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Effects of In utero Exposure to Diagnostic Ultrasound (5 MHz) on Developing Rat Ovaries

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SUMMARY

Ultrasound waves are widely used for diagnostic purposes. One of its diagnostic use, which has gained popularity during the recent years is in the field of obstetrics. It is used routinely for the estimation of fetal well being, status of placenta and amniotic fluid. In the past attempts have been made to investigate the adverse effects of ultrasound waves on adult and embryonic tissues. The present study was carried out to see the effects of 5 MHz frequency ultrasound waves on the development of rat ovaries and compared with the control. Two experimental groups B and C received 6 and 9 exposures of ultrasound waves, respectively, during their entire gestation. After parturition, the female litters were selected (subgroups B1 and C1) and dissected at puberty. Their ovaries were studied for gross features. The mean paired ovarian weight of control was 0.09±0.02 g, while the values for experimental groups B1 and C1 were 0.06±0.02 g and 0.07±0.02 g, respectively. The relative tissue weight index in control was 0.06±0.01 and in experimental groups B1 and C1 were 0.04±0.01 and 0.04±0.01, respectively. These results showed statistically significant reduction of mean paired ovarian weight and relative tissue weight indices as compared to control.

INTRODUCTION

Ultrasound scanning is very important in many branches of medicine. It is used for physical therapy, hyperthermia treatments, lithotripsy and particularly has found widespread application in obstetrics and gynaecology. In United States more than half of all pregnancies are examined ultrasonically.[1] The potential applicability of ultrasound was first recognised in 1966 by Ian Donald[2] and since then its use has gained popularity. In many western countries sonographers arrange "baby look" and "fun ultrasound" programmes by which prospective parents are offered an early "meet the baby" opportunity alongwith photographs and home videos.[3] In our country almost every pregnant woman reporting to antenatal clinic in hospitals, is advised ultrasound scanning at least thrice during the whole gestational period in normal pregnancy.

Ultrasound is capable of producing many bioeffects on tissues. Its thermal, mechanical, cavitational and chemical effects are very well established.[4,5,6]

Various epidemiological studies were performed to see the biological effects of ultrasound. A comprehensive research to evaluate the short and long term risks after exposure to diagnostic ultrasound in utero was performed in United States during 1968 to 1984. A cohort of 425 children exposed to diagnostic ultrasound and 381 controlled children were studied for adverse effects at birth and again at 07 and 12 years of age. No biologically significant differences between exposed and unexposed children were found.[7] Randomised controlled trial in Perth, Australia suggested that the increased proportion of growth restricted fetuses had received five ultrasound exposures of 3.5 MHz and 5 MHz frequency during intrauterine period.[8]

Experimental studies have also been performed on animal in vivo and on mammalian cells in vitro. Child et al[9] conducted a study on rat fetuses and confirmed that no effect on fetal weight, number of living fetuses could be attributed to ultrasound exposure. The embryotoxicity of ultrasound exposure during pregnancy has been

investigated in mice, using 1 Mhz frequency continuous wave ultrasound. Results showed that there was a slight increase in the incidence of malformed fetuses and occurrence of multiple malformations in individual fetuses as intensity of the ultrasonic exposure increased.[10] The bioeffects of ultrasound exposure of 7.5 MHz frequency on monkeys during gestational period showed significant reduction in birth weight and crown rump length.[11]

There is little information, regarding the effects of ultrasound waves on intrauterine development of gonads specially the ovaries. As replicating cells are in general more susceptible to external stimuli,[12] so it may be speculated that the probability of damage following ultrasound exposure might be higher in developing ovaries as compared to adult tissues. Therefore, the present study was designed to see the effects of ultrasound waves on female rat ovaries, exposed to ultrasound during fetal period.

MATERIALS AND METHODS

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

The rats were provided with commercial chick feed No.1.

Every 1 kg of chick feed No: 1 contained 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 following ingredients were added for every 05 kilograms of the chick feed.

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

This feed was given to the rats ad libitum. Continuous supply of fresh water was also provided. Temperature of the animal house was maintained between 22°C to 25°C with 12 hourly light and dark cycles.[13] The rats were allowed to acclimatize for two weeks before the start of experiment.

At the end of two weeks the rats were weighed and an average weight gain of 25 grams per rat was noted. The female rats were then randomly divided into three groups:

1. **Control (Group A):** 10 female rats were selected for this group.
2. **Experimental (Group B):** 15 female rats were selected for this group.
3. **Experimental (Group C):** 15 female rats were selected for this group.

Conception of Rats

For the conception, three female and one male rats were placed in a cage for six days and then the male was removed. In this manner all the forty female rats were allowed to conceive simultaneously.

The pregnancies were confirmed by the examination of vaginal plug, the appearance of which was counted as day first of gestation. The total gestation period in rats ranges from 20 to 22 days which in this study was divided into three trimester; each of 07 days.

Further experimental procedure was as follows.

1. **Control (Group A)**
   10 pregnant rats of this group were allowed to complete their gestational period without exposure to ultrasound waves.

2. **Experimental (Group B)**
   15 pregnant rats of this group received ultrasound waves exposure, of 5 MHz frequency, twice weekly i.e. 06 exposures during entire gestation. The time for each exposure was five minutes in each rat.

3. **Experimental (Group C)**
   15 pregnant rats of this group received ultrasound waves exposure of 5 MHz frequency, thrice weekly for a period of five minutes each time i.e 09 exposures during entire gestation.

Procedure of Exposure to Ultrasound Waves

Toshiba, model SAL 32A linear array probe with 5MHz frequency was used. The rats were taken in groups from Zoology Department, University of Punjab (new campus), Lahore to department of surgery, Shaikh Zayed Hospital, Lahore. Skin infront of abdomen and pelvis of each experimental rat was shaved for smooth conduction of ultrasound waves. With the help of an assistant the rats were laid down on a metallic board by holding fore and hind limbs. After applying coupling agent (liquid paraffin), the 5 MHz frequency transducer was applied on the abdomen and the transducer was rotated slowly over the abdomen for five minutes.

This technique allowed whole body exposure of embryos and fetuses of rats, to ultrasound waves since the size of embryos and fetuses of rats are quiet small as compared to size of ultrasound transducer so there was high chance of exposure to ovaries besides other organs.

Further methodology

After the parturition of group A, B and C, only female litters were selected for further procedures which was as follows:

Subgroup A1, B1 and C1.

The female offsprings from group A,B and C were then subgrouped as groups, A1, B1 and C1, respectively and were placed in separate cages with proper labeling. They were nourished and allowed to grow and all the subgroups were dissected at day 70 after weighing each rat. Their ovaries were removed and were placed on blotting paper, to make them free of surrounding fluid. The detailed study of gross features of ovaries was then carried out. The following gross parameters were considered.

1. **Body Weight**
   The body weight of each animal was recorded at the end of experiment just before they were sacrificed.

2. **Gross Appearance of Ovaries.**
   The shape, colour, vascularity and weight of paired ovaries were recorded soon after dissection.

3. **Relative Tissue Weight Index (RTWI).**
   This was calculated by the following formula:-

$$\text{RTWI: } = \frac{\text{Mean weight of paired ovaries (g)}}{\text{Mean body weight (g)}} \times 100$$

Statistical Analysis

The paired ovarian 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

All the animals of control and experimental groups were found to be active and healthy at the time of sacrifice. Their feeding behaviour was normal and showed no sign of any ailment. No gross congenital abnormality in control and experimental animals was observed.

Gross appearance of ovaries

The ovaries were soft in consistency and well vascularised in control as well as in experimental animals. The ovaries looked pinkish in control but reddish in both experimental groups.

Mean paired ovarian weight and relative tissue weight index (RTWI)

The mean weight of paired ovaries in control was found to be 0.09±0.02g, while the values for the experimental groups B1 and C1 turned out to be

0.06±0.02g and 0.07±0.02g, respectively (Table 1). The mean weight of paired ovaries in both the experimental groups reduced significantly as compared to control values whereas the comparison between groups B1 and C1 showed non-significant difference, p>0.05 (Table 2).

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

Group	n	Body weight (g)	Paired ovarian weight (g)	RTWI
A1 (Control)	10	155.1±4.20	0.09±0.02	0.06±0.01
B1 (Experimental)	15	162±4.45	0.064±0.02	0.04±0.01
C1 (Experimental)	15	163.2±4.82	0.07±0.02	0.04±0.01

Values given are Mean±SD

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

Source of variation	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	Variation ratio (F)
Between levels	0.00489	2	0.002445	8.85***
Residual	0.01022	37	0.0002762	
Total	0.01511	39		
A1 V B1	0.004704	1	0.004704	17.03***
A1 V C1	0.0027307	1	0.0027307	9.88**
B1 V C1	.000333	1	0.000333	1.206++

A1 = Control group
B1 = Experimental group received 6 exposures of ultrasound in utero.
C1 = Experimental group received 9 exposures of ultrasound in utero.
Significant differences are indicated by asterics:
** = P < 0.01
*** = P < 0.001
++ indicate non significant difference P > 0.05
Based on one way ANOVA.

The relative tissue weight index in control was found to be 0.06±0.01, while the values for the experimental groups B1 and C1 turned out to be 0.04±0.01 and 0.04±0.01, respectively (Table 1). The relative tissue weight indices in both he experimental groups showed significant reduction as compared to control situation, whereas the comparison between experimental groups B1 and C1 showed non-significant difference, p>0.1 (Table 3).

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	0.002	2	0.001	11.6***
Residual	0.00319	37	0.0000862	
Total	0.00519	39		
A1 V B1	0.0023207	1	0.0023207	26.922***
A1 V C1	0.0016007	1	0.0016007	18.569***
B1 V C1	0.0000833	1	0.0000833	0.966**

A1 = Control group
B1 = Experimental group received 6 exposures of ultrasound in utero.
C1 = Experimental group received 9 exposures of ultrasound in utero.
Significant differences are indicated by asterics:
*** =P < 0.001
++ indicate non significant difference P > 0.05
Based on one way ANOVA.

DISCUSSION

Ultrasound exposure of 5MHz frequency to rat's fetuses in the present study, resulted in significant reduction of mean paired ovarian weight as well as the relative tissue weight indices as compared to the control values. In this study two experimental groups received 6 and 9 exposures of ultrasound waves. The comparison between the two experimental groups revealed non-significant difference. These results indicate that increasing the number of exposure of 5 MHz frequency ultrasound waves during gestation did not produce more adverse effects on mean paired ovarian weight and relative tissue weight indices in rats.

Ultrasound exposure is well known for its adverse effects on various adult body tissues in general.[14,15] Adverse effects of ultrasound on embryonic tissues are also known. For instance, destruction of embryonic tissues and distortion of development of amphibian embryos with 0.88 MHz frequency ultrasound waves.[16]

Regarding fate of development of embryonic ovaries exposed to ultrasound, there is practically no

published data. Therefore, results of the present study are helpful for establishing a base line criteria for the safety of ultrasound during gestation. In the present protocol the exposed foetuses were allowed to grow till day-70, then all of them were scarified to study their ovaries. Further work is needed as to reveal that fate of such experimental female rats regarding their reproductive function and the evaluation of their offsprings.

CONCLUSION

The results of the present investigation produced significant reduction in the paired ovarian weight of rats. Ultrasound waves may be responsible for similar biological effects in human tissues as well. It is therefore recommended that unnecessary exposure to ultrasound waves should be avoided during gestation. Its frequent use without proper indication should be discouraged during pregnancy.

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