

Mean Diameter of Non-Growing Ovarian Follicles at Different Age of Bangladeshi Females

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The human ovary contains different type of non growing follicles (primordial, intermediate and primary). With increasing age the size of non-growing follicles are reduced. Measurements of the diameters of non-growing follicles are essential for onset of infertility. Therefore this study was aimed at to measure the diameter of the non-growing ovarian follicle in different age group of Bangladeshi female to have idea about the onset of infertility. This study was cross sectional analytical type conducted in the department of Anatomy, Rangpur Medical College, Rangpur. 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) contain 12 ovaries, intermediate (group B: 31-38 years) contain 12 ovaries and old (group C: 39-50 years) contain 8 ovaries. Three slides were prepared from each ovary. The non-growing follicles were classified as the primordial or type B (oocyte surrounded by the single layers of flattened granulosa cells), transitory or intermediate or type B/C (oocyte surrounded by mixture of flattened and cuboidal granulosa cells), primary or type C (oocyte surrounded by cuboidal granulosa cells) and type D (oocyte surrounded by more than one layer of cuboidal granulosa cells). The numbers of type B, type B/C, type C, type D were counted in eight fields of each slide with light microscope and the diameters of those follicles were measured with ocular micrometer. Then the diameters of follicles were compared between different age groups. Mean diameter of non-growing follicles (type B, B/C) were higher in group B ($18.29 \pm 4.09 \mu\text{m}$ and $27.92 \pm 10.30 \mu\text{m}$ respectively) but the mean diameter of type C and D follicles were higher in group A (34.75 ± 4.66 and $49.26 \pm 5.78 \mu\text{m}$ respectively) followed by group B and group C. But there were no significant difference in the diameter of different types of non-growing follicles between and within the groups. The present study may be concluded that mean diameter of ovarian follicles was decreased in type C and D follicles in relation to age. This may be due to aging process and follicular atresia.

[Dinajpur Med Col J 2014 Jul; 7 (2):83-88]

Key words: Ovary, non-growing ovarian follicles

Introduction

Follicular growth may begin at any time of the female's reproductive life, as early as infancy and continues

throughout puberty, during the ovarian cycle and pregnancy, until the reproductive period.¹ In reproductive age the cortical region consists of follicles of different stages:

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primordial or quiescent, primary or preantral follicle, secondary or antral (also called vesicular) and preovulatory or Graafian follicle.² Follicles have been classified into 4 groups as proposed by Lintern- Moore(1974)³ for the human ovary type B follicles in which oocytes are surrounded by flattened granulosa cells; type B/C follicles in which oocytes are surrounded by a mixture of flattened and cuboidal granulosa cells; type C follicles in which oocytes are surrounded by a single layer of cuboidal granulosa cells; type D follicles in which oocytes are surrounded by more than one layer of cuboidal granulosa cells but where epitheloid cells had not differentiated in theca layer. The type B, B/C, C and D are described as non growing follicles (NGFs). Follicles of types B and B/C mostly had a diameter 30.0 and 42.5 μm . For follicles of types C and D, the diameters were between 37.5 and 52.5 μm and 50.0 and 90.8 μm respectively.⁴ Several factors are required for oocyte and follicular growth follicle-stimulating hormone (FSH), growth factors (epidermal growth factor, insulin-like growth factor I (IGF-I) and calcium ions (Ca^{2+})).⁵ Oxidative stress (mean an unbalance between oxidant and antioxidant system called by Tatone et al.2008)⁶ may be key element in inducing GC apoptosis via alteration of the cellular ionic environment, which activates a $\text{Ca}^{+2}/\text{Mg}^{+2}$ sensitive endonuclease resulting in activation of the apoptotic cascade.⁷ Tatone et al.(2008)⁶ mentioned that most relevant concept of ageing, age associated malfunction results from physiological accumulation of irreparable damage to biomolecules as an unavoidable side effect of normal metabolism and follicular vascularization.

Ovarian function related clinical conditions such as infertility; non-neoplastic follicular cyst and malignancy are common in our country. To our knowledge, there is no data about the size of non-growing ovarian follicles at different age of Bangladeshi

females, who are suffering from above mention clinical condition. So this study can be used as a potential reference for the diameter of non-growing ovarian follicles at different age of Bangladeshi females.

Methods

The study was carried out in the Department of Anatomy of Rangpur Medical College, Rangpur from July 2008 to June 2009. Thirty-two ovaries were collected from Bangladeshi females from age 15 years 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.

For convenience of the study age grouping was done for differentiating various features in relation to age according to the EL-Toukhy(2002).⁸ 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 from group C (25%).

After fixation in 10% formalin solution each ovary was cut into approximately 1 mm slabs perpendicular to the long axis of ovary.⁹ 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. From each slide three follicles of each type B, B/C, C, D follicles were identified properly and their diameters were measured under light microscope using an X 40 objective and an X 10 eyepiece using an ocular micrometer. Reading was converted to the values by procedure in method of standardizing ocular micrometer with stage micrometer. For this purpose the largest follicular diameters and another diameters perpendicular to the previous one was done and the mean of the two diameters was taken as the diameter of the follicles (fig – 2).

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 (13.0 version) for windows software.

Results

Table I showed that there is a trend of decrease in the mean diameter of type C and D follicles from group A to Group B to Group C. But in case of type B follicles the mean follicular diameter was lower in group C than group A but highest diameter was found in group B. Again for type B/C follicle the mean diameter was highest in group B followed by group C then group A. But the statistical analysis showed no significant differences among the different types of follicles between and within in three different age groups ($P > 0.05$).

Table I: Mean diameter (in μm) of non-growing follicles in different age groups

Follicular type	Age in groups (Years)			Level of significance
	A (15-30 years)	B (31-38 years)	C (39-45 years)	
Type B	14-26.25 17.35 ± 3.39	14-24.50 18.29 ± 4.09	10.5-24.50 16.91 ± 5.70	0.77
Type B/C	21-31.50 25.82 ± 3.21	20-59.50 27.92 ± 10.30	22.75-33.25 27.57 ± 4.32	0.75
Type C	29.50-42 34.75 ± 4.66	28-39.50 33.09 ± 3.57	30-39.66 33.00 ± 3.12	0.54
Type D	42-59.50 49.26 ± 5.78	35-74.38 45.38 ± 10.36	35.50-70 44.26 ± 12.00	0.50

Results are shown as ranges and mean \pm SD

Statistical Analysis

Age Groups	Follicular types (P value)			
	Type B	Type B/C	Type C	Type D
A vs. B	0.82 ^{NS}	0.79 ^{NS}	0.63 ^{NS}	0.52 ^{NS}
B vs. C	0.84 ^{NS}	1.00 ^{NS}	1.00 ^{NS}	1.00 ^{NS}
A vs. C	1.00 ^{NS}	0.63 ^{NS}	0.62 ^{NS}	0.58 ^{NS}

Note:

Type B oocyte surrounded by flattened granulosa cells.

Type B/C oocyte surrounded by a mixture of flattened and cuboidal granulosa cells.

Type C oocyte surrounded by more than one layer of cuboidal granulosa cells.

Type D oocyte surrounded by more than one layer of cuboidal granulosa cells but where epitheloid cells had not differentiated in theca layer.

NS = Not Significant

* = Significant (p<0.05)

** = Moderately Significant (p<0.01)

*** = Highly Significant (p<0.001)

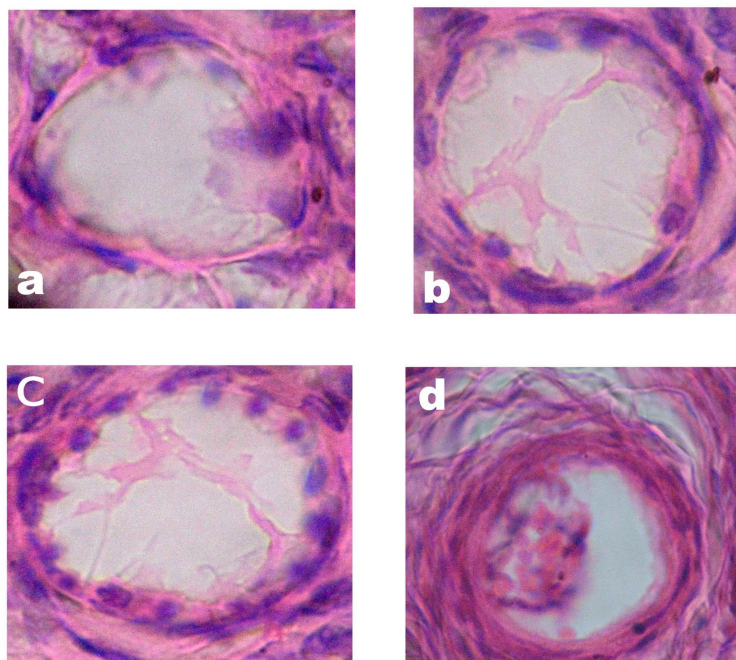


Fig 1 - Photomicrograph showing different types of the ovarian follicles with Haematoxylin-Eosin Stain(H&E) X 40.

- Follicles in which oocytes are surrounded by flattened follicular cells.
- Follicles in which oocytes are surrounded by mixture of flattened and cuboidal cells.
- Follicles in which oocytes are surrounded by single layer of cuboidal and follicular cells.
- Follicles in which oocytes are surrounded by more than one layer of cuboidal follicular cells

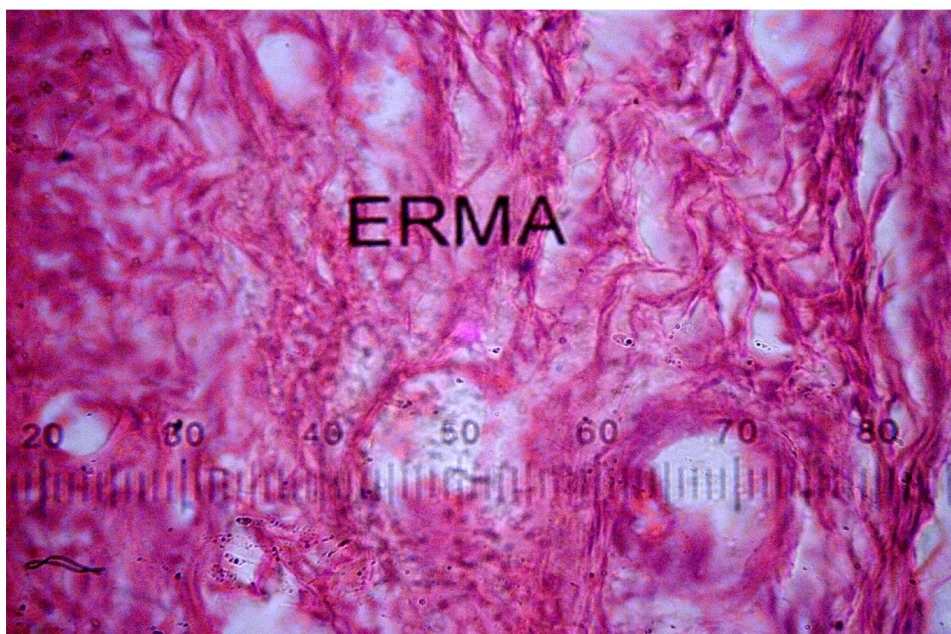


Fig 2 - Photomicrograph showing method of measurement of follicular diameter by ocular micrometer (X 40).

Discussion

In this study mean diameter of different types of non-growing follicles showed different results. It is noteworthy that the diameter of follicles was increased progressively from type B to D in women of all groups A, B and C. This may be due to increase in the number and size of granulosa cells as well as the diameter of the oocyte itself. When comparison was done between the groups (A, B and C) for each type of follicle (type B, B/C, C and D), highest mean diameter of type B and B/C follicles was found in group B but highest mean diameter of type C and D follicles were found in group A. But none of the difference reached significant level for any types of follicles both between and within the groups. The above mentioned result was similar to Gougeon and Chainy(1974).⁴ They stated that an intraovarian mechanism may be operating to regulate the initiation of

follicular growth and maintain a sufficient number of follicles necessary for further follicular development. Follicles of type B and B/C may exert some inhibitory influence on the initiation of growth of type C follicles. This inhibitory influence could be directly proportional to the size of the pool of the small follicles and regulate the rate of follicular depletion by follicular growth. Follicular depletion with increased age may also be caused by follicular atresia.

Conclusion

In relation to age the mean diameter of ovarian follicles was decreased in type C and D follicles which is indicative of ovarian aging. Further study with larger samples and measurement of all follicles are suggested for definite conclusion.

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