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RESEARCH ARTICLE

ULTRASOUND MEASUREMENT OF ANTRAL FOLLICULAR COUNT AND OVARIAN VOLUME IN NORMAL (FERTILITY –PROVEN) AND INFERTILE SOUTH INDIAN WOMEN.

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Abstract

Infertility is a major distress to many couples in the world, causing increased numbers of them to approach medical care. Infertility or otherwise subfertility is the failure of a couple to conceive after 1 year of regular, unprotected intercourse. Ovulatory disorder is one of the most common reasons of female factor infertility accounting to 30% of all cases.[1] Ovarian reserve denotes the capacity of the ovary to provide egg cells capable for fertilization. To asses the individual quantitative ovarian reserve various tests are available like Day3 FSH, AMH, Antral follicular count and ovarian volume measurement. Antral follicular count is referred as a number of oocytes and follicles in ovaries which is morphologically healthy. Anti mullerian hormone is produced by healthy ovarian follicles and serves as a marker of quantity of healthy follicles and oocytes in ovaries. Ovarian volume is assessed by ultrasound and helps in assessment of ovarian function. The aim of this study is to compare the ovarian reserve of fertility proven women with women presenting with subfertility using the tests like antral follicular count and ovarian volume measurement in a tertiary care teaching hospital in Thoothukudi,Tamil Nadu, South India.

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Review Of Literature:-

Ovarian Reserve:-

Ovarian reserve is the size and number of resting or non –growing primordial follicle population that presumably determines growing follicles numbers and the reproductive potential of the oocytes. Its evaluation on Day 2 plays a major role in the assessment of response of the ovaries to exogenous gonadotropin administration in terms of the number of oocytes that may be produced in the present cycle planned for an ART.

A strong association between increasing age of the infertile women and decreasing fertility rate has been documented well. Chronologic age of the mother is the strongest predictor of ovarian reserve and also the major determinant of are productive success.

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Screening Tests For Ovarian Reserve:-

Measurement Of Day 3 Fsh:-

This test is based on the fact that increase in serum basal FSH levels correlates with the decreased fecundability seen in women in their late 30s. A value more 12mIU/ml is worrisome and usually is associated with lesser success rates in ART.

If more than 25mIU/ml confirms with menopausal level denoting ovarian failure due to decreased ovarian reserve.

Basal Estradiol Level:-

The estimation of estradiol level in day 3 of cycle correlates more with the follicular growth rather than the number of antral follicles.

Serum Inhibin B:-

Inhibin B is secreted by ovarian granulosa cells from the preantral follicle stage and reflects the overall granulosa cell function of the group of follicles that are recruited to undergo gonadotropin–dependent growth .The Patients with inhibin B levels of less than 400 pg/mL have a significantly less number of follicles.

Serum Antimullerian Hormone (Amh):-

The granulosa cells of both pre-antral and small antral follicles produce AMH. It can be measured during any phase of the menstrual cycle.Its function is to inhibit the initiation of growth of the primordial follicle. In women with regular cycle, the serum level of AMH declines with age and it becomes undetectable by menopause. Also as the primordial follicle count decreases, the serum AMH concentration also decreases and hence making this hormone an ideal parameter for the early diagnosis of ovarian reserve depletion. Decreased levels of AMH in the early follicular phase correspond to poor ovarian reserve in ART cycles.

Normal AMH-1.5-4.0

Low AMH-0.5-1

High AMH>4

Antral Follicular Count:-

Antral follicular count is measured by using transvaginal ultrasound in the early follicular phase. All ovarian follicles measuring between 2 -10 mm in both ovaries are counted and the total value obtained is called basal antral follicle count(AFC).It is suggested that counting all antral follicles of size 2-10mm in diameter is the preferable method.The intra and inter observer variability that occur while estimating the AFC can be largely reduced by a newer technique called three dimensional automated follicular tracking.

Ovarian Volume:-

The human ovary is an organ which changes in size and activity throughout life. At birth, the ovary is ~1cm in length and weighs <0.3g. The ovary decreases slightly in volume at 1 month of age, probably due to the clearance of maternal estrogen from the female neonate (Haber and Mayer 1994). Ivarson *et al* in 1983 demonstrated that there was an increase in mean volume of ovary from 0.7cm3 to 5.8cm3 through 10 years to 17 years of age. Adult ovaries are ovoid, measure approximately 3-5cm by 1.5-3cm by 0.6-1.5cm and weigh 5-8g. There are no major changes in ovarian volume during reproductive years until the premenopausal period (Christensen *et al*, 1997). Following menopause, the ovaries will shrink to about one-half of their size as seen in the reproductive period. They weigh 3-4g (Thatcher and Naftolin,1991). Andolf *et al* showed that the size of ovaries decreases in menstruating women over 40 years of age and that parity has no influence over this (Andolf *et al* 1987)Tepper *et al* found a linear relationship between menopause age and ovarian volume. The mean ovarian volume dropped from 8.6cm3 a year after the menopause to 2.2cm3 15 years into menopause.

Measurement Of Ovarian Volume By Transvaginal Ultrasound:-

Women having a mean ovarian volume of <3cm3 have a higher chance of follicular stimulation failure. 37

The size of ovaries plays an integral role in the diagnosis of OHSS and is useful for grading the severity of it. The ovaries are measured in three planes and the ovarian volume was calculated using the ellipsoid formula V=D1xD2xD3x0.523.

D1, D2 ,D3 are the three maximal longitudinal antero-posterior and transverse diameters respectively. Recently a few investigators have suggested using computerized three dimensional transvaginal ultrasound as a tool for predicting both over and under-responsiveness to superovulation.

Other Clinical Implications Of Ovarian Volume Measurement:-
Ovarian Volume As A Marker For Ovarian Cancer:-
Van Nagell *et al*, in his study performed transvaginal scan on 8500 asymptomatic women, diagnosed that if the volume of an ovary is >20cm3 in premenopausal and >10 cm3 in postmenopausal women it was abnormal. In addition they looked for the presence of internal papillary projections.

Aim Of The Study:-
1. To establish the role of AFC as a function of ovarian reserve in fertility-proven and in sub fertile Indian women.
2. To know the cut-off value of antral follicle count in normal and infertile women in South Indian population.
3. To asses if there is a difference in the ovarian volume between fertile and infertile women in South Indian population

Settings and design:-
case-control study done at govt.thoothukudi medical college hospital, thoothukudi, a tertiary care teaching hospital in tamilnadu, south india.

Inclusion criteria:-
for cases:-
1. primary infertility (ii) no ovarian abnormality (polycystic ovary, ovarian endometriomas) as assessed by transvaginal usg.
2. no evidence of uterine malformations or uterine pathology,
3. no evidence of endocrinal disease
4. no evidence of previous ovarian surgery

for controls **:-**
1. proven natural fertility by having at least one pregnancy carried to term
2. regular menstrual cycles,
3. no evidence of endocrinal disease
4. no history of ovarian surgery

Exclusion criteria:-
1. h/o ovarian abnormality like polycystic ovary, ovarian endometriomas
2. history and any evidence of uterine malformations or uterine pathology,
3. h/o endocrinal disease
4. h/o previous ovarian surgery
5. hormonal contraception stopped > 3 months before entering the study protocol.

Sample size:-
sample size for frequency in a population – 30 cases and 30 controls .

Study method:-
The basal ovarian volume and afc were measured by endovaginal ultrasound
Carried out on the second or third day of the menstrual cycle.

All follicles 2-10 mm size range of well-defined anechoic cysts with smooth margins and absence of internal septations or nodularity were measured and counted in each ovary. The sum of follicular count in both ovaries was labeled as antral follicular count.

The ovaries were measured in three planes and the ovarian volume was calculated using the prolate ellipsoid formula v=d1xd2xd3x0.523. D1, d2 ,d3 are the three maximal longitudinal antero-posterior and transverse diameters

The results were analysed after being grouped as follows:

1. Age
Age group 1 25– 30yrs
Age group 2 31 -35 yrs
Among infertile group 15 in group 1 , 15 in group 2
Among fertility proven control group 16 in group 1 , 14 in group 2
The mean standard deviation of the both infertile and control group showed that there exists a statistical significance among the two groups with response to age.

Ovarian volume:-
Group 1:- 9-11
Group 2 :- ≥11
Among infertile group 15 in group 1 , 15 in group 2
Among control group 11 in group 1, 19 in group 2
The mean standard deviation of the both infertile and control groups that was found revealed that there is no statistical significance among the two groups with regard to ovarian volume.

Group statistics group	n	Mean	Standard deviation	Standard error mean	Significance P
Ovarian volume 1(infertile)	30	10.86	1.639	0.299	0.184
0(control)	30	11.36	1.211	0.221	0.185

Antral follicular coun t:-
Group 1 – count >8
Group 2- count ≤8
Among infertile group 6 in group 1 , 24 in group 2
Among control group 30 in group 1 , 0 in group 2
The mean standard deviation of the both infertile and control group reveals that there exists a statistical significance among the two groups with response to antral follicular count

Group statistics	N	Mean	Standard deviation	Standard error mean	Significance P
1(infertile)	30	6.67	1.688	0.308	0.000
2(control)	30	11.23	2.112	0.386	0.000

Result:-
On comparative analysis of biophysical and sonographic variables in infertile and fertile patients, the given table shows that there is no change in ovarian volume in both the cases and the controls but there is significant change in antral follicular count in both groups when a cutoff of 8 was used.

Variables	Cases(n=30) (mean±sd)	Controls(n=30) (mean±sd)	P value
Age(years)	31.30±2.466	29.80±2.355	0.0134
Bmi(kg/m2)	22.70±2.672	22.22±2.194	0.4568
Antral follicle count(afc)	6.67±1.688	11.23±2.112	0.0001
Total ovarian volume(cc)	10.86±1.639	11.36±2.112	0.4113

Discussion:-
Our observation indicates that the number of antral follicles is lower in sub-fertile patients than in fertile group (25 - 35 yrs), with a significantly lower median afc in women of the former group ($p<0.001$). The range of afc in females presenting with complaints of infertility was 4-12(median value of 8) reported in western studies. The cut off value in indian women is at a lower base line than that noted in the western literature. This variability in the value of afc is most probably due to the differences in the race, socio-economic and geographic background of indian and western populations. A cut off value of 8 may be used to prognosticate patients undergoing assessment for female factor infertility. The results of this study indicate that afc is a valuable predictor of fecundity in south indian women of child bearing age in terms of capability to conceive on a two point scale (i.e. Positive or negative). Also noted in our

study was that there was no statistically significant evidence to show that the ovarian volume was different in the fertility proven and sub-fertile women.

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