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Mean Number of Non-Growing Ovarian Follicles at Different Age of Bangladeshi Females - A Postmortem Study

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Abstract

Context: *The human ovary contains a fixed number of non-growing primordial follicles maximal at 5th month of gestational age that decreases bi-exponentially with age, culminating in the menopause at an average age of 50-51 years. Number of follicular count is important for determination of ovarian aging. For this reason this study was aimed at determining the mean number of total non-growing follicles in different age groups of Bangladeshi female (cadaver) which will serve as a baseline data for the gynaecologist and obstetrician about the onset of infertility in our country.*

Materials and Methods: *A cross sectional analytical type of study was conducted in the Department of Anatomy of Rangpur Medical College from July 2008 to June 2009. Thirty two ovaries were collected during postmortem from Bangladeshi female aged between 15 and 45 years. The samples were classified into the young (group A- 15-30 years), intermediate (group B 31-38 years) and old (group C - 39-50 years). Three histological slides were prepared from each ovary. Total numbers of non-growing follicles were counted in eight fields of each slide with light microscope. The numbers of follicles were compared between different age groups.*

Result: *Mean number of total ovarian follicles were more in young group, but the number progressively and significantly decreased in intermediate and old groups (P< 0.01 in groups Avs B, P< 0.001 in groups B vs C and P< 0.001 in groups A vs C).*

Conclusion: *From the present study it may be observed that the total follicular number was decreased with increase of age. This may be due to ageing process, hormonal influence, environmental and genetic cause. Estimation of the total number of follicles present in the ovary (ovarian reserve) could be helpful for assisted reproductive techniques such as IVF.*

Key words: *Ovary, follicle.*

Introduction

The greatest number of non-growing follicles containing primary oocyte (several millions) is present at about 20th weeks of gestation. At birth this number is about 1-2×10^6 and then the rate of atresia/apoptosis is relatively constant until age 37.5 years. So that at puberty approximately 400,000 oocytes are present, these number reduced to about 25,000 oocytes at 37.5 years[1]. From this point, atresia accelerates until the average age of menopause (51 years) when only 1000 oocytes (follicles) remain[2].The total ovarian reserve (non-growing follicular population) decrease due to the anatomic, physiological and endocrine changes that occurs within the ovary. The age related depletion of the non growing follicles occurs as a result of two process, atresia and entry in growth phase. In human ovary, the percentage of atresia has been estimated to be about 50% at birth, decreases from birth to approximately 30 years of age. Up to 30 years of age, the loss of non growing follicles mainly occur

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due to growth phase[3].There is no data about the distribution and number of non-growing follicles in infertile women.

So this study was aimed at to predict the number of non-growing ovarian follicles present in the ovaries at different age.

Materials and methods

The present study was carried out on 32 human ovaries. The ovaries were collected from Bangladeshi females from age 15years to 45 years during post mortem. Specimens of ovaries was collected from the unclaimed dead bodies of road-traffic accident within 24 to 36 hours of death autopsied in the morgue of the Gazipur Sadar Hospital and the Department of Forensic Medicine of Rangpur Medical College, Rangpur after completion of all legal formalities. Before collection, the approximate age of the cadaver was recorded from departmental record book. Soon after collection, each specimen was gently washed in tap water on a dissection tray. Then the ovaries were duly tagged with an identifying number on a container and preserved in 10% formalin solution for histological study.

The study was carried out in the Department of Anatomy of Rangpur Medical College, Rangpur from July 2008 to June 2009.For convenience of the study, age grouping was done for differentiating various features in relation to age according to EL-Toukhy[4]. So the collected ovaries were grouped according to age in group A (age range 15-30 years), group B (age range 31-38 years) and group C (age range 39-45 years).As 32 of right ovaries were collected for counting the total number of different types of follicles, after age grouping, 12 ovaries belong to group A (37.5%), 12 ovaries belong to group B (37.5%) and 8 ovaries belong to group C (25%).

Gougeon[3] classified primordial, intermediate and primary follicles according to morphologic criteria as non-growing follicles (NGFs).

Gougeon and Chainy[5], Miller[6] stated that number of non-growing follicles show relative symmetry between right and left ovaries from the same individual. So in this study only right ovary was taken for histological study.

After fixation in 10% formalin solution each ovary was cut into approximately 1 mm slabs perpendicular to the long axis of ovary[7]. Every eighth slab was taken in a simple random technique; so that 3 blocks were prepared from each ovary thus three slides were studied from each ovary and examined under light microscope using an X10 objective and an X10 eyepiece. For convenience of counting of the NGFs each section was divided into 8 parts. For this purpose a transparent plastic sheet which was divided into 8 equal parts, placed over the slide (fig-1 & 2). Then the numbers of follicles were counted from each part. As three slides were prepared from three tissue blocks for each ovary, so total 96 slides and 768 fields were studied from 32 ovaries of different age groups. Then the average number of non growing follicles was calculated which represent the number of NGFs for each ovary.

After collecting the data means, standard deviations (SD) etc were calculated for various parameters. Appropriate statistical analysis of Variance (ANOVA) test were done using computer based SPSS (13 version) for windows software.

Results

The observations and results of histological studies of different age groups were described in table-I & Fig.-3. It was observed from this table that the mean ± SD of numbers of ovarian follicles were found 87.58±28.16, 53.50±12.00 and 23.00±4.14 in group A, B and C. The lowest number was found in group C and highest number in group A. However the difference reached significant level among the A, B and C group. Level of significance were A vs B ($P<0.01$), B vs C ($P<0.001$) and A vs C ($P<0.001$).

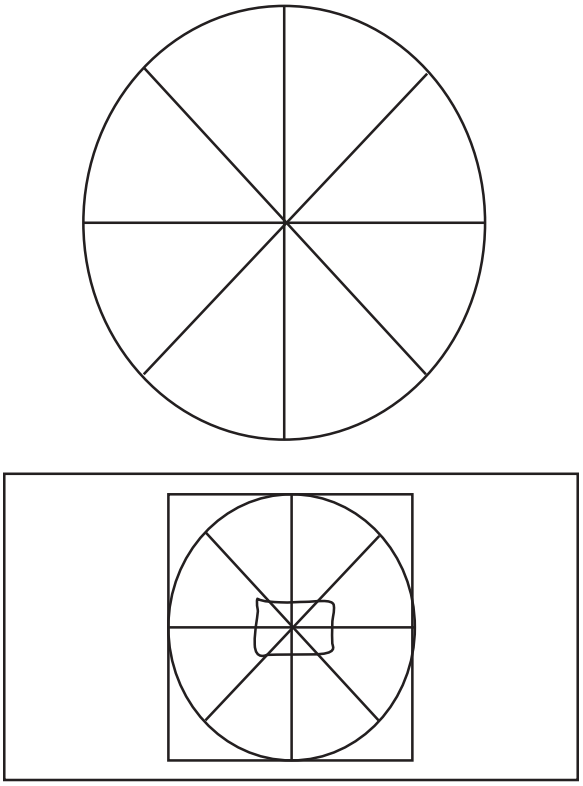

Fig.-1: *Diagrammatic representation of the process of examining the number of follicles in a ovarian section by placing a transparent sheet over the slide which was divided into eight equal parts*

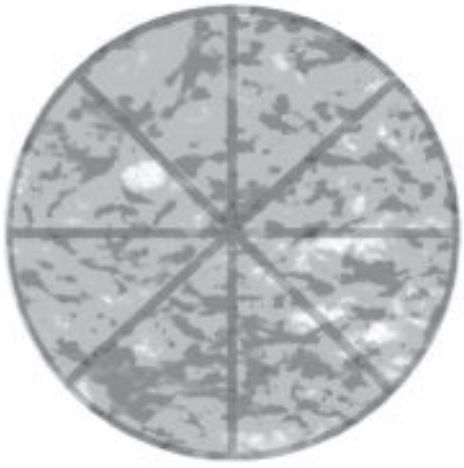

Fig.-2: *Photomicrograph showing the division of histological slide for examining the number of follicles. One division shows the location of a microscopic field in which the ovarian follicles were counted.*

Table-I

Mean number of total ovarian follicles in different age Groups

Groups	Range	Mean follicular number+SD
A (15-30 years)	43-125	87.58+28.16
B (31-38 years)	38-70	53.50+12.00
C (39-45 years)	19-30	23.00+4.14

Statistical Analysis

Groups	P value
A vs. B	0.004**
B vs. C	0.000***
A vs. C	0.000***

NS = Not Significant

* = Significant (p<0.05)

** = Moderately Significant (p<0.01)

*** = Highly Significant (p<0.001)

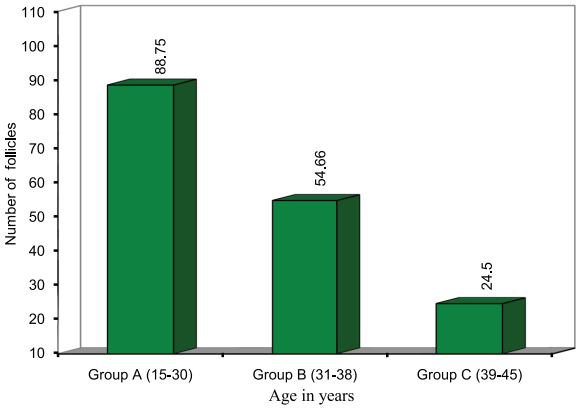

Fig 3: *Bar diagram showing mean number of total follicles in different age groups*

Discussion

Reproductive aging in women is a continuous process that begins prior to birth and extends throughout the reproductive period up to menopausal transition. The aging ovary appears to be characterized by depletion of primordial follicle.The results of the present histomorphological study from ovaries of different age groups showed statistically decreasing mean number of ovarian follicles from younger to middle to old age groups. Several authors[2,8,9] worked on

the depletion of ovarian follicles from different age group, their result coincided with present study. Age dependent decreases of follicles are not uniform from birth to menopause. The rate of follicular disappearance increase with age[10] the rate is more than double when follicular number falls to the critical figure of 25000 at the age of 37 years[2]. The accelerated rate of atresia at age 37-38 is often associated with increase in FSH and decreased fecundity[11,12].

Conclusion

Ovarian reserve of NGFs count decrease with increase age due to hormonal influence, environmental and other pelvic inflammatory diseases. For further study more sample size and modern technology can be used to count the NGFs which may be helpful during assisted reproductive techniques such as IVF.

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