A MICROSCOPIC STUDY OF AGE-RELATED CHANGES OF HUMAN OVARIAN FOLLICLES

Ritu Saloi[1]

[1]Associate Professor, Department of Anatomy, Tezpur Medical College, Tezpur, Assam.

ABSTRACT

BACKGROUND
Ovarian cancer is one of the leading cancers in Indian women. Ovarian pathology can manifest in various ways, e.g. menstrual abnormalities, cystic disease, infertility, benign and malignant tumours of the ovary, etc. The study was undertaken to observe the age-related changes of the follicular number of human ovary and to study if there is any difference between the right and left ovary with respect to the total number of follicles and compare it with the established findings of previous workers, which will help the clinicians to adopt appropriate diagnosis and treatment of the various clinical conditions associated with the ovaries.

MATERIALS AND METHODS
The study on human ovary was conducted in the Department of Anatomy, Gauhati Medical College, Guwahati. The histological characteristics of 42 pairs of normal human ovaries of different age groups were studied (14 pairs in each age group). The ovaries were divided into three groups, viz. group A or pre-reproductive, group B or reproductive and group C or postmenopausal. The results were statistically analysed and t-test was done to find out the significant difference of mean value.

RESULTS
No difference was found in the follicular number between the right and left ovaries in any age group. However, there was statistical difference in the number of follicles between age groups A and B, A and C and B and C.

CONCLUSION
The study showed that there were certain differences in the number of follicles of the ovary in the three groups. The study also revealed that there was no difference in follicular number between the right and left ovary in all the three age groups. It was observed that the total number of follicles of the ovary undergo age-related changes. The results were statistically analysed and compared with the findings of previous workers.

KEYWORDS
Ovary, Ovarian Follicles, Pre-Reproductive, Postmenopausal, Reproductive.

BACKGROUND
The paired ovaries, which are considered the home of the follicles are the primary female sex organs. They serve a dual purpose namely the production of ova and secretion of hormones, namely oestrogen and progesterone, which control the secondary sex characters and furnish the endocrine background to the cyclic changes of mature sex life and of pregnancy. The ovaries are continuously undergoing complex changes in structure during mature sexual life. These changes are associated with profound alteration in the function and indeed in the structure of the secondary sex organs (the uterus, vagina and the mammary glands).[1]

During childhood, a close correlation between follicle growth, hormone response and hormone production seems to exist. Certain diseases and treatment with cytotoxic agents or radiation to the abdomen influence ovarian development and follicular growth. Chromosomal abnormalities specially Turner's syndrome, trisomy 18 or 21, alter normal ovarian development by reducing the pool of available follicles and inhibiting follicular growth.[2]

Regrettably, however, the ovaries becomes completely depleted of follicles in menopause. In fact, this depletion of the follicular population of the ovaries begins very early in embryonic life, continues throughout childhood, increases at each ovarian cycle along the menarche and is finished at menopause.[3]

The total number of ovarian follicles is determined early in life and the depletion of this pool leads to reproductive senescence. The dynamics of ovarian follicle development have interested endocrinologists and developmental biologists for many years. With the advent of assisted reproductive techniques in humans, the possibility of regulating follicle development in vivo and in vitro has gained clinical relevance.[4]

Ovarian pathology can manifest in various ways, e.g. menstrual abnormalities, cystic disease, infertility, benign and malignant tumours of the ovary, etc. Disease can be

defined and measured only in terms of deviation from normal structure and function. Hence, a clear conception and population data of the ovarian follicles is a prerequisite for the diagnosis and treatment of ovarian disease.[5]

The present study has been undertaken with the aim that the results of the study will help the clinicians to adopt appropriate diagnosis and treatment of the various clinical conditions associated with the ovaries.

Aims and Objectives
1. To study the follicular number of human ovary in different age groups.
2. To study if there is difference between the right and left ovary with respect to the total number of follicles in the different age groups.

MATERIALS AND METHODS
The study on human ovary was conducted in the Department of Anatomy, Gauhati Medical College, Guwahati. The ovaries were divided into three groups according to the different ages namely group A or pre-reproductive (newborn to 13 years), group B or reproductive (14 to 49 years) and group C or postmenopausal (50 years and above). Specimen of ovary were collected from cadavers in the Department of Forensic Medicine, Gauhati Medical College, Guwahati, within 24 hours of death following all legal formalities where autopsies were done. The ovaries with no obvious pathological change and decomposition were taken for the study. The cases with history of poisoning, crushing injury to the ovary and diseased ovaries were excluded from the study. Proper family history of the cadavers were taken from the relatives. The ovaries were taken along with the uterus and fallopian tube to have a better idea of the right and left ovaries. Specimens were also dissected from fresh full-time intrauterine dead babies and neonatal dead babies, collected from the Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital, Guwahati. The ovaries were dissected out from the uterus and fallopian tube. They were first washed in normal saline, dried in blotting paper and weighed in an electronic weighing machine. For preparation of slides, tissue blocks were fixed in 10% formalin for 24-48 hours. The fixed tissue were processed for embedding in paraffin and sectioned at 5 μm thickness in a 'rotary microtome.' The sections of the tissue were stained by routine haematoxylin and eosin and examined under both low and high power microscope to see the follicles of the ovary in each age group.

For measurement of the total number of ovarian follicles per sq. mm. area of the microscopic field, 28 best prepared slides were taken from each group (14 slides of right ovary and 14 of left ovary) and examined under light microscope in low power magnification. The stained tissue section on the slide was divided into three equal parts by a computer generated, photographically produced equal-sized room over a transparent plastic sheet by drawing three lines, which radiated from the centre towards the periphery at 10 o'clock, 2 o'clock and 6 o'clock position. This sheet was fixed on top of the coverslip by an adhesive tape. The centre of

this sheet corresponded with the centre of the tissue section. For each triangular area, one microscopic field was selected near the centre for study. Thus, from each slide, three different fields were chosen for counting the number of ovarian follicles. Therefore, from each group, 84 fields were taken for the study. The counting was done within a counting circle specially devised for this purpose. A counting circle of 5 mm 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. Counting the follicles under low power objective, each microscopic field contains full section of follicles. However, many other follicles are only partially 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 a stage micrometer.

For statistical analysis, the software quick "p value calculator" (www.socscistatistics.com/pvalues) was used. The comparison between the right and left ovary was done by unpaired Student's t-test.

Study Group	Age Range (in Years)	No. of Samples (n=42 x 2=84)
Group A (pre-reproductive)	Newborn to 13	14 × 2
Group B (reproductive)	14 to 49	14 × 2
Group C (postmenopausal)	50 and above	14 × 2
Table 1. Study Group Distribution in Different Age Groups		

RESULTS AND OBSERVATIONS
In this study, the mean number of follicles of the right ovary (mean ± SE) per sq. mm area of the microscopic field was 82.55 ± 6.77 in group A, 42.35 ± 6.05 in group B and 1.37 ± 1.6 in group C. The mean number of the follicles of the left ovary per sq. mm of the microscopic field was 83.17 ± 6.44 in group A, 42.85 ± 5.78 in group B and 1.28 ± 2.00 in group C. The difference in number of follicles of the ovary were statistically significant in between group A and B, A and C and B and C. However, there was no statistical significant difference in the number of follicles in between the right and left ovaries.

In the reproductive age group, primordial follicles were fewer than that found in the pre-reproductive age groups. In sections of ovaries of the postmenopausal age group, primary follicles were not found. Primary follicles were found in largest numbers in the reproductive age, but they were absent in the postmenopausal age group. The secondary follicles were numerous in sections of the reproductive ovaries. In the pre-reproductive age group, they were fewer than primary follicles, and in the senile group, they were not found at all. Large tertiary or Graafian follicles were seen in large numbers in the sections of ovaries of the reproductive age group. Graafian follicles were also seen in few numbers in sections of ovary in the postpubertal age group. But, they

were not seen in the younger age groups. In the postmenopausal age groups, tertiary follicles were not seen. Smaller atretic follicles were seen in large numbers in the younger age groups. Both small and large atretic follicles were also seen in the reproductive age groups, but in the postmenopausal age groups, they were scanty.

Group	Number of Follicles Per sq. mm		t0.05	p0.05	Result
	Right Ovary Mean ± SE	Left Ovary Mean ± SE			
A n1=14, n2=14	82.55 ± 6.77	83.17 ± 6.44	-0.25	0.403	Not significant at p<0.5
B n1=14, n2=14	42.35 ± 6.05	42.85 ± 5.78	-0.223	0.412	Not significant at p<0.5
C n1=14, n2=14	1.37 ± 1.6	1.28 ± 2.00	0.29	0.38	Not significant at p<0.5
Table 2. Calculation of t and p Values to Find Out The difference in the Total Number of Follicles between Right and Left Ovaries in Different Age Groups					

Interpretation- In table 1, the t and p value were calculated to find out any difference in the number of follicles in between the right and left ovaries in each age groups. Since, the p value for each age group A, B and C is less than 0.5, it can be concluded that the number of follicles in the right and left ovaries are not significant, i.e. there is no statistical difference in between right and left ovaries.

Group A- 0-13 years.

Group B- 14-49 years.

Group C- 50 years and above.

n_1- number of slides of right ovary.

n_2- number of slides of left ovary.

Age Groups	Total No. of Follicles, Mean ± SD
A (n=28)	82.86 ± 6.78 (78.00 - 88.00)
B (n=28)	42.6 ± 5.48 (37.00 - 47.00)
C (n=28)	1.302 ± 0.57 (0 - 1)
Table 3. Mean ± SD of the Total Number of Follicles in Different Age Groups	

Interpretation- The total number of follicles per square mm area of the microscopic field were 82.86 ± 6.78 in the group A, 42.6 ± 5.48 in group B, 1.302 ± 0.57 in group C.

Group A- 0-13 years.

Group B- 14-49 years.

Group C- 50 years and above.

| Age Groups	t0.05	p0.05	Result
A vs. B	24.417	0.00001***	Significant at p <0.5
A vs. C	66.089	0.00001***	Significant at p <0.5
B vs. C	37.385	0.00001***	Significant at p <0.5
Table 4. Calculation of t and p Values to Find Out the Difference in the Total Number of Follicles in Different Age Groups			

Interpretation- By calculating the t and p value at 5% level of confidence, it is found that the p value is less than 0.5, whereas t value is very high for group A vs. B, A vs. C and B vs. C. Therefore, we can conclude that there is statistical difference in the number of follicles between groups A and B, A and C and B and C. *** = significant.
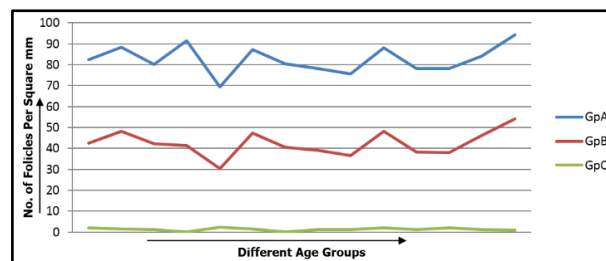
Figure 1. Graphical Representation of the Number of Follicles of Right Ovary in Different Age Groups
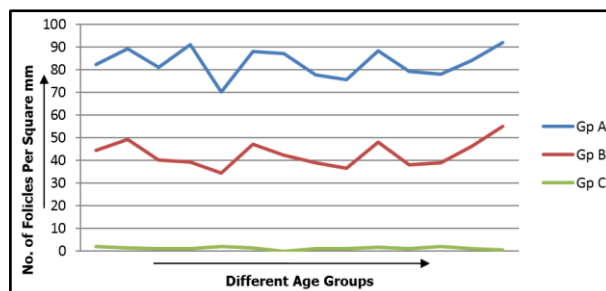
Figure 2. Graphical Representation of the Number of Follicles of Left Ovary in Different Age Groups
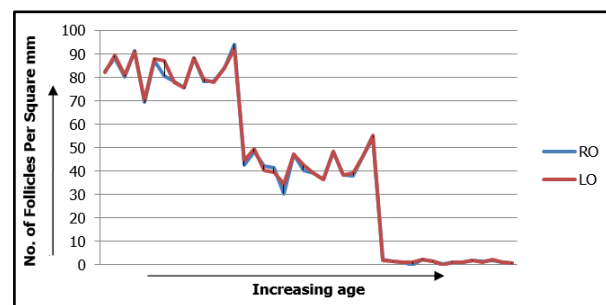
Figure 3. Graphical Representation of the Number of Follicles of Right and Left Ovaries in Terms of Increasing Age

DISCUSSION

Forabosco et al[6] in a study of 5 neonatal ovaries, mentioned that in the ovarian cortex, follicles accounted for 10-25% of the total volume. The total follicle number ranged from 1,30,000 to 3,85,000 per ovary with an average of 2,66,000 with 95% being represented by primordial follicles.

According to Sadler TW,[7] the total number of primary oocytes at birth vary from 6,00,000 to 8,00,000. During childhood, most of the oocytes becomes atretic, only approximately 40,000 are present by the time of puberty and fewer than 400 will be ovulated. He further mentioned that with each ovarian cycle, a number of follicles begin to

develop, but only one reaches full maturity and the rest degenerates and becomes atretic.

Qasim R et al[8] studied 65 ovaries of Bangladeshi female cadavers and found the mean total number of follicles per square mm in the right ovaries were 82.67 ± 2.07 in 5-12 years age group, 43.33 ± 8.17 in 13-45 years age group, 10.00 ± 5.47 in 46-51 years age group and 1.17 ± 1.94 in 52-65 years age group.

Perven HA et al[9] in their study mentioned that the mean number of follicles of the right and left ovaries per sq. mm of the microscopic field were 55.87 ± 7.82 and 54.07 ± 7.78 in 10-13 years age group, 81.96 ± 5.25 and 80.34 ± 4.83 in 14-45 years age group and 28.42 ± 5.50 and 26.95 ± 5.60 in 46-52 years age group.

Ahmed[10] studied 62 ovaries and found the mean number of growing follicles of the right and left ovary 13.59 and 15 in 2-13 year's age, 14.68 and 15.66 in 14-45 years age and 9.57 and 9.25 in 46-80 years age group, respectively.

Richardson SJ et al[11] selected 17 women aged 45-55 years who were undergoing elective total abdominal hysterectomy and salpingo-oophorectomy. One ovary from each women was serially sectioned for determination of follicle numbers. The mean number of primordial follicles in the ovaries of women who were still menstruating regularly was 10-fold higher than that in perimenopausal women (1392 ± 355 (± sem) vs. 142 ± 72). Follicles were virtually absent in the postmenopausal ovaries. Comparison of these data with those obtained by others in younger women suggests that follicular depletion accelerates dramatically in the last decade of menstrual life. These results support the view that declining follicular reserve is the immediate cause of both the perimenopausal and menopausal transitions and indicate that the rate and, therefore, the regulation of follicular depletion change during the final phase of reproductive life.

In a study carried out by Gougeon et al,[12] the effect of ageing on the number of Non-Growing Follicles (NGF) and Early-Growing Follicles (EGF) was studied in humans through use of a database obtained by pooling two subsets of ovarian pairs (2 x 43 pairs) collected in two distinct populations. Extrapolation of the fitted model suggested the presence of approximately 4,02,000 healthy NGF per ovary at birth and a total exhaustion of the follicular stock at around 74 yrs. of age. These results support the view that depletion of the NGF pool is caused mainly by atresia in younger women, but mainly by entrance of NGF into the growing pool in older women. The mechanisms triggering accelerated entrance into the growth phase of NGF are discussed in relation to the previously reported increase in FSH plasma levels that starts in the late thirties, approximately and precedes the menopausal period by several years.

In the present study, the highest mean (±SD) number of follicles were observed in the pre-reproductive age group (group A) and the lowest were observed in the postmenopausal age group (group C). The findings of the present study was similar to that mentioned by Qasim R et al.[8] But, the mean (±SD) values of the present study was higher than that of Ahmed.[10] The mean number of follicles of the right and left ovaries per square mm in the reproductive age group was similar to that of Perven HA et al.[9] Our findings also agreed with Richardson SJ et al[11] who stated that follicular depletion accelerates dramatically in the last decade of menstrual life and follicles were virtually absent in the postmenopausal ovaries.

CONCLUSION

Female reproductive ageing has been proposed to proceed as an ongoing decline in ovarian reserve determined by remaining ovarian follicle number. It remains unclear whether reproductive ageing progresses simply as a predetermined function of remaining ovarian follicles or as an age-dependent process in humans. The present study has highlighted the changes in follicular number with respect to different ages. To have a better idea of the ovarian follicles, counting of the total number of follicles is necessary, which requires serial sections of the whole ovary at regular intervals, which is a long process. This study may be used as a base for further advanced studies on the ovarian follicles, which may help in the treatment of ovarian diseases including problems like infertility.

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