

Effects of Ultrasound on Follicular Atresia Count

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Abstract

Background: To observe the morphological effects taking place in ultrasound treated rabbit's ovaries by observing the total atretic follicles count.

Methods: This experimental study was conducted at National Institute of Health Islamabad and Army Medical College Rawalpindi. Forty adult, non pregnant female rabbits (*Oryctolagus Cuniculus* strain) weight 900 gm-1500 gm were used. They were divided in four groups, I, II, III & IV, each containing ten rabbits, Group I was taken as control. Ovaries of others were exposed to ultrasound frequency of 3.5 MHz and intensity of 96 dB for two minutes for 3, 6 and 12 days. After euthanasia, ovaries were dissected, fixed and processed. Atretic follicles from the largest cross section stained by hematoxylin and eosin were counted. Analysis of variance (ANOVA) was used for statistical comparison among groups.

Results: The current study showed increase in total atretic follicles count especially in group III

Conclusion: Exposure to ultrasound produces morphological changes in ovaries.

Key words: Ultrasound, Rabbit, Ovary, Follicle, Atretic.

Introduction

In recent years the diagnostic dominance of ultrasound has been strongly recommended because of its predetermined safety, and as a result it is being used manifold since its first use by Dussik in 1937.¹ Ultrasound effects include those on nucleic acid bases, uracil and thymine of DNA,² the cell death³, hind limb paralysis⁴ and ultrasound mediated disruption of cell membrane.⁵

The frequency employed in Obstetrics /Gynaecology varies from 2 - 3.5 M Hz⁶ and intensity ranges from 5 m W/cm² to 10 W/cm².⁷ Waves are propagated through the body with the speed of

1540m/sec. Fifty percent of waves are reflected back from the different densities of the tissues of the body producing visual images on monitor. Fifty percent of waves absorbed by the body tissues produce primary effects⁸ and secondary biological effects.⁹

Ovary, stockroom of ova, the genome of potential generation and the central appendage of the female reproductive system, lies with uterus in between the urinary and alimentary system. It is prone to endure morphological and histological change either isonated directly or indirectly.

Follucogenesis is a continuous process. Follicles degenerate and disappear through a process called ovarian follicular atresia. This process continues in prenatal, natal and post natal life. Lee¹⁰ showed gamma irradiated apoptosis of follicles. Literature on the effect of ultrasound on atresia of follicles is patchy. Frequency 1 MHz, Intensity 25 w/cm (spatial peak) for 30 sec used by O' Brien¹¹ showed it to be disrupting testicular tissue by affecting both spermatogenesis and spermiogenesis and by disrupting the interstitial tissue. Bailey¹² exposed mouse ovaries with frequency 1 MHz, intensities ranging from 5 to 100 W/cm² and time 300 to 15 sec. showed general cellular disruption with preference for degenerating follicles but no mention of numerically affected follicles. Additional studies were required with commercially available ultrasound machines with routine obstetrics and gynaecology probes and exposures.

This study was designed to observe the numerical response of ovary to diagnostic ultrasound by observing the atretic follicle numbers

Material and Methods

Forty adult, non pregnant female rabbits (*Oryctolagus Cuniculus* strain) weight 900 gm - 1500 gm were, fed on gram, fodder, leafy vegetables, water and libitum. Temperature was maintained at 30^o C with dark/light cycle of 10/14 hrs in the animal house of NIH, Islamabad which was used for the study.

Animals were divided into four groups. Group I was taken as control and others were exposed to ultrasound

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Fig.1 Ultrasonography in progress.

The skin in front of genital tract was shaved by hair removing cream for smooth conduction of ultrasound. 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. The animal was laid supine on a rack inclined at 20° to the horizontal during sonography (Fig.1). Falco unit 100. Model no. 410477 with 3.5 MHz R40 curved Array and HiD probe 410638 manufactured by Pie Medical (Netherlands) were used. After euthanasia, ovaries were dissected, labelled, fixed, processed and stained by haematoxylin and eosin. Atretic follicles were counted from the largest cross section of ovary.

Analysis of Variance (ANOVA) was used for comparison among groups. p value < 0.05 was considered as significant. Computer Software SPSS version 10.0 was used for data analysis.

Results

Left Ovary

The mean atretic follicle count ± S.D in groups I, II, III, IV were 7.80 ± 1.65, 6.50 ± .68, 7.60 ± 1.49 and

6.00 ± 1.13 respectively. When ANOVA was carried out it was observed that the difference was statistically insignificant (p=0.760) (Table 1 and Fig 1).

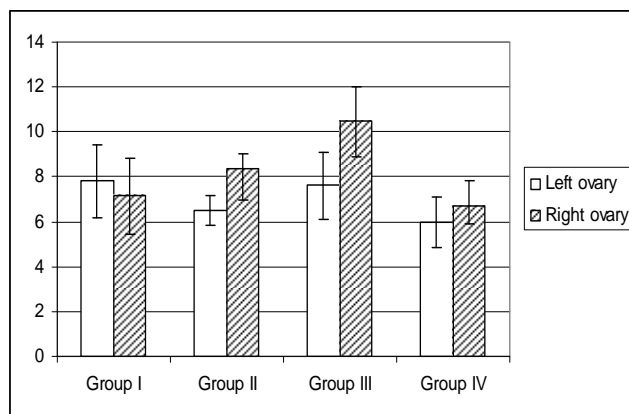


Fig.2 Atretic follicle count

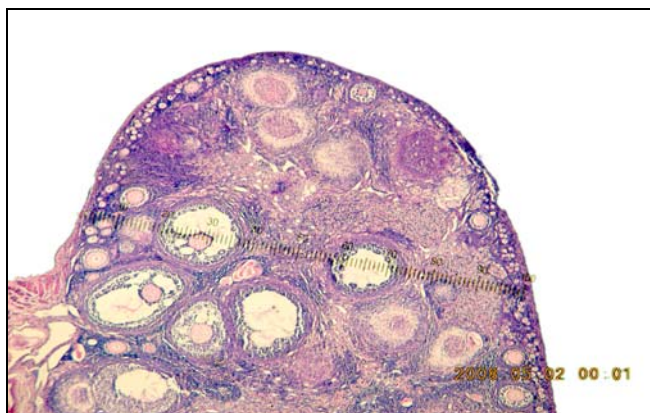


Fig.3 Photomicrograph of an ovary (AIII4L) showing atretic follicles.

Right Ovary

The mean atretic follicle count ± S.D in groups I, II, III, IV were 7.16 ± 1.74, 8.37 ± 1.41, 10.50 ± 1.57 and 6.71 ± .8081 respectively. When ANOVA was carried out it was observed that the difference was statistically non significant (p=0.251) (Table 1 and Fig 2).

Table.1 Total Atretic count

Atretic	Group I		Group II		Group III		Group IV		p-Value
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	
Left ovary	7.80	1.65	6.50	.68	7.60	1.49	6.00	1.13	0.76
Right ovary	7.16	1.740	8.37	1.41	10.50	1.57	6.71	.8081	0.25

Discussion

The mammalian ovary is physiologically dynamic and anatomically heterogeneous, consisting of several distinct structures. The cortex is a tough connective tissue stroma consisting of collagen, with layers of follicles, corpora lutea, covered by tunica albuginea which is epithelized by germinal epithelium. The medulla composed of loose connective tissue with blood vessels, nerves and hilar cells¹³. Female rabbits are always in estrus, and induced ovulator¹⁴. The size, number and life span of these structures vary.

Apoptosis plays an essential role in normal germ cell endowment and follicular dynamics in ovary and eventually follicular atresia. The apoptosis in antral follicles process is mediated by granulosa cells Ross¹⁵ and in primordial, primary follicles through oocyte Morita¹⁶ they overall affect the ovarian reserve and hence the fertility.

In our study, although the histomorphometric analyses of atretic follicle numbers in section were statistically non significant but overall there was increase in atretic follicle count especially in Group III. This finding correlates with the findings of Lee¹⁷ in which he correlated with neutophils.

The absorption coefficient value of ultrasound in the ovarian tissue is highest in the cortex than medulla. The absorption coefficient produces primary effects of heat and mechanical effects of cavitation¹⁸ which affect the parenchyma and stroma of the organ. These shearing forces on the cell membrane can change its antigenic specificity, responsible for inflammatory reaction¹⁹ or disturb the organelle which can result in its disturbances, disruption and cell death (provided the lysosomal wall ruptures). Or may disturb the genetic coding for early apoptosis and hence follicular atresia leading to increase in atretic follicle count.

In conclusion, ultrasound does have side effects if used repeatedly and for longer duration. Therefore, misuse of this diagnostic tool should be avoided.

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