction in the weight of reproductive organ may or may not be related to alteration in body weight. Other reproductive risk parameters that are important for evaluation of lead toxicity are sperm's concentrations, sperms motility and morphology.¹³

The objective of this study was to find that there is any relationship between volume of testes and fertility by determining the number of sperms

MATERIALS AND METHODS

An experimental study carried out at Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences (DUHS), Karachi, from November 2009 to August 2010 (10 months). Sixty Four adult male albino rats were selected for study and obtained from Animal House, IBMS, and DUHS and maintained at food and water ad libitum. The animals were randomly divided into two groups: A and B and each group consist of 32 animals. Each group further subdivided into four groups (each subgroup consisted of 8 animals) on basis of period of treatment these were 1, 3, 5 and 8 weeks.¹⁴ The animals were kept in separate cages.

Control Group-A was divided into 4 subgroups (A1, A2, A3 and A4) and this group received injection normal saline 1 cc intraperitoneally (IP) daily for their respective period of treatment.

Lead Group-B was divided into 4 subgroups (B1, B2, B3 and B4) this group received lead chloride in dose of 10 mg/kg body weight in the distilled water IP daily for 8 weeks.

On the day of completion of period of treatment, animals were sacrificed under deep ether anesthesia. The testes and epididymis were carefully separated.

Two variables were used to assess the testicular activities the volume of testes and number of

sperms.

The volume (V) of testes was calculated by using vernier caliper. Testes were dissected after sacrificing animals and they were kept in Petri dish and their dimension obtained. The length (L), breadth (B) and thickness (T) of testes were calculated with help of vernier caliper and recorded. These values were put into a formula as mentioned by Setchell and Waites, 1964¹⁵, and the volume of testes was obtained. The Setchell and Waites, 1964¹⁵ formula for calculating the volume testes was $V = \frac{4}{3} \times \pi \times L/2 \times W/2 \times T/2$. Here pi (π) is a constant figure and its value is 3.141. In this study Setchell and Waites equation was used.

The epididymis was used for obtaining the sperms. A small nick was given to cauda epididymis with the help of sharp scalpel and was cut and placed in Petri dish containing 5 ml of normal saline. The cut epididymis was left for few minutes in Petri dish so that the sperms from epididymis could swim out of epididymis and collected in saline. The number of sperms was counted with the help of Neubauer ruling method. A small drop of fluid from Petri dish was taken with help of the hemoglobin pipette and charged Neubauer Chamber and number of sperms were counted.¹⁶

The level of significance (P) was calculated by the help of student's t-distribution table with the help of which P- value, read against the table degree of freedom (d. f.). The significance level was considered as $P \leq 0.05$.

OBSERVATIONS AND RESULTS

The present study was designed to observe the effect of testicular volume on the male fertility in albino rats. For that purpose the volume of testes was calculated and to support relationship between the volume of testes and fertility, the number of sperms was also counted. The observations and their results were observed, recorded and compared with their respective control groups and previous studies.

The volumes of testes were calculated by recording the length, width and thickness of testis. The

length, width and thickness were observed by using vernier caliper as shown in figures.

The method of measuring the length of testes is shown in figure-1. The values of length of testes were recorded on excel sheath. The method of measuring the width of testes is shown in figure-2. The values of width of testes were recorded on excel sheath. The method of measuring the thickness of testes is shown in figure-3. The values of thickness of testes were recorded on excel sheath.

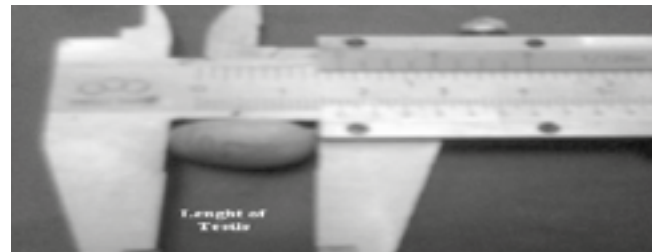


Figure- 1. Photograph showing the method of calculating Length of testes in albino rats



Fig-2. Photograph showing the method of calculating Width of testes in albino rats



Figure-3. Photograph showing the method of calculating thickness of testes in albino rats

The volumes of testes were calculated by applying the Setchell and Waites, 1964¹⁵ formula that was $V = \frac{4}{3} \times \pi \times L/2 \times W/2 \times T/2$. Mean values of volume of testes were calculated and recorded as shown in table-I.

The mean \pm SEM of volume testes in group A and B after eight week of treatment were $0.77142 \pm 0.04778 \text{ cm}^3$ & $0.11768 \pm 0.01673 \text{ cm}^3$ respectively. The volume of testes of group B was significantly decreased as compare to group B ($P = 0.000$).

Mean \pm SEM number of sperm cells million / ml of

seminal fluid of different groups at different time interval were recorded and shown in table-II. The mean \pm SEM number of sperm cells million / ml in groups A and B after eight week of treatment was 7.65 ± 186706.553 & 1.84 ± 132792.770 respectively. Number of sperms in group B were significantly decreased as compared to group A ($P = 0.000$).

Groups	First Week (1) cm^3	Third Week (2) cm^3	Fifth Week (3) cm^3	Eight Week (4) cm^3
A	0.68520 ± 0.05339	0.73353 ± 0.04615	0.75174 ± 0.03541	0.77142 ± 0.04778
n = 20	n = 5	n = 5	n = 5	n = 5
B	0.63461 ± 0.03850	0.36174 ± 0.02900	0.18050 ± 0.02722	0.11768 ± 0.01673
n = 20	n = 5	n = 5	n = 5	n = 5
P- Value**	A1 VS B1 0.455	A2 VS B2 0.000	A3 VS B3 0.000	A4 VS B4 0.000

Table-I. Mean* Volume of Testes cm^3 of different groups at different time interval in albino rats

* Mean \pm SEM ** calculated against 95% confidence interval
P value < 0.05 means statically significant

Groups	First Week (1) million / ml	Third Week (2) million / ml	Fifth Week (3) million / ml	Eight Week (4) million / ml
A	7.04 ± 260162.548	7.34 ± 288540.599	7.52 ± 387954.469	7.52 ± 387954.469
n = 20	n = 5	n = 5	n = 5	n = 5
B	5.59 ± 193972.752	4.19 ± 166096.297	3.26 ± 196360.547	1.84 ± 132792.770
n = 20	n = 5	n = 5	n = 5	n = 5
P- Value**	A1 VS B1 0.001	A2 VS B2 0.000	A3 VS B3 0.000	A4 VS B4 0.000

Table-II. Mean* number of sperm cells million / ml of seminal fluid of different groups at different time interval in albino rats

* Mean \pm SEM ** calculated against 95% confidence interval
P value < 0.05 means statically significant

DISCUSSION

On the basis of this study this result could be acquired that there was a relation between the volume and fertility in male albino rats.

Normal or nearly normal volume of testis is necessary for the proper spermatogenesis. Different methods for measuring the testicular volume are Prader orchidometry, punched-out orchidometry or ultrasonography and by using vernier caliper. The measurements volume of testes is an important landmark for assessment of spermatogenesis. The testicular volume is significantly correlated with the other parameters of testicular function like number, motility & morphology of sperms, along serum FSH, LH and testosterone levels.¹⁴

Lead is a testicular toxicant and interferes with reproductive capability. Some of studies indicated there is a decrease in human sperm quality in the last 50 years; this finding opens the new field to detect the environmental agents and pollutants containing metals.¹⁸

The lead intoxication induced significant reduction in the width of germinal epithelium and number Sertoli cells in lead exposed animals. The number of primary spermatocytes and spermatogonia were decreased after treatment. These findings showed that lead induced morphometric changes were irreversible. Reduced width of germinal epithelium that was seen in this study seems to be due to damage of germinal cells as it was reported previously by other researcher.^{18,19}

Lead induced apoptosis of the germinal cells which was reported by Adhikhari & colleague²⁰ 2001 is possible mechanism for loss of germinal epithelium. Apoptosis has been identified as the major mechanism of toxicant-mediated germ cell death and altering the size of tissue. The fibrosis and necrosis resulted into loss of normal tissues and reduction in tissue and organ mass.

Reduction in testicular volume was the other finding, and it seems that the estimation of volume of testis is a good criterion for defining the testicular atrophy mostly seen in animals treated with high doses toxicant. Some authors used the length and width of as the indices for the estimation of testicular functions after injecting anabolic-androgenic steroid injection, and than it was concluded that this hormone responsible for reduction of testicular parameters.²¹

Batra & coworkers¹⁸ observed significant reduction in type A spermatogonia after lead toxicity associated with decrease of other germ cell populations. In another study complete arrest of spermatogonia was seen in lead treated rats.^{16,18}

The lead blocked the some of key enzymes require the spermatogenesis and altered the production of spermatozoa. The lead is responsible for the degeneration and necrosis of male sex cells decreasing the number of germ cells reduced the diameter of seminiferous tubules. Lead increases the fibrosis.

In this study decreased in volume of testes was resulted in decreased of number of sperms. This finding is correlated with the results of Mastuo N & colleague. According their findings exposure to that the lead was responsible for reduction in the volume of intertubular tissue in the testes of adult rats. Low doses of lead decreased the mice sperms concentrations in the epididymis, while high doses lead reduced the number of sperms, percentage of motile sperm and increased the production of abnormal sperm within the epididymis. Earlier findings showed there was reduction in the vitality of spermatozoa

(asthenozoospermia) and abnormal spermatozoa (teratozoospermia) in the people who were exposed to lead during their occupations and also reduction in sperms count.

CONCLUSION

Based on this study it is concluded that chronic exposure to lead was resulted in loss of tissue mass and decreased in volume of testes with loss of spermatogenesis. Reduced in spermatogenesis was responsible in reduced male fertility.



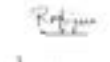
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AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Hina Khan	Help in collecting semen and calculation of volume of testis	
2	Dr. Mazhur-UI-Hauqe	Help in compiling of data on SPSS	
3	Dr. Muhammad Rafique	Corresponding Author Did All works	
4	Dr. Asad Raza Jiskani	Helip in Analysis of data on SPSS	