Original Article

Microscopic study on the total number of follicles of ovary in Bangladeshi female cadavers

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ABSTRACT

Context: The ovaries are paired female reproductive and endocrine glands. It is an unique organ in the female body. It undergoes various changes under the influence of hormones which started from prepubertal period and continue up to post menopausal period. Reproductive life span is determined by the histological changes of ovarian follicles. The study was carried out to counting the total number of follicles in relation to age.

Study design: Cross sectional, descriptive type of study.

Place and period of study: Department of Anatomy, Sir Salimullah Medical College (SSMC), Dhaka from July 2010 to June 2011.

Materials: 65 ovaries of Bangladeshi female cadavers age ranging from 5 years to 65 years.

Methods: The samples were divided into four different age groups. They were group A or prepubertal group (5-12years), group B or reproductive group (13-45 years), group C or perimenopausal group (46-51years) and group D or postmenopausal group (52-65years). Histological studies were carried out only in right ovary on 24 fresh samples, 6 samples from each group.

RESULTS: The mean ($\pm$SD) total number of the follicles in the right ovaries were 82.67 ± 2.07, 43.33 ± 8.17, 10.00 ± 5.47 and 1.17 ± 1.94 per square millimeter in group A, B, C & D respectively. The differences of the mean ($\pm$SD) number of the follicles were highly significant ($P<0.001$) among the all age groups and was significant ($P<0.01$) when compared between group C with group D.

CONCLUSION: Age related changes were observed in the total number of follicles of the ovary.

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The ovaries are paired female reproductive organ which are situated in the lesser pelvis; one on each side of the uterus[1]. In the young adult, they are almond shape, solid and white in colour, approximately 3cm long, 1.5cm wide and about 1cm thick [2]. It undergoes various changes under the influence of hormones which starts from prepubertal period and continues up to post menopausal period [3].

Follicles are the functional unit of the ovary. Most of the follicles (90%-95%) are primordial follicles. As the follicles increase in size, it passes through three developmental stages: primary follicles, secondary follicles and tertiary (mature or graafian) follicle. Those follicles which do not ovulate, degenerate by a process called atresia. Following ovulation of the large dominant follicle, the follicle wall collapse into folds and the units transforms into a corpus luteum. The corpus luteum ultimately degenerates becoming a white scar of dense connective tissue, the corpus albicans[4] .

Ovary fulfills two major objectives: generation of fertilized ovum and the preparation of endometrium for implantation through the sequential secretion of estrogen and progesterone hormones[5]. These activities are integrated into cyclic repeatitive process of follicular maturation, ovulation with formation and regression of corpus luteum under the control of the hypothalamo-hypophyseal system [6].

Regrettably however, the ovaries as organs that become completely depleted in menopause. In fact, this depletion of the follicular population of the ovaries begins very early even in embryonic life, continues throughout childhood, increases at each ovarian cycle along the menarche and is finished at menopause[7].
Besides these, ovary possesses many pathological disorders including cyst, inflammation, carcinoma. There are a great variety of hormonal disorder, most commonly due to endocrine imbalances, complications of pregnancy and neoplastic proliferation[8].
Considering the enormous anatomical and physiological overload, the reproductive lifespan is ultimately determined by the number of follicles. By applying the accurate and efficient methods for estimating the number follicles are paramount for ovarian aging process[9]. The study of the follicular numbers can provide also important information about the function of the ovary, particularly in the relationship between folliculogenesis and the factors that regulate it[10].

MATERIALS &METHODS OF THE STUDY

Parameter studied- The total number of follicles of the ovary

The present study was performed on 65(sixty five) pairs of postmortem human ovaries. Samples were collected from unclaimed dead bodies within 12 to 36 hours of death which were autopsied on different dates in the morgue of the Department of Forensic Medicine of Sir Salimullah Medical College (SSMC) and Dhaka Medical College (DMC),Dhaka. 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. Samples were distributed into four groups**[11]**

Study group distribution in different age groups

Study groups	Age range (in years)	No. of samples (n = 65x2=130)
Group A (prepubertal)	05-12	07x2
Group B (reproductive)	13-45	30x2
Group C(perimenopausal)	46-51	15x2
Group D (postmenopausal)	52-65	13x2

Procedure for the histological study

For histological study, formalin fixed right sided ovaries were taken. Six fresh 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 (Fig.1a). A 5mm^3 rectangular enblock was taken from each part of the ovary for preparation of tissue block (Fig.1b). Thus four pieces of blocks were taken for subsequent processing.

Four slides were prepared from each sample. Thus a total of twenty four (6×4=24) histological slides were made from each group. Finally, ninety six (24×4=96) slides were prepared for the whole study.

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.
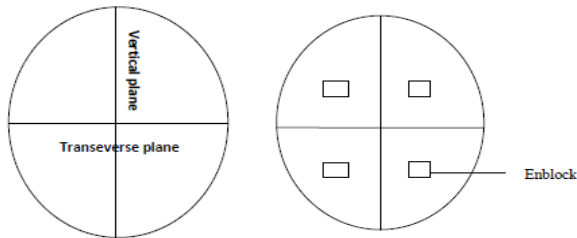

Fig. 1 Diagrammatic representation showing the cutting plane (a) & enblock of ovary for histological study

Counting the total number of the follicles per sq mm in the right ovary

From each slide, three different fields were chosen for counting the number of the follicles. The slide was divided into three equal parts by computer generated photographically produced

100 divisions of the stage micrometer = 1000 μm
So 1 divisions of the stage micrometer = 1000 ÷ 100μm

equal sized room on a transparent plastic sheet by drawing three lines (Y shaped) which radiate from the centre towards the periphery at 10 o'clock, 2 o'clock and 6 o'clock positions. This sheet was fixed on the top of the cover slip by an adhesive tape. The centre of this sheet corresponded with the centre of the tissue section. From each triangular area, one microscopic field was selected near the centre for the study. The counting was done within a counting circle specially devised for this purpose.

A counting circle of 5mm diameter was printed on a transparent plastic sheet. This counting circle was cut to fit into the eyepiece of the light microscope. Thus a black circular outline was superimposed over the actual microscopic field near the centre of the meeting point of three drawing lines(Y shaped). Counting the follicles under low power objective (X10), each microscopic field contains full section of follicles. However many other follicles were only partly included. From three counts of three different fields of each slide, an average count was calculated for each slide. The count was then converted into number per square mm by conversion measurement by means of an ocular micrometer and stage micrometer.
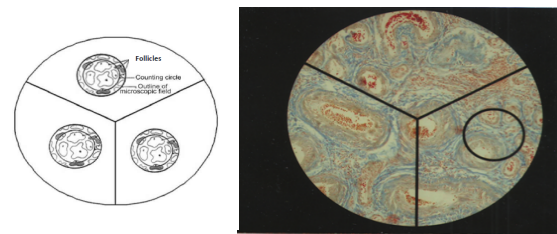

Fig.2 representation of the use of counting circle in Diagrammatic (a) and Photomicrograph (b) counting the number of the follicles.

Standardization for counting number of follicles per unit area of the microscopic field:

Inner diameter of the counting circle corresponded with 52 divisions of the stage micrometer under 10x objective and 10x eyepieces.

So 52 divisions of the stage micrometer = $(1000 \times 52) \div 100\mu m = 520\mu m$
There fore radius of the circle = $(520 \div 2)\ \mu m = 260\ \mu m$

There fore area of the circle = $\pi\, r^2$

$$= 3.1416 \times (260\ \mu m)^2$$
$$= 3.1416 \times 67600$$
$$= 212372.16\ \mu m$$

As 1 sqmm
$$= 1000 \times 1000\ sq\mu m$$
$$= 1000000\ sq\mu m$$

If it is thought that number of follicles counted within the circle is R
Then 212372.16 sq µm contained R number of follicles
So, 1 sq µm contained   R ÷ 212372.16 numbers of follicles
Therefore 1000000 sq µm contained (R ÷ 212372.16) × 1000000 numbers of follicles

$$= R \times 4.7\ \text{number of follicles}$$

Therefore   N $= R \times 4.7$
Where   N   = number of follicles per sq mm area of the
                               microscopic field.

RESULTS

In the present ovary, the mean (±SD) total number of the follicles in the microscopic field were 82.67 ± 2.07 per square millimeter in group A, 43.33 ± 8.17 per square millimeter in group B, 10.00± 5.47 per square millimeter in group C and 1.17 ± 1.94 per square millimeter in group D. The differences of the number of the follicles were significant (P<0.01) between group C & D and highly significant (P<0.001) between the other age groups. The results are shown in Table 1and Fig 3.

Table 1   Mean (±SD) total number of follicles in the right ovary in different age groups

Age group	Total number of follicles Mean±SD		
A (n=6)	82.67±2.07 (80.00-85.00)		
B (n=6)	43.33±08.17 (30.00-50.00)	Group A : Age 5-12 years	
C (n=6)	10.00±05.47 (5.00-20.00)	Group B : Age 13-45 years Group C : Age 46-51 years	
D (n=6)	1.17±01.94 (0.00-05.00)	Group D : Age 52-65 years	

	P value
A vs B	<0.001***
A vs C	<0.001***
A vs D	<0.001***
B vs C	<0.001***
B vs D	<0.001***
C vs D	<0.01**

Figures in parentheses indicate range. Comparison between age groups done by One-way ANOVA (PostHoc), ns = not significant, */**/*** = significant.
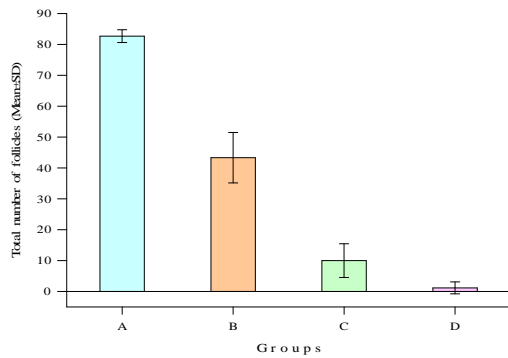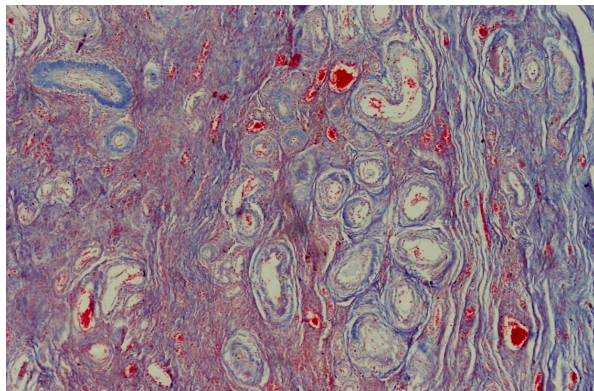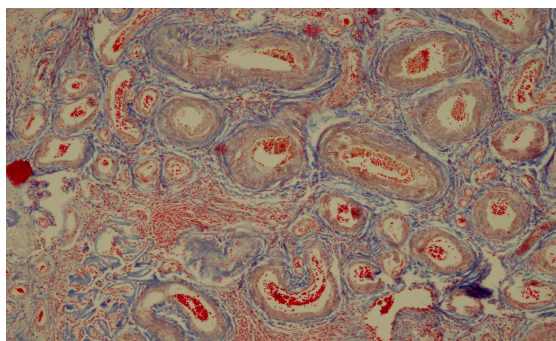
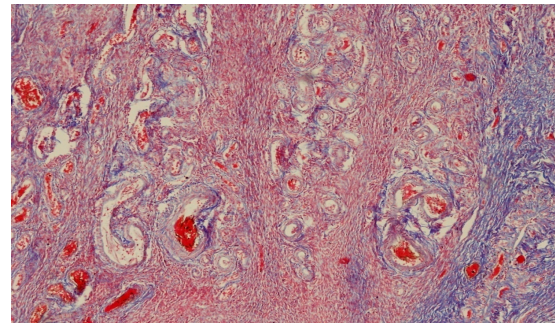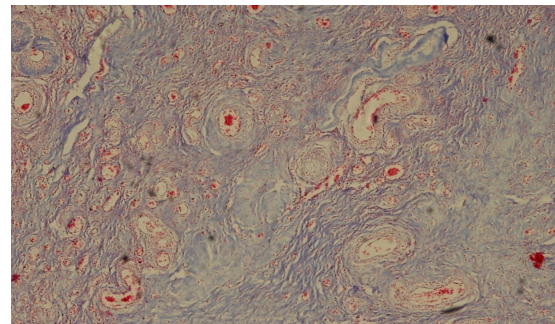**Fig. 3** Mean (±SD) total number of follicles in different age groups

Group A

Group B

Photomicrograph showing the total number of follicle in group A (prepubertal) and group B(reproductive) [Mallory-Azan]stain.

Group C

Group D

Photomicrograph showing the total number of follicle in group C (perimenopausal) and group D(postmenopausal).

DISCUSSION

In the present study, the highest mean (±SD) number of the follicles were established in prepubertal age (group A) and the lowest were in postmenopausal age (group D).

According to Standring[2] 40,000 ovarian follicles are present at puberty. Ryan, Berwokitz and Barbieri stated that by the first menses, the number of follicles is approximately 200,000. By 52 years of age, most women have loss all functional ovarian follicles and menopause has begun, menopausal ovary becomes devoid of functional follicles.

Larsen[5] reported that by the onset of puberty approximately 30000 ovarian follicles are present. Ultimately 50 years later, this is finally exhausted, no follicles are present.

Gartner &Hiatt[13] described that near the end of the 5th fetal month, ovary contains about 5 to 7 millions oogonia. About 1 million of the oogonia become surrounded by follicular cells and survive to the time of birth. Of the 1 million oogonia that survive to become primordial follicles, 600000 become about 400000 follicles.

Faddy and Gosden[14] found that according to simple bi-exponential of ageing, 100000 follicles remain in the great majority in 21.5-26.5 years of age. The rate of follicle disappearance increases at age 37.5 years (or when 25000 follicles remain). So that the numbers fall to approximately 1000 at 51 years, the median age of menopause.

Seeley, Stephens & Tate[15] mentioned that by the fourth month of prenatal life, the ovaries may contain 5 million oogonia. By the time of birth, many of the oogonia have degerated and about 2 million of them are present. From birth to puberty, the number of primordial follicles declines to around 300,000-400,000; of these only about 400 continue oogenesis and are released from the ovary**.**

The mean (±SD) values of the present study in reproductive age agreed with Standring, Larsen, Gartner & Hiatt and Faddy & Gosden . However, in prepubertal age, the values were lower than that of the findings of Ryan, Berwokitz & Barbieri and Seeley, Stephens & Tate. This discrepancy might be due to the differences in the methods of collection, fixation, staining and magnification.

CONCLUSION

In the present study, it was observed that significant change of the total number follicles of the ovary occur with advancing age.

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