

Proportional Volume of Follicles and Stroma of Ovary in Bangladeshi Female Cadaver in Different Age Groups

Rubina Qasim¹, Humaira Naushaba², Md. Enayet Ullah³, Hasna Hena⁴

Abstract

Context: Under the influence of hormones, ovary undergoes various changes especially in the volume of follicles as well as stroma. This change starts from prepubertal period and continue up to post-menopausal period. This repetitive growth and regression of ovarian follicles predispose to develop many abnormalities of ovarian structures and functions. This study is designed to evaluate the proportional volume of follicles and stroma in relation to different age groups.

Materials & Methods: A cross sectional, descriptive type of study was carried out in the Department of Anatomy, Sir Salimullah Medical College, Dhaka from July 2010 to June 2011. The present study was performed on 24 postmortem right sided ovaries, age ranging from 5 to 65 years. The samples were divided into four different age groups. They were group A (5-12years), group B (13-45 years), group C (46-51 years) and group D (52-65years). The structural component was determined by Hennig's method and calculation was done with the point counting technique.

Results: The proportional volume of follicles and stroma were $(67.50 \pm 5.21)\%$ & $(32.50 \pm 5.21)\%$ in group A, $82.33 \pm 3.83\%$ & $17.67 \pm 3.83\%$ in group B, $(70.00 \pm 0.63)\%$ & $(30.00 \pm 0.63)\%$ in group C and $(24.50 \pm 0.55)\%$ & $(75.50 \pm 0.55)\%$ in group D.

Key words: proportion, follicles, stroma

Introduction

The ovaries are paired ovoid reproductive glands known as female gonads.¹ They are attached to the posterior layer of the broad ligament below and behind the corresponding uterine tube.²

The ovary consists of spindle shaped cells, fine collagen fibers and ground substance which together constitute the ovarian stroma. In peripheral zones of stroma known as cortex have numerous different stages of follicles and corpora lutea.³ In central zone of stroma known as medulla have a mass of contorted blood vessels and associated nerves.⁴

In neonate, ovarian cortex is packed with primordial follicles with relatively little stroma. During reproductive years, cortex contains follicles in different stages of development whereas after menopause follicles are absent and the ovarian cortex become narrow.⁵ Ovary contains fixed pool of primordial follicles, maximal at 5th month of intrauterine life and numbering around 701,000 at the time of birth. From this number, the pool becomes 250,000 –300,000 at time of menarche and then declines with increasing age. At 37-38 years, it contains about 25,000 follicles; Then the follicular depletion accelerates and in menopause only few follicles remain.⁶ So, the fertility decline in women after the age of 30 years and striking decrease is observed after 35 years of age and completely lost by 45 years of age. This age-related decline in fertility results from several factors that contribute to overall reproductive failure including inadequate endometrium and poor oocyte quality.⁷ There is a significant correlation between follicles and ovarian

¹Associate professor, Department of Anatomy, East West Medical College & Hospital

²Professor & Head, Department of Anatomy, Green life Medical College & Hospital

³Professor & Head, Department of Anatomy, East West Medical College & Hospital

⁴Professor & Head, Department of Anatomy, East West Medical College & Hospital

Correspondence: Rubina Qasim, Email: drrubinaqasim@gmail.com

stroma. The number of follicles with stroma accurately reflects ovarian volume which may further determine the ovarian reserve and reproductive age.⁸ By applying the accurate and efficient methods for estimating the ovarian volume of the follicles and stroma, ovarian aging process can be assessed.⁹

Now a days, infertility is a common problem among the female. Infertility rate is gradually increasing day by day in all over the world and one of the main causes of it is abnormalities of the ovarian functions as well as the ovarian diseases.⁸ Follicular depletion and reduction with increasing ovarian stroma leading to decrease the fertility of a women. The decreased fertility with increasing female age appears to diminish the quality of existing oocytes and the number of growing follicles.⁷ The number of growing follicles with volume of stroma indicates the development state of the oocyte.¹⁰ So, in assisted reproductive technology, the volume of follicles with stroma is resourceful information that gives an indication of ovarian reserve and these will help to manage the infertility problem and treatment with assisted reproductive technology. Therefore, the volumes of follicles and stroma count may be a sensitive parameter for assessing the ovarian reserve and use as a single test for predicting the ovarian response with induction of hormone.⁶ This volume is also very helpful to assess the hormonal status, ovarian functions, causes of dysfunctional uterine bleeding and infertility as well as inflammation, hyperplasia & neoplasia of the ovary.¹¹

Studies regarding the proportional volume of follicles and stroma of ovary are less available in Bangladesh. The present study is designed to study in Bangladeshi women of different age groups. It will provide an essential data on the ovary of women in Bangladesh. This study will also provide resourceful information for improving the accurate diagnosis and management of infertility problems with assisted reproductive technologies.

Materials & Methods

The present study was performed on 24 postmortem human ovaries, age ranging from 5 to 65 years. Samples were collected from unclaimed dead bodies within 12 to 36 hours of death. Approximate age & sex were noted down from the morgues record

book at the time of collection of samples. Then the samples were brought to the Department of Anatomy, Sir Salimullah Medical College.

The collected samples were divided into four groups; group A- prepubertal group (5-12years), group B- reproductive group (13-45years) , group C- perimenopausal group (46-51years) and group D- postmenopausal group (52-65 years).

Procedure for histological study

For this study, formalin fixed right sided ovaries were taken. Six samples were selected from each group. With the help of a sharp BP blade, ovary was opened by a median vertical cut extending from the one pole to another pole of the ovary and then another cut which sectioned the ovary transversely at its middle. A 5mm³ rectangular enblock was taken from each part of the ovary for preparation of tissue block (Figure 1). Thus, four pieces of blocks were taken for subsequent processing.

Preparation of the slides

Tissue blocks were refixed in Carnoy's fluid in a plastic container. The tissues were washed in running tap water. Then dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut at 5µm thickness and were stained with Mallory–Heidenhains Aniline blue (Mallory-Azan) stain.

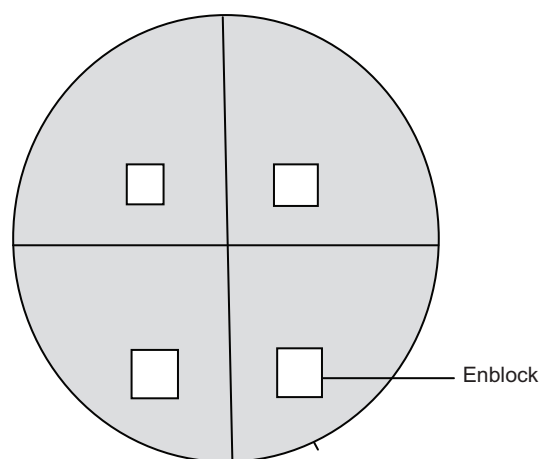


Fig. -1: Diagrammatic representation showing the enblock of ovary

Cortex of the ovary consists of follicles and connective tissue stroma. The proportional volume of the structural component was determined by Hennig's method with the point counting technique. Point counting Zeiss I integrating eyepiece was prepared in a transparent plastic sheet. Then it was cut & placed into the eyepiece and focused on the midpoint of each area of tissue section.

Integrating eyepiece: This eyepiece counting a point network of 25 points spaced at 70µm, arranged within a circle, which delimits the counting field.

Point counting technique¹²: The counting was done under light microscope at low magnification (100 X objective, 100 X eyepiece) on the slides. The position of each point falling on any structural component was recorded for each field. Then the eyepiece was rotated 90° keeping the field constant. Again, the position of each point was recorded. Thus 50 points were recorded for each field. Three such fields or 150 points were studied on each section. The total number of points were summed up and expressed as a percentage of the total number of points fitting the structural component of the ovary. This percentage represents the proportion of follicles and stroma of the ovary.

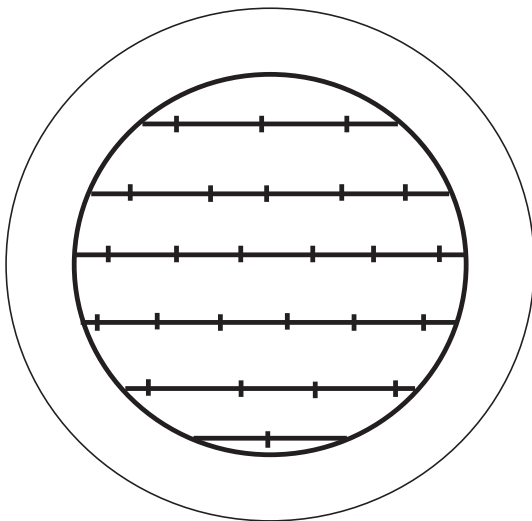


Fig.-2a: Showing an integrating eyepiece, containing a network of 25 points.

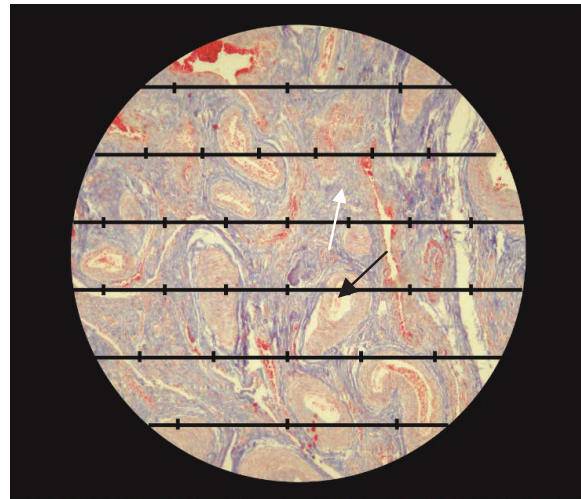


Fig.-2b: Photomicrograph showing measurement of the proportional volume of follicles and stroma by 'point counting technique' white arrow indicates the stroma and the black arrow indicates the follicles.

Ethical Clearance

This research work was approved by the Ethical review committee of Sir Salimullah Medical College (SSMC).

Statistical processing of data

The data collected from the histological slides were processed and statistical analysis were done by one-way ANOVA test. All the statistical analyses were done by using the SPSS version 16.0

Results

The mean ± SD proportional volume of follicles and stroma were (67.50±5.21)% & (32.50±5.21)% in group A, (82.33± 3.83)% & (17.67±3.83)% in group B, (70.00 ± 0.63)% & (30.00±0.63)% in group C and (24.50± 0.55)% & (75.50± 0.55)% in group D. The differences of the mean value of proportional volume of follicles and stroma in the right ovary were highly significant (P<0.001) between the different age groups except between group A & C where the difference was not statistically significant (P>0.10).

Table I
Percentage proportion of follicles and stroma in the right ovary in different age groups

| Age group | Percentage proportion (%) | |
|-----------|------------------------------|-----------------------------|
| | Follicles Mean±SD (range) | Stroma Mean±SD (range) |
| A | 67.50±5.21 (57.00-70.00) | 32.50±5.21 (30.00-43.00) |
| B | 82.33±3.83 (80.00-89.00) | 17.67±3.83 (11.00-20.00) |
| C | 70.00±0.63 (69.00-71.00) | 30.00±0.63 (29.00-31.00) |
| D | 24.50±0.55 (22.00-31.00) | 75.50±0.55 (73.00-92.00) |
| | <i>P value</i> | <i>P value</i> |
| A vs B | <0.001*** | <0.001*** |
| A vs C | >0.10 ^{ns} | >0.10 ^{ns} |
| A vs D | <0.001*** | <0.001*** |
| B vs C | <0.001*** | <0.001*** |
| B vs D | <0.001*** | <0.001*** |
| C vs D | <0.001*** | <0.001*** |

Figures in parentheses indicate range. Comparison between age groups done by One way ANOVA (PostHoc), ns = not significant, *** = significant.

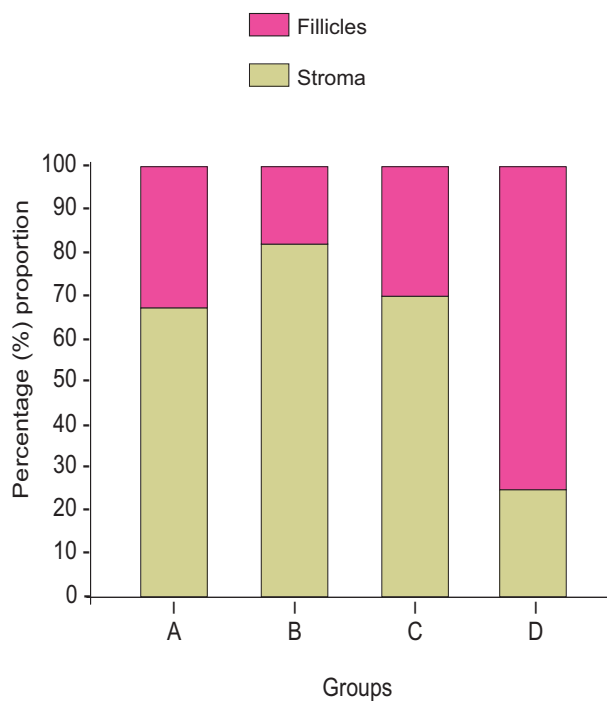


Fig.-3: *Percentage proportion of follicles and stroma of the right ovary in different age groups*

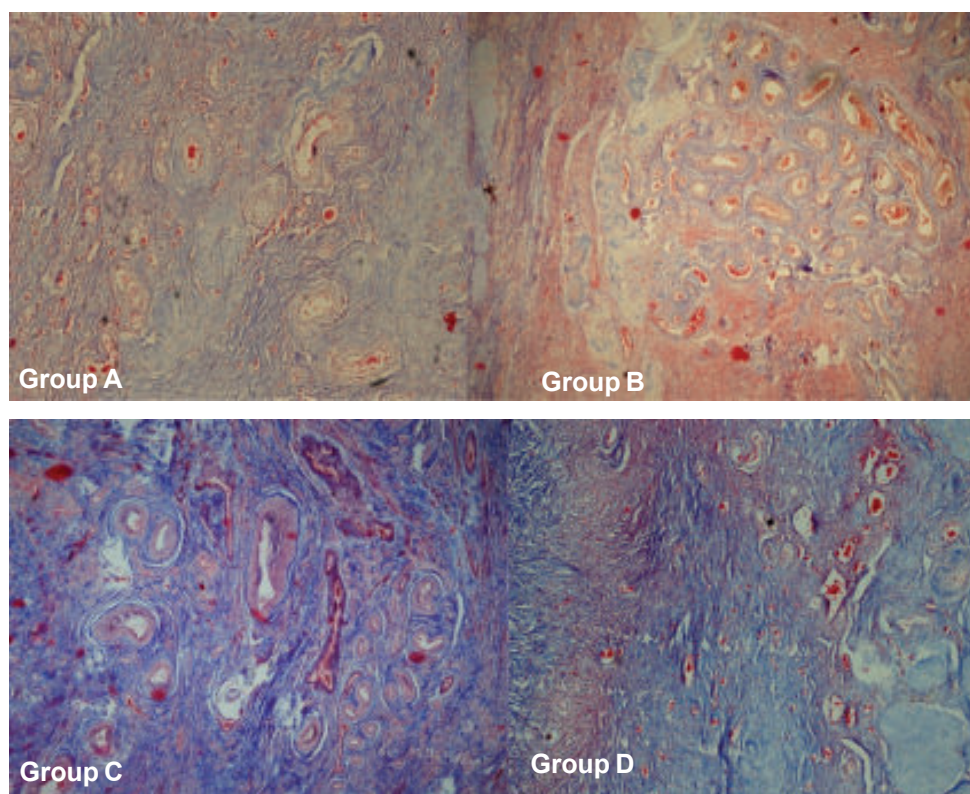


Fig.-4: *Photograph showing the proportional volume of follicles and stroma in different age group*

Discussion

The highest mean proportional volume of follicles was $82.33 \pm 3.83\%$ in group B (reproductive group) & lowest was $24.50 \pm 0.55\%$ in group D (post-menopausal group). But the highest mean proportional volume of stroma was $75.50 \pm 0.55\%$ in group D (postmeno- pausal group) & lowest was $13.67 \pm 3.83\%$ in group B (reproductive group). The mean values of the present study corresponds with the different researchers of different countries.^{2,10,13,14} The total number of follicles is higher at birth. Gradually it begins to decline with advancing age. After thirty-five years of age, the number of the follicles strikingly reduces. They are completely lost after menopause accompanied with increase in the proliferation of stromal cells.⁷ Finally, the ovary becomes devoid of follicles after menopause and filled with stroma. The present study was a modest effort and new beginning to collect the data on volume of follicles and stroma of ovary of Bangladeshi female.

Conclusion

Study findings revealed that proportional volume of follicles was reduced and the volume of the stroma increased with advancing age.

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