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Effect of In utero Ultrasound Exposure (5MHz) on Developing Rat Testes

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SUMMARY

The frequent use of diagnostic ultrasound in various branches of medicine, particularly in Obstetrics & Gynaecology has led to consider the safety of ultrasound waves during gestation. Since the embryos and fetuses are at risk for any teratological insult, ultimately leading congenital abnormalities, therefore this study was designed to see the bioeffects of 5 MHz frequency ultrasound waves on the development of testes. Two experimental groups S_2 & S_3 received 6 & 9 exposures of ultrasound waves, respectively of 5 minutes duration each time, during their entire gestation. After parturition the male offsprings were selected and were grown to puberty and then dissected. Their testes were removed and were studied for gross features. The mean testicular weight in control (subgroup S_1) was 2.64±0.29, while in experimental subgroups S_2 & S_3 was 1.99±0.29 & 1.8±0.29, respectively. The relative tissue weight index in control (group S_1) was 1.64±0.18, while in the experimental groups (S_2 & S_3) was 1.15±0.08 and 1.1±0.1, respectively. These results showed statistically significant reduction of mean testicular weight and relative tissue weight indices as compared to control ($p<0.001$). The comparison between experimental subgroups S_2 & S_3 showed statistically non significant difference ($p>0.05$). The conclusion drawn from this study is that ultrasound exposure of 5 MHz produced reduction in mean testicular weight but increased number of exposures did not produced more severe effects.

INTRODUCTION

Ultrasound waves are made up of increase and decrease in pressure with frequency greater than 20,000 cycles per second. Ultrasound waves are used for various diagnostic purposes in medical as well as surgical departments. During the recent years it has become an important diagnostic tool in the field of obstetrics and gynaecology. In 1966, Ian Donald was the first one to recognise the potential applicability of ultrasound in gynaecology and obstetrics.[1] Due to its widespread use in this speciality it seems probable that the most sensitive target that are exposed to ultrasound are the tissues of embryo and fetus, so the potential for inducing adverse effects are greater here. The increasingly widespread use of diagnostic ultrasound necessitate the importance of its safety considerations.

Bioeffects of ultrasound

Ultrasond is capable of producing many bioeffects on tissues and it is now generally accepted that the two main mechanism which may produce biological changes are heat and cavitation.[2] Various studies have been performed in the past to see the adverse effects of ultrasound waves on different organs. In a study pregnant mice were exposed to 2 MHz frequency, continuous wave ultrasound on day 8 of gestation. The most significant finding was a decrease in mean uterine weight.[3] In another study in Manipal, India, the pregnant Swiss albino rats were exposed to diagnostic ultrasound of 3.5 MHz for 10 minutes on day 3.5, 6.5 and 11.5 of gestation. Exposure on day 3.5 of gestation resulted in a small increase in the resorption rate and a significant reduction in fetal body weight. A low fetal weight and an increase in the number of growth retarded fetuses were produced by exposure on day 6.5 of gestation. A statistically nonsignificant increase in the incidence of micro-ophthalmia was induced in fetuses exposed on day 6.5 and 11.5 of gestation.[4]

In an investigation it has been shown that

ultrasound in the diagnostic range appears to cause detectable effect on DNA and growth patterns of aminal cells. There was loss of contact inhibition with a criss crossed growth pattern.[5] It has also been shown that ultrasound exposure of 1 MHz frequency induced statistically significant increase in mutation frequency, invitro, in mammalian cells.[6] Non thermal bioeffects of ultrasound on lungs were studied and haemorrhagic lesions in mouse lungs were found on low frequency output.[7]

Experimental studies have also been performed on animals in which no bioeffects of ultrasound waves were observed. In the University of Illinois, Pregnant hybrid mice were exposed to continuous wave ultrasonic energy at a frequency of 1 MHz for 20 seconds on the eighth day of gestation. Fetal weight (day 18 of gestation) and postmortem pup weight (21, 29 and 42 days post conception) were observed. No significant differences in weight were observed.[8] A similar study using 3 MHz frequency ultrasound waves exposure for 10 minutes/day to pregnant rats on gestational days. 4 to 19 showed no effects on fetal weight and skeletal or visceral malformations.[9]

Since there is little information on the bioeffects of ultrasound waves on developing testes, the present study was designed and conducted to see such effects on developing testes at 5 MHz frequency.

MATERIALS AND METHODS

Forty adult (70-75 days old) female albino rats weighing 200-300 gms and fifteen male rats weighing 400-450 gms of Wistar strain were selected for this study. The rats were obtained from department of animal nutrition, Agricultural University Faisalabad. They were housed in a spacious air-conditioned room of the animal house of Zoology Department, University of Punjab, Quaid-e-Azam Campus, Lahore.

The animals were provided with commercial Chick Feed No. 1. Every 1 kg of this feed contained the following ingredients.

1.	Maize	150 gm
2.	Rice broken	280 gm
3.	Wheat	250 gm
4.	Cotton meal	20 gm
5.	Corn G meal	20 gm
6.	Canola meal	40 gm
7.	Guar meal	30 gm
8.	Soya Bean meal	100 gm
9.	Fish meal	60 gm
10.	Molasses	30 gm
11.	Lime stone	10 gm
12.	Di cal phos	7 gm
13.	L-Lysine	0.8 gm
14.	DL-Meth	0.7 gm
15.	Premix	1.5 gm

The feed was supplemented by the following ingredients, which were added for every 5 kg of the chick feed.

1.	Wheat flour	2½ kg
2.	Molasses	1 kg
3.	Fish meal	100 gms

The following factors were maintained.

A. The feed was given to the rats ad-libitum.
B. Continuous supply of fresh water was provided.
C. Temperature of animal house was maintained between 22°C 25°C with 12 hourly light and dark cycles.

The rats were allowed to acclimatize in this environment for two weeks before the start of the experiment. After this period of two weeks the rats were weighed and an average weight-gain of 25 gm/rat was noted. The female rats were than divided randomly into three groups.

Group No. 1 (Control)

10 female rats were selected for this group.

Group No. 2 (Experimental)

15 female rats were selected for this group.

Group No. 3 (Experimental)

15 female rats were selected for this group.

Conception of rats

For the purpose of conception, three female and one male rat were placed in a cage for six days.

Then after this period the male was removed. This allowed all the forty female rats to conceive at the same time.

The pregnancies were confirmed by the examination of vaginal plug. The appearance of vaginal plug was counted as day 1st of gestation. The total period of gestation in the rats ranges from 20-22 days. This period was divided into three trimesters in this study, each of seven days duration.

Further experimental procedure was as follows:

Control Group No. 1

Ten pregnant rats of this group were set aside without exposure to the ultra sound waves during their gestational period.

Experimental Group No. 2

15 pregnant rats of this group received ultra sound waves exposure of 5MHz frequency, twice weekly during their entire gestation. The time for each exposure was 5 minutes in each rat.

Experimental Group No. 3

15 pregnant rats of this group received ultra sound waves exposure of 5 MHz frequency thrice weekly during entire gestation. The time for each exposure was 5 minutes in each rat.

Procedure of exposure to ultra sound waves and further methodology

The ultrasound machine selected for this experiment was Toshiba, Model SAL 32A Linear array probe with 5MHz frequency transducer. Before exposure the skin of abdomen and pelvis of each rat was shaved off. The rats were taken to the Department of Surgery, Shaikh Zayed Hospital, Lahore. With the help of an assistant, the rats were laid down on a metallic board by holding fore and hind limbs. Then a coupling agent (liquid paraffin) was applied. The 5MHz transducer was applied on the abdomen and pelvis and rotated slowly for 5 minutes. After the parturition of group 1, 2 and 3 one male offspring from each animal was selected at random for further procedure. The male offsprings were sub grouped as groups S1, S2 and S3 respectively. They were placed in separate cages, which were properly labeled. They were nourished and allowed to grow. All these subgroups were dissected at day 70. Each rat was weighed before dissection, their testes were removed and placed on a blotting paper to make them free of surrounding fluid. The detailed study of gross features of the testes with the following gross parameters was performed.

1. **Body Weight:**
   The body weight of each rat was recorded just before they were sacrificed.

2. **Gross Appearance of Testes:**
   The shape, colour, vascularity and weight of paired testes were recorded immediately after dissection.

3. Relative Tissue Weight Index (RTWI):

   This was calculated by the following formula.

$$RTWI = \frac{\text{Mean Weight of Paired Testes (g)}}{\text{Mean Body Weight (g)}} \times 100$$

Statistical analysis

The paired testicular weight and relative tissue weight index was analysed statistically by one way ANOVA. The p value < 0.05 being significant for all analysis.

RESULTS

General physical examination

At the time of sacrifice, all the animals belonging to control and experimental groups were found to be healthy and active. No sign of any ailment was observed. The feeding behaviour was also noted to be normal. No gross congenital abnormality in control and experimental animals was seen.

Gross appearance of testes

The testes were firm, oval and pale pink in color in both control and experimental animals. No apparent difference on gross examination was detected in all groups.

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Gross appearance of testes

The testes were firm, oval and pale pink in color in both control and experimental animals. No apparent difference on gross examination was detected in all groups.

Mean testicular weight and relative tissue weight index (RTWI).

The mean testicular weight in control group (S1) was 2.64±0.2 g while the values in experimental groups S2 and S3 were 1.9±0.2 g and 1.8±0.2 g, respectively. The relative tissue weight index in control (S1) was 1.64±0.18, while in experimental groups S2 and S3 was 1.15±0.08 and 1.1±0.1, respectively (Table 1). Both of these parameters *i.e.* mean paired testicular weight as well as RTWI in groups S2 and S3 showed statistically significant reduction (P>0.001) as compared to control. The comparison between experimental groups S2 and S3 showed statistically non-significant difference P>0.05 (Tables 2, 3).

Table 1: Body weight, paired testicular weight and relative tissue weight index (RTWI) of 70 days old control and experimental animals.

Group	n	Body weight (g)	Paired testicular weight (g)	RTWI
S1 (Control)	10	161.2±5.13	2.64±0.2	1.64±0.18
S2 (Experimental)	15	165.7±3.35	1.9±0.2	1.15±0.08
S3 (Experimental)	15	163.5±5.26	1.8±0.2	1.1±0.1

Values given are Mean±SD

Table 2: Effect of diagnostic ultrasound (5 MHz) on paired testicular weight.

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	Variation ratio (F)
Between levels	4.818	2	2.409	57.908*
Residual	1.5394	37	0.0416	
Total	6.3574	39		
S1 V S2	3.336	1	3.336	80.19***
S1 V S3	4.284	1	4.284	102.98***
S2 V S	0.074	1	0.074	1.77++

S1 = Control group
S2 = Experimental group received 6 exposures of ultrasound in utero.
S3 = Experimental group received 9 exposures of ultrasound in utero.
Significant differences are indicated by asterisk:
***= $p < 0.001$
++ indicate non significant difference $P > 0.05$
Based on one way ANOVA.

DISCUSSION

This study was conducted to evaluate the adverse effects of ultrasound waves on developing tests. Two experimental groups S2 and S3 were exposed to 5 MHz ultrasound waves, 6 and 9 times respectively, during their entire intrauterine period. The time for each exposure was 05 minutes. The results showed statistically significant reduction of mean paired testicular weight ($p<0.001$) as well as relative tissue weight indices ($p<0.001$) in both experimental groups, compared with control group S1. The comparison of these parameters between the two experimental groups showed statistically non significant difference ($p>0.05$). These results indicate that ultrasound exposure does produce adverse effects on testes regarding their paired weight in both experimental groups, but increasing the number of exposure did not produce more severe deleterious effects.

Table 3: Effect of Diagnostic Ultrasound (5 MHz) on Relative Tissue Weight Index

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	Variation ratio (F)
Between levels	1.994	2	0.997	96.8***
Residual	0.381	37	0.0103	
Total	2.375	39		
S1 V S2	1.424	1	1.424	138.29***
S1 V S3	1.741	1	1.741	169.08***
S2 V S	0.0199	1	0.0199	1.933++

S1 = Control group
S2 = Experimental group received 6 exposures of ultrasound in utero.
S3 = Experimental group received 9 exposures of ultrasound in utero.
Significant differences are indicated by asterisk:
***= $p < 0.001$
++ indicate non significant difference $P > 0.05$
Based on one way ANOVA.

Various adverse effects of ultrasound on different body tissues of animals have been identified.[5,7,10] It bioeffects on developing testes, using 1 MHz frequency showed decrease in testes size and daily sperm production in mice.[11] The results of this investigation support the findings of

the present study, however the detailed evaluation of microscopic structure of testes is also required and is being carried out presently and will be reported soon.

CONCLUSION

Ultrasound waves have deleterious effects on developing testes of rats. Although the reduction of paired testicular weight is observed in this study, further work is required to evaluate the reproductive function of the rats exposed to ultrasound during gestational period and also the detailed evaluation of their subsequent offsprings in order to establish the safety of frequent ultrasound exposure during gestation.

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