ANTRAL FOLLICLE COUNT: A COMPARATIVE STUDY AMONG FERTILE AND INFERTILE WOMEN OF DISTRICT DERA ISMAIL KHAN, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

Objective: *The objective of the current study was To compare the AFC between fertile and infertile females of Dera Ismail Khan.*

Materials and Methods: *This study was conducted in order to determine the follicle count with the help of ultrasound of fertile and infertile females of D.I Khan. A total of 120 females were selected out of which 55 were fertile and 65 infertile and ultimately 100 females were left after fulfilling the inclusion and exclusion criteria. All the measurements were done by the same observer using colour Doppler Sonoscape. The multi-frequency probes used were the transabdominal convex probe with a mid frequency of 2-6MHz and transvaginal probe with a mid frequency of 5-9MHz. The examination was performed first transabdominally on a filled urinary bladder and transvaginally after the bladder was emptied. All the follicles 2-10mm in size were counted in each of the ovary and the total was obtained by sum of the follicle count of the two ovaries.*

Results: *Antral follicle counts in fertile and infertile group: The data pertaining to antral follicle counts in fertile and infertile patients are presented in table 1 and figure 1, 2 & 3 respectively. From the table it was found that highly significant ($P \leq 0.01$) differences were observed in antral follicle counts in fertile and infertile patients. AFC were recorded higher (11.47) in fertile patients as compared to infertile patients (6.92). Comparison of AFC in fertile and infertile groups according to age: The antral follicle count according to age in fertile and infertile groups. It shows that maximum number of follicles was found in the youngest age of 18-26 years i.e. 16.51 while minimum number of 4.61 in the oldest age group in fertile patients. In case of infertile group the count was 9.15 in the youngest age group i.e. highest as compared to other age groups (2 in the oldest age group). It was further observed that with increase in age, the follicle counts decreased*

INTRODUCTION

A couple may be considered infertile if, after two years of regular sexual intercourse, without contraception, the woman has not become pregnant. Due to the fact that there are inherent difficulties in estimating the prevalence of infertility it has generally

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been accepted that one in four women are affected at sometime. The causes of infertility can be either due to male or female factors. The common causes are tubal blockage, polycystic ovarian syndrome i.e. anovulatory infertility, pelvic inflammatory disease, uterine problems, age related factors, previous tubal ligation, endometriosis and advanced maternal age.[1] Although there are a number of tests used to evaluate the infertile couples, but were divided into five categories which are semen analysis, post coital tests, ovulation assessment, tubal and uterine evaluation and last but not the least is laparoscopy.[2] But semen analysis, serum progesterone level in mid luteal phase and evaluation of tubal patency were considered as the initial basic tests for infertile patients.[3]

The term ovarian reserve refers to the remaining or residual repertoire of oocyte-granulosa cell, at a required age, which will be available for procreation. Despite all the basic tests used for infertile couples the most important test regarding success of ovulation induction is to determine ovarian reserve at first then go for other invasive tests.[4] Estimation of ovarian reserve will help in predicting the remaining reproductive life span and expected success of assisted reproductive techniques (ART) like in vitro fertilization (IVF).[5,6,7] In theory, this would help clinicians to personalize patient management by selecting an appropriate treatment protocol, stressing the need for early initiation of treatment or counseling against initiation of treatment.[4] The primordial follicles even at rest are continously entering the growing pool throughout life. Follicles occur at different stages of development in the ovary, and large number of them of different sizes can be observed at any point of the menstrual cycle. Follicle Stimulating Hormone (FSH) is an absolute requirement for the development of large antral pre ovulatory follicles. High levels of FSH usually give rise to continued growth of some of the follicles. The Gonadotropin Releasing Hormone (GnRH) secreted by hypothalamus stimulate the anterior pituitary which produce both (LH) luteinizing hormone and (FSH) follicle stimulating hormone. When ovary is stimulated by gonadotropins to secrete steroid hormones like estrogen and progesterone. These steroids stimulate endometrial proliferation and produce their effect on many end organs.

Among the different tests required for the assessment of ovarian reserve, Basal antral follicle count is of profound importance. Basal antral follicle count is a measure of resting follicles in both the ovaries in the start of proliferative phase of menstrual cycle by transvaginal ultrasound. Their size is approximately 2-6mm and according to recent studies, are the predictors of ovarian response in ovarian hyperstimulation36. Antral follicles >14 are good predictor of ovarian hyper response. 3D or 2D ultrasound has no advantage over one another in order to assess ovarian reserve.[8,9,10,11,12,13,14]

Antral Follicle Count is defined as number of follicles which are less than 10mm in diameter detected in the early follicular phase with the help of transvaginal ultrasound.[15] The number of follicles less than 18mm are called antral follicles.[16] The number of antral follicles in the ovaries is directly related to the primordial follicle pool from which they were recruited. So the quantitative aspect of ovarian aging is represented by the antral follicle count. As a result of development and advancement in ultrasound technology we can now easily count antral follicles.[17] The precise and exact definition of antral follicles varies ranging between 2-10 and 2-5mm; similarly the threshold for the definition of low AFC is different according to different studies.[18] AFC is considered to be a better predictor of distinguishing between old patients for a good or a bad prospect for pregnancy as compared to age or FSH.[19] The best way to measure AFC is by a transvaginal ultrasound in the early part of follicular phase, by taking mean of two measurements which are at right angles. Sum of total number of follicles in both the ovaries is taken for total AFC. The antral follicles may range from 8-10, 2-6 or 7-10mm. The diameters may be different as there is no consensus regarding the size of follicles which still will represent ovarian reserve.[14] Antral follicles with a size of 2-6mm declines with age correlating with FSH and CCCT but follicles with a size of 7-10mm remains constant, so the former is a more reliable marker of ovarian reserve. Smaller antral follicles with a size of 6mm better reflect ovarian reserve than total AFC, if 2-10mm of follicle size are counted. So the large antral follicles reflect remaining follicle pool.[18] Ovarian follicles with a size more than 2 mm are very sensitive and responsive to FSH and are defined as "recruitable". The total number of 2–10 mm follicles in both the ovaries represents the AFC which can be visualized and measured with transvaginal

ultrasound. Hence the AFC reflects the extent of the pool of follicles on which the exogenous FSH will act.[20] Like the total AFC, the small antral follicles decrease significantly with age but the pool of large follicles does not practically change until about 45 years of age.[21]

OBJECTIVE

The objective of the current study was To compare the AFC between fertile and infertile females of Dera Ismail Khan.

MATERIALS AND METHODS

This study was conducted in order to determine the follicle count with the help of ultrasound of fertile and infertile females of D.I Khan. A total of 120 females were selected out of which 55 were fertile and 65 infertile and ultimately 100 females were left after fulfilling the inclusion and exclusion criteria.

Inclusion criteria:

For fertile females:

The inclusion criteria for fertile women were age ranging from 18years and above, married, regular menstrual cycles varying from 21 to 35 days, no evidence of endocrinological disease, natural fertility proved by having had at least one pregnancy carried to term, the first pregnancy occurred spontaneously within 1 year after the start of unprotected intercourse and hormonal contraception stopped > 2 months before entering the study protocol.

For infertile females:

For infertile females the inclusion criteria were age from 18years and above having regular menstrual cycles, married female living with her husband for the past 1 year and primary infertility.

Exclusion criteria:

For fertile females: Exclusion criteria for fertile females: History of Hypertension, Diabetes Mellitus, drug abuse, smoking, malignancy, ovarian surgery, pregnancy, lactation, ovarian induction and ovaries containing cysts.

For infertile females: Exclusion criteria for infertile females: History of Hypertension, Diabetes Mellitus, drug abuse, smoking, malignancy, ovarian surgery, abnormal ovarian morphology and the presence of polycystic ovaries or ovaries containing cyst assessed by ultrasound at the time of recruiting subjects for the study.

Sample collection procedure:

A written informed consent was obtained from all the subjects. Scan of the ovaries was carried out on any day in the start of menstrual cycle. All the measurements were done by the same observer using colour Doppler Sonoscape. The multi-frequency probes used were the transabdominal convex probe with a mid frequency of 2-6MHz and transvaginal probe with a mid frequency of 5-9MHz. The examination was performed first transabdominally on a filled urinary bladder and transvaginally after the bladder was emptied.

Antral follicle count:

All the follicles 2-10mm in size were counted in each of the ovary and the total was obtained by sum of the follicle count of the two ovaries.[22,23, 24,11,12,13,14,17]

Statistical analysis:

The data feeding and study was done on computer package SPSS (Statistical Package for Social Sciences) Version 16.0 for Windows. The results were given in the text as mean, standard deviation of significantly quantitative variables (age, weight, height, ovarian volume and ovarian follicle count). Mean and standard deviation of quantitative variables were evaluated by using student t-test for mean differences and found the correlation coefficient of ovarian volume, ovarian follicle count against age.25 In all statistical analysis, only p-values <0.05 were considered significant. The results were presented as tables and graphs.

RESULTS

Antral follicle counts in fertile and infertile group:

The data pertaining to antral follicle counts in fertile and infertile patients are presented in table 1 and figure 1, 2 & 3 respectively. From the table it was found that highly significant ($P \leq 0.01$) differences were observed in antral follicle counts in fertile and infertile patients. AFC were recorded higher (11.47) in fertile patients as compared to infertile patients (6.92).

Comparison of AFC in fertile and infertile groups according to age:

The mean values of antral follicle counts in fertile and infertile groups are given in table 1 and figure 3. The table 2 shows the antral follicle count according to age in fertile and infertile groups. It shows that maximum number of follicles was found in the youngest age of 18-26 years i.e. 16.51 while minimum number of 4.61 in the oldest age group in fertile patients. In case of infertile group the count was 9.15 in the youngest age group i.e. highest as compared to other age groups (2 in the oldest age group). It was further observed that with increase in age, the follicle counts decreased gradually.

Table 1: Comparison of antral follicle counts (AFC) in fertile and infertile patients.

PARAMETER	FERTILE	INFERTILE	T-VALUE	PROB
Antral follicle count(AFC)	11.47±0.69	6.92±0.37	4.92	0.0000

Table 2: Comparison of antral follicle counts (AFC) in fertile and infertile patients of various age groups

Age(years)	Fertile	Infertile
18-26	16.51	9.15
27-34	12.93	5.81
35-42	7.85	4.60
43-50	4.61	2
SD	5.28	2.96

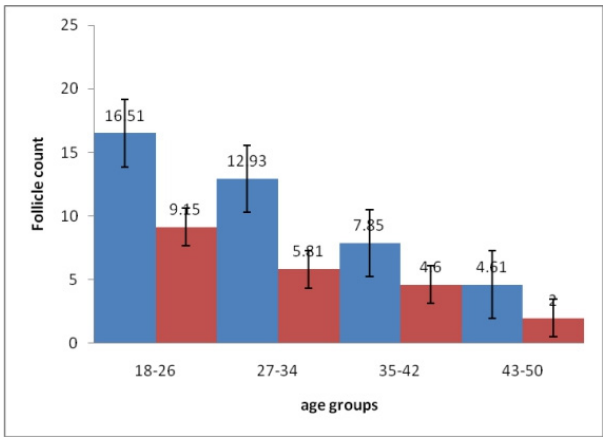

Fig 1: Comparison of antral follicle count in fertile and infertile patients of various age groups.

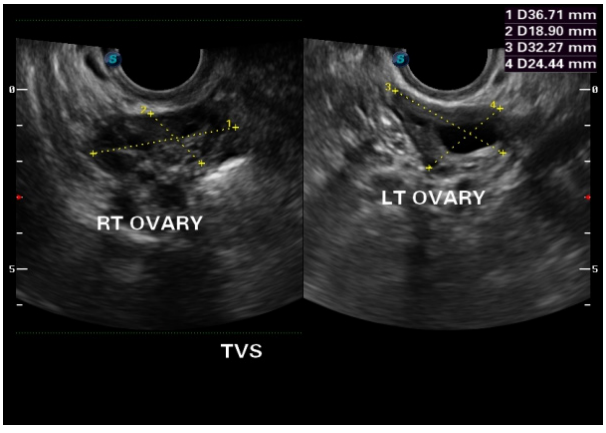

Fig 2: Transvaginal ultrasound image of ovaries (arrows) showing follicles in fertile group.

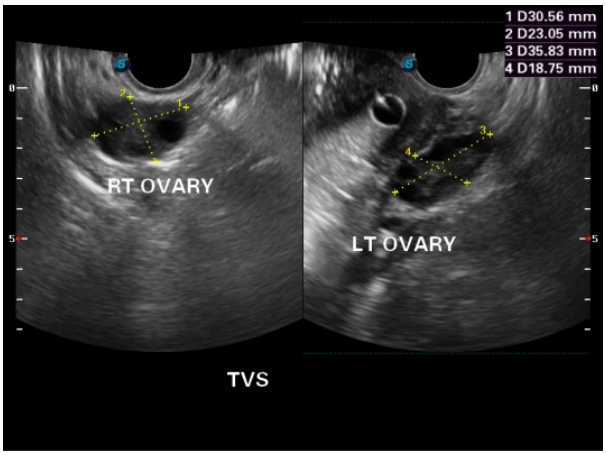

Fig 3: Transvaginal (TVS) ultrasound image of ovaries rarely showing any follicles in infertile group

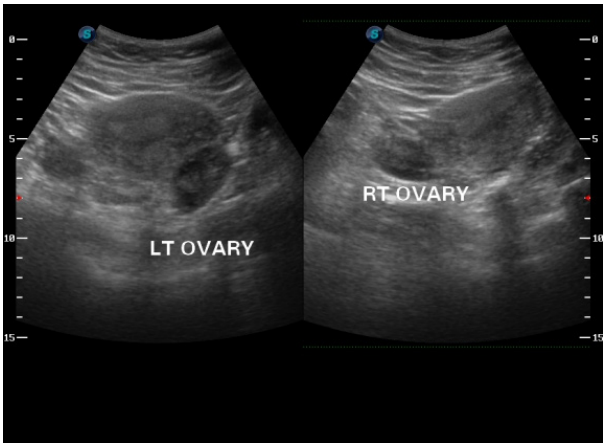

Fig 4: Transabdominal (TAS) ultrasound image not clearly showing Ovarian follicles in fertile group.

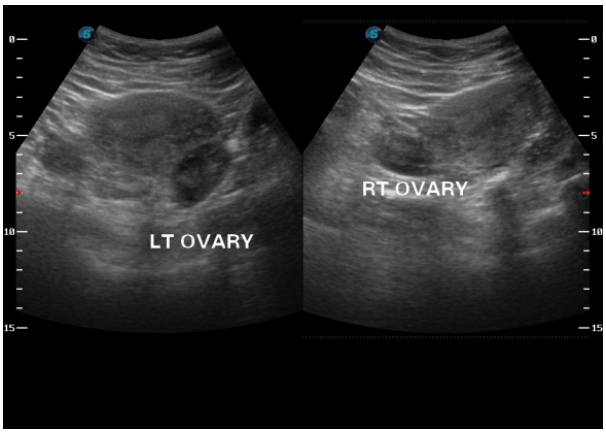

Fig 5: Transabdominal (TAS) ultrasound image not showing ovarian follicles in infertile group.

DISCUSSION

In our study we compared the follicle count of the ovaries in fertile and infertile females according to age. Our results showed that as the age of the female increases both the volume of the ovaries and follicle count decreases. Our results clearly showed that antral follicle count has a significant decrease with increasing age in both fertile and infertile population. Our study showed the AFC to be highly significantly different in both fertile and infertile females and same results were shown by Ng EH in his study.[23] In a review by Carvalho et al., age was considered as the most important clinical marker and the first to be considered in the assessment of ovarian reserve.[26] None of the study was successful in selecting an ovarian reserve marker with both sensitivity and specificity so in the same review article AFC alone was considered good in order to predict poor response to IVF stimulation as compared to combination of markers. The significance of AFC have been extensively studied in infertile patients and Tomas et al., concluded that AFC was the best predictor of ovarian response.[27] AFC had the best predictive value, followed by FSH and age of women in a prospective study of 128 women who underwent first IVF cycle after ovarian stimulation.[23] Other studies also confirmed AFC as very important for prediction of ovarian response. In our study we studied the AFC in fertile women in order to compare it with infertile females. The count was highest in the youngest age group of 18-26 years (16.51) as compared to the same age group in infertile females (9.15) but the count was much lower (4.61) in the oldest age group of 43-50 years. In a study conducted by Himabindu et al., showed AFC to be better, cost effective and routinely done test then AMH for the prediction of ovarian response and also to be more reliable in females above 35 years of age11 which was also shown in our study.[11,14,17,18,20] Scheffer et al., showed that antral follicles with a diameter of 2-10mm, measured by TVS in the early follicular phase has the best correlation with age in normal fertile females.[13] In our study AFC was 16.51 in fertile as compared to 9.15 in infertile females which is a very significant difference. We have also shown that the mean follicle count in fertile females was 11.47 whereas infertile had a follicle count of 6.92. In a Chinese population the follicle count was 9.0 in females with known fertility, while a study conducted in Karachi showed follicle count to be 7.35 in fertile and 5.7 in sub fertile females.[22,23] Another study done in India showed mean follicle count to be 8.57 in females enrolled for ICSI (Intra Cytoplasmic Sperm Injection).[11] These variations are a proof that there obviously is a difference in the follicle count depending on the genetics, climate, socioeconomic status and also age of the females.

CONCLUSION

This study concluded that the follicle count determined by sonography was significantly decreased in infertile women. Furthermore, additional studies with hormonal levels are required to drive improvements in the diagnosis and management of ART processes in future years.

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