ORIGINAL ARTICLES

ENDOCRINOLOGICAL RESPONSE OF RABBIT AFTER EXPOSURE OF OVARIES TO DIAGNOSTIC ULTRASOUND

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ABSTRACT

Objective: To study the serum progesterone and estradiol level in rabbit after exposed of their ovaries to ultrasound waves.

Study Design: Experimental study.

Place and Duration of Study: NIH, Islamabad, Army Medical College Rawalpindi from Mar 2006 to Sep 2006.

Materials and Methods: Forty, adult, non pregnant female rabbits (Oryctolagus cuniculus strain) weighing 900 gm to 1500 gm were used. They were divided in four groups, each containing ten rabbits, Group I was taken as control. Ovaries of other three groups (group II, III and IV) were exposed to ultrasound (frequency of 3.5 MHz and intensity of 96 dB for two minutes) for 3, 6 and 12 days respectively. Two milliliter of blood samples was withdrawn after 24 hours of last exposure. Progesterone and Estrogen levels in serum were analyzed by Chemiluminescence's method. Initial serum Progesterone and Estrogen levels in rabbits not exposed to ultrasound were compared with ultrasound exposed serum levels.

Results: The estradiol level decreased significantly in group IV (P= 0.014) and progesterone level increased significantly in group III (P=0.011).

Conclusion: The current study has revealed that exposure to ultrasound produces changes in hormonal level, if used for longer duration.

Keywords: Ultrasound, Progesterone, Estrogen, Ovary, Rabbit.

INTRODUCTION

Ovary, the vital organ of the female reproductive system, lies along with uterus in between the urinary and alimentary systems. It is liable to undergo morphological, histological and physiological changes when isonated either directly or indirectly [1].

Female rabbits are always in estrus [2], vascularised theca interna cells are the major source of the estrogen through granulosa cells. Ovulation changes the vascularity, the theca cell regress, but the granulosa cells proliferate to form luteal tissue and start secreting estrogen and progesterone [3].

At ovulation in response to copulation or pseudopregnancy the follicular wall collapses following expulsion of ova. Bleeding from the theca externa into follicular lumen leads to the formation of the corpus hemorragicum. The cells of the granulosa and theca interna layers undergo dramatic morphologic changes. They become luteal cells, increase in size and become filled with lipid droplets. A lipid soluble pigment, lipochrome in the cytoplasm of the cells gives them yellow appearance and transforms them into corpus luteum. At the ultrastructural level, the cells demonstrate features associated with steroid secreting cells. Two types of cells are identified granulosa lutein cells and theca lutein cells. They start secreting progesterone and estrogen [4].

The diagnostic superiority of ultrasound has been strongly suggested because of its stipulated safety, and consequently it is being used manifold since its first use by Dussik in 1937 [5]. Ultrasound effects were studied on animals where it; damages DNA the nucleic acid bases, uracil and thymine [6], damages the cells leading to death [7], damages the spinal cord leading to limb paralysis [8] and damages the cell membrane and its disruption [9].

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Received: 11 Aug 2008; Accepted: 17 Nov 2008

Literature on the effect of ultrasound on endocrine gland is fragmentary. There is no placental hormone study where additional studies were urged [10]. In a study on thyroid gland, there was a partial loss of epithelial cells of the thyroid follicle following ultrasound [11] and ultrasonic irradiation led to impaired iodine uptake, hypofunction and reduced iodothyronine synthesis [12]. Keeping the above observation in mind further studies were required on steroiodogenesis.

Therefore this study was planned to see the biochemical response of ovaries if any, to diagnostic ultrasound by observing the serum progesterone and estradiol levels.

MATERIALS AND METHODS

Forty, adult, non pregnant female rabbits (Oryctolagus cuniculus strain) weight 900 gm – 1500 gm were used for study. They were fed on gram, fodder, leafy vegetables, water ad libitum, temperature maintained at 300 C with dark/light cycle of 10/14 hrs in the animal house of NIH, Islamabad.

Animals were divided in four groups. Group I was taken as control to others were exposed to ultrasound. Each animal was laid down supine on rack inclined at about 20° to the horizontal during sonography (Figure). Ovaries of rabbits were exposed to frequency of 3.5 MHz , intensity 96 dB for two minutes for 3, 6 and 12 days in group II, III and IV respectively. Two ml of blood was taken from the marginal vein of the pinna after 24 hours of last exposure. Progesterone and Estrogen ovarian hormonal study except, in human levels in serum were analyzed by Chemiluminescence's method, by using DPC, an automated quantitative immunoassay analyzer Immulite 1000. Initial serum Progesterone and Estrogen levels in rabbits not exposed to ultrasound were compared with ultrasound exposed serum levels.

Data Analysis

Paired samples t-test was used to compare serum levels of the zero and the final day within the groups whereas; Analysis of Variance (ANOVA) was used for comparison among groups. P value < 0.05 was considered as significant. Computer Software SPSS version10.0 was used for data analysis.

RESULTS

Serum Estradiol-17 B (E2)

Mean serum estradiol initial level before exposure to ultrasound in groups I, II, III and IV was 87.59 ± 5.52, 94.01 ± 7.82, 80.06 ± 3.62, 104.52 ± 7.73 respectively and ultrasound exposed serum level was104.15 ± 9.22, 95.51 ± 9.66, 85.36 ± 6.82, 76.33 ± 2.18 respectively. When compared within groups it was statistically significant in group IV (P= 0.014). ANOVA showed non significant result between the groups at both levels (P>0.05), but it was close to significance (Table-1).

Serum Progesterone

Mean serum progesterone initial levels not exposed to ultrasound in groups I, II, III and IV were 1.04 ± 0.20, 0.75 ± 0.096, 0.71 ± 0.083 and 0.81 ± 0.051 respectively and

Table-1 Estradiol (Pmol/L) level in different groups

Groups n=10	Initial level Mean ± S.E	Final level Mean ± S.E	P-value
A-I	87.59 ± 5.52	104.15 ± 9.22	0.114
A-II	94.01 ± 7.82	95.51 ± 9.66	0.628
A-III	80.06 ± 3.62	85.36 ± 6.82	0.431
A-IV	104.52 ± 7.73	76.33 ± 2.18	0.014
P-value	0.077	0.060	

Table-2 Progesterone (nmol/L) level in different groups

Groups n=10	Initial level Mean ± S.E	Final level Mean ± S.E	P-value
A-I	1.04 ± 0.20	1.14 ± 0.21	0.049
A-II	0.75 ± 0.096	0.97 ± 0.15	0.765
A-III	0.71 ± 0.083	1.03 ± 0.092	0.011
A-IV	0.81 ± 0.051	0.93 ± 0.090	0.506
P-value	0.197	0.780	

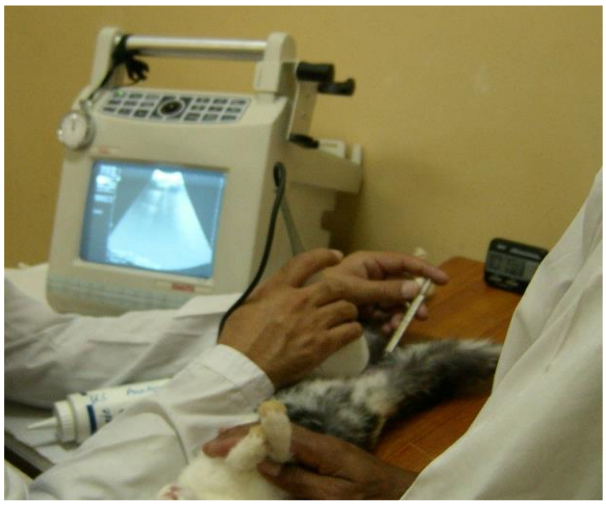

Figure: Ultrasound in progress

ultrasound exposed serum level was1.14 ± 0.21, 0.97 ± 0.15, 1.03 ± 0.092, 0.93 ± 0.090 respectively. When compared within groups it was statistically significant in group III (P= 0.011) (P<0.05). ANOVA showed non significant result between the groups at both levels (P>0.05) (Table-2).

DISCUSSION

The serum estradiol result showed statistically significant difference between zero day level and final day estradiol level in group-IV within groups, when compared among groups it was not significant but the level were at higher side. Serum progesterone level showed statistically significant difference between zero day level and final day level when compared within groups in group-III. When compared among groups it was insignificant. However all values in experimental groups were at augmented level. These reduced estradiol and augmented progesterone levels after ultrasound exposure correlate to the findings of Lee [13]. When whole body of rats and mice were gamma irradiated, estradiol level in both rats and mice were significantly lower than control group. Where as, in mice progesterone level was high than control. It was assumed this might be due to inactivation or activation of enzymes, responsible for the manufacturing of estradiol and progesterone in theca and granulosa cells. The concept of assuming radiation effects are similar to ultrasound was augmented by the study of Bailey et al [14]. They concluded their study on the note that the morphological alterations due to ultrasound are much the same as that resulting from ionizing radiation. Similar results in rats were also found after low doses of gamma irradiation by Freud and sod moriah [15].

The ultrasonic waves are propagated through the body with the speed of 1540m/sec. The fifty percent of waves are reflected back from the different densities of the tissues of the body producing visual images on monitor and fifty percent of waves are absorbed by the body tissues [16].

The absorption coefficient value of ultrasound in the ovarian tissue is highest in the cortex than that of medulla [17]. The absorption coefficient produces primary effects of heat and mechanical effects of cavitation which can affect the parenchyma and stroma of the organ. These shearing forces on the cell membrane can change its antigenic specificity [18], responsible for inflammatory reaction or disturb the organelle which can result in its disturbances in the hormonal synthesis of estradiol and progesterone or cell death (provided the lysosomal wall ruptures). Steroid hormones are synthesized in the mitochondria of theca and granulosa cells of ovary are distributed in the body by blood [3]. Any insult during this stage may derange hormonal balance.

CONCLUSION

The current study has revealed that ultrasound can derange hormonal balance if used repeatedly and for longer duration. Therefore, cautioned use of this diagnostic tool is recommended.

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