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Original Research Article

Assessment and Correlation of Endometrial Biopsy, III Day Serum Luteinizing Hormone, Ovarian and Endometrial Ultrasonography Findings in Infertile Women

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ABSTRACT

Ovulatory defects are found in 40% of infertile women in about 15% of the infertile couples. This requires a thorough history and proper evaluation of ovulatory functions.
Aims: to study the LH levels between 3rd-5th menstrual day, endometrial dating in premenstrual period along with follicular growth monitoring done on ultrasonography.
Methods: Blood sample evaluation between 3rd-5th menstrual day was done to monitor the LH levels. Endometrial thickness and follicular growth monitoring were monitored daily from 9th menstrual day onwards. Also, on LH+6, LH+10, endometrial biopsy was taken with the Pipelle suction catheter. The findings were then correlated.
Conclusion: There is an increased rate of primary infertility in women in age group 25- 40 years. Majority of these were cases of Polycystic ovarian syndrome with increased BMI and increased LH with increased endometrial thickness. Also we found 63.4% of cases with predominant hormonal imbalance, they were consistent with the findings if endometrial dating.

Keywords: female infertility, ovulatory defects.

INTRODUCTION

Reproductive health is defined as "a condition in which the reproductive process is accomplished in a state of complete physical, mental, social and spiritual well being and not merely the absence of disease or disorders of the reproductive process." It implies that the people have the ability to reproduce, to regulate their fertility and to practice and enjoy sexual relationships. [1]

Infertility is defined as the inability to conceive after 1 year of regular unprotected intercourse. The infertility evaluation is typically initiated after 1 year of trying to conceive, but in couples with advanced female age (>35 years), most practitioners initiate diagnostic evaluation after an inability to conceive for 6 months. [2]

The two types were identified: primary infertility, which is the inability of couples to conceive despite cohabitation and exposure to pregnancy for a period of one year, while the secondary infertility, is the inability of couples to impregnate following previous pregnancy, irrespective of the outcome of pregnancy, despite cohabitation and exposure to pregnancy for one year. [3]

Female infertility has been caused by many factors such as endometriosis, anovulation from polycystic ovarian disease, hyperprolactinemia with or without galactorrhea, hypogonadotropic hypogonadism, premature ovarian failure and cervical and endometrial factors. [1] In terms of ovarian reserve, a typical woman has 12% of her reserve at age 30 years and has only 3% at age 40 years. 81% of variation in ovarian reserve is due to age alone, making age an important factor in female infertility. [4] Ovulatory defects are present in 40% of infertile women and in approximately 15% of couples with infertility. Often a defect in ovulatory function manifests itself in menstrual disturbances and can be identified by history in the majority of women. [2] Ultrasound evaluation in the follicular phase is used to identify uterine polyps, fibroids, and congenital cavitary anomalies such as a septate uterus. At the same time, information on ovarian volume and antral follicle counts can be obtained, making pelvic ultrasound part of the initial workup for infertility. [2]

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility and is characterized by the symptoms of hyperandrogenism, oligo- or amenorrhoea, polycystic ovarian morphology, central obesity and insulin resistance. LH has been assessed as a diagnostic test to discriminate between women with PCOS and healthy controls. [5] Elevated LH is an inextricable feature of PCOS, found in about half of PCOS patients, the prevalence reported from 35-77%. The cause of high variability in prevalence of elevated LH is due to differences in the timing of LH sampling. Most studies measure LH in the early follicular phase, but due to the intercycle variation of LH, the prevalence of high LH concentrations in PCOS is probably underestimated. The optimal timing for measuring LH concentrations is when LH is minimally suppressed, and this is between at least 2 weeks from the start of the menstruation and at least 3 weeks before the next period. This phase is called the "specific oligomenorrhoeic phase", and only exists in cycles longer than 35 days. [5] The endometrium in PCOS has a wider spectrum compared to that of normal endometrium and has a higher incidence of hyperplasia. Higher prevalence of endometrial hyperplasia in such women is attributed to persistently high levels of oestrogens without progesterone. [6] Increased gonadotrophin releasing hormone pulsatility, luteinizing hormone hyper secretion is one of the hallmarks of PCOS. Increasing levels are observed in about 70% of PCOS patients with elevated LH pulse amplitude and increased LH pulse frequency leading to a two to three fold elevation in serum LH level versus follicle stimulating hormone (FSH) serum level. [6] Luteal phase deficiency has been described as a condition in which endogenous progesterone is not sufficient to maintain a functional secretory endometrium ad allow normal embryo implantation and growth. LPD is only clinically relevant if it is consistently present in most cycle. The short luteal phase was initially described as an interval of < 8 days from the LH peak to the onset of menstrual flow. The short luteal phase has been associated with low follicular follicle stimulating hormone (FSH). Abnormalities in gonadotropin releasing hormone (gnRH), FSH and LH pulsatility may be found in recovery from hypothalamic amenorrhea and may result in diminished luteal oestrogen and progesterone secretion. [7] Investigation of endometrium has often required dilation and curettage or via an office endometrial biopsy. Sonographic assessment of endometrial thickness is frequently used in the monitoring during infertility treatment. Ultrasound measurements of endometrial thickness were originally carried out by measuring the distance from anterior stratum basalis to the posterior stratum basalis, and dividing by 2 to give a single-layer measurement. The endometrial thickness in premenopausal patients varies

with menstrual cycle. A range of 6-12mm during the menstrual cycle was observed. [8] The ultrasound appearance of the endometrium differs throughout the cycle. Histological examinations of endometrial tissue samples from both fertile and infertile women are performed to yield information revealing histological evidence of ovulation, for postovulatory dating of the secretory phase specimen and for presence or absence of endometrial abnormalities that may be responsible for infertility. [9]

Transvaginal ultrasonography is used to monitor ovulation. This is performed since the onset of fertile type mucus and / or luteinizing hormone surge such as detected by the rapid assays. Scanning is first performed on each other day until observation of 16-mm follicles, then daily until evidence of ovulation. The day of ovulation is defined as the24 hour time-lag between the visualization of a mature follicle at one scan and the appearance of ruptured follicle or an early corpus luteum, and/or free fluid in the cul-de-sac at the next scan. [10]

Owing to the anovulation, the endometrium in PCOS is exposed to the prolonged mitogenic effects of estrogen, unopposed by the inhibitory effects of progesterone present in the luteal phases of normal menstrual cycles. [11] The endometrial biopsy, with evaluation of morphological changes, has been considered superior to alternatives such as serum P measurements, because of the pulsatile nature of P secretion and a belief that these morphological changes represent the cumulative effect of cycle-specific patterns of ovarian hormone secretions. [12] Obesity plays a significant role in determining the severity of clinical manifestations and metabolic disorder. It has been seen that in patients with PCOS the body mass index (BMI) is correlate with an increased rate of hirsutism, menstrual irregularity and infertility. [13]

AIMS AND OBJECTIVES:

1) To study the demographic characteristics and type of infertility.
2) To study their serum LH levels in the 3^{rd}-5^{th} menstrual day.
3) To study their endometrial dating in premenstrual period.
4) To study the follicular growth monitoring on ultrasonography.
5) To find the correlation in the above findings if any.

MATERIALS AND METHODS

The study was done at a tertiary care hospital. The patients attending the infertility clinic between the months of June 2016-August 2016 were considered.

It was a prospective study. About 52 samples were evaluated.

Inclusion criteria:

1) No pregnancy after 12 months of attempted conception.
2) Regular menstrual cycles, every 24-34 days.
3) Aged 18-40 years.
4) No hormonal treatment in the last menstrual cycle.

Exclusion criteria:

The women not fulfilling the criteria mentioned above were excluded.

Fifty two women, complaining of infertility for at least one year, voluntarily agreed to participate in the study. Informed written consent was taken. The study was proceeded with after obtaining the permission from Institutional Ethical Committee.

Blood samples were drawn from patients between days three and five of the menstrual cycle for basal plasma levels of LH (normal range:1.5-10 U/L). From menstrual day nine onwards, follicular diameter and endometrial thickness were measured daily by trans abdominal ultrasonography. When the dominant follicle reached 16 mm, daily LH level measurements were started, until LH peak was demonstrated. The following day was taken as the first day of luteal phase (LH+1).

The patients were then asked about the menses cycle. If no delay was found in their menstruation, then the endometrial thickness was measured right away by the gynecologist by trans abdominal ultrasound.

In the same way the gynecologist performed the endometrial biopsy on LH+6 and LH+10 with the Pipelle suction catheter. In the first of these the suction catheter was introduced with its subterminal orifice directed towards the right uterine wall and in the second to the left. Tissue was immediately placed in a fixative and processed for histological evaluation.

Histological analysis was done by the pathologist. The pathologist was blinded to the purpose of the study, including the fertility status and the menstrual day when it was performed.

Statistics:

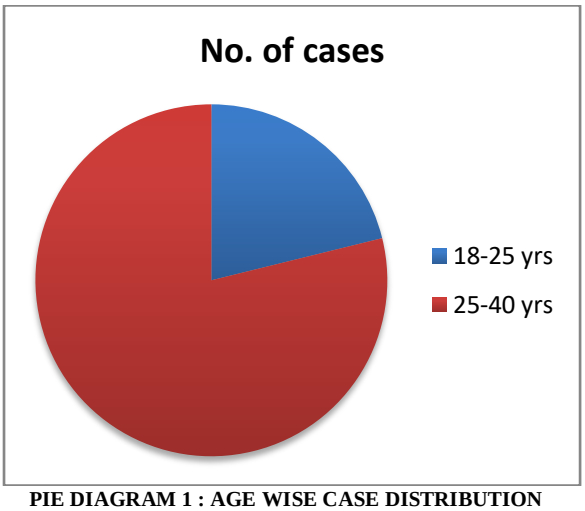
The various parameters were tabulated and statistical analysis was done.

The data was statistically analyzed by using the SPSS software version 20. P<0.05 was considered significant. Student's t- test was used to compare the normally distributed data.

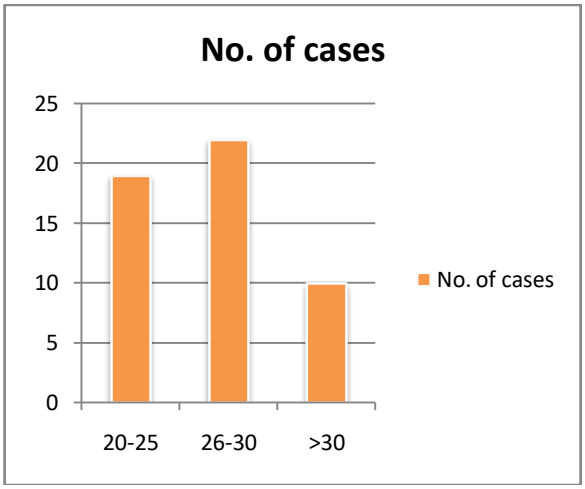
Type	No. of cases
Primary infertility	47
Secondary infertility	5

OBSERVATIONS AND RESULTS:

TABLE 1 : TYPE OF INFERTILITY



PIE DIAGRAM 1 : AGE WISE CASE DISTRIBUTION

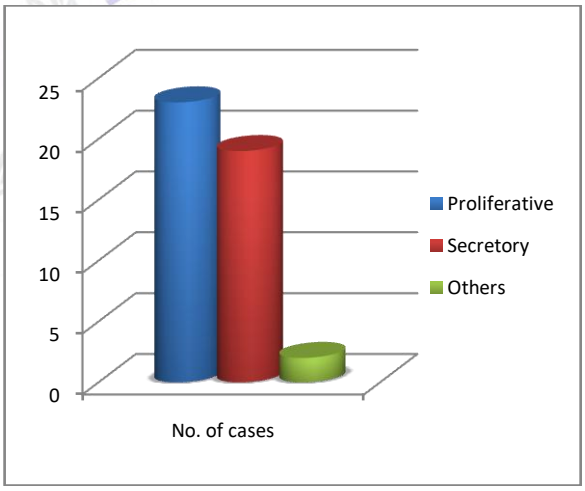


COLUMN DIAGRAM 1: CASES DISTRIBUTED ACCORDING TO THE BMI

In our study majority of the patients were in the BMI range 26-30.

TABLE 2: CASE DISTRIBUTION ACCORDING TO ENDOMETRIAL DATING FINDINGS.

	Proliferative	secretory	Others
No. of cases	23	19	10



COLUMN DIAGRAM 2

On performing endometrial biopsy, majority of the patients were in proliferative phase.

TABLE 3: CASE DISTRIBUTION ON THE BASIS OF 3rd-5th day serum LH levels.

	<12IU/ml	>12 IU/ml
No. of cases	19	33

TABLE 4: ENDOMETRIAL THICKNESS ON ULTRASONOGRAPHY.

	<10 mm	>10 mm
No. of cases	23	29

TABLE 5: OVULATION ON ULTRASONOGRAPHY.

	With ovulation	Without ovulation
No. of cases	20	32

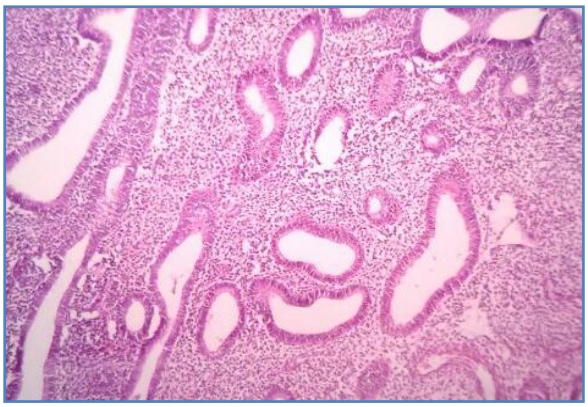


Fig 1: H&E 40X , Altered glands to stroma ratio. Tubular to dilated glands in a fairly oedematous stroma with few foamy cells.

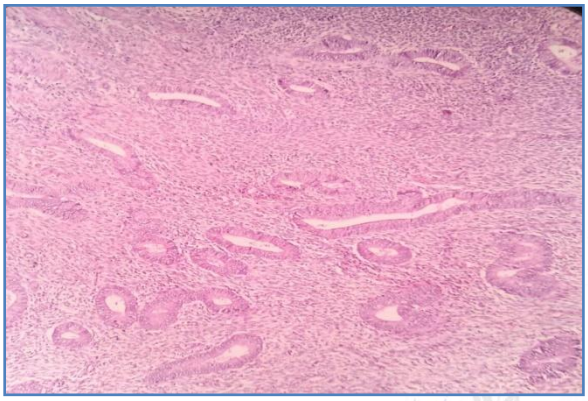


Fig 2: H&E 40X, Round to tubular glands lined by columnar epithelium in the compact stroma.

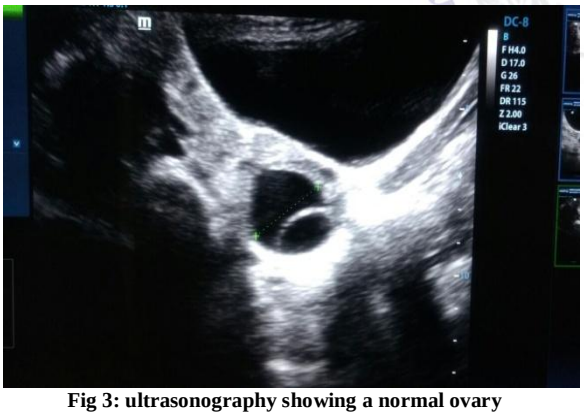


Fig 3: ultrasonography showing a normal ovary

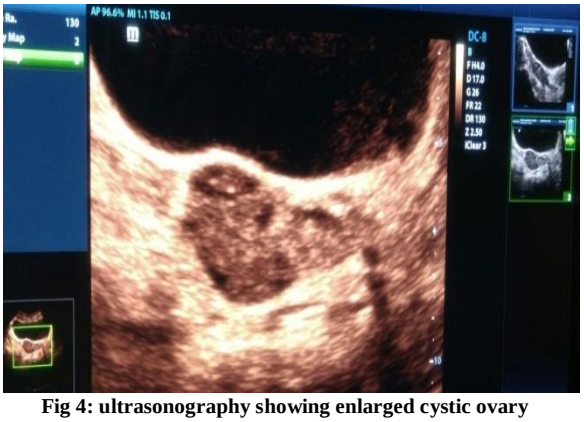


Fig 4: ultrasonography showing enlarged cystic ovary

Majority of the women were in the age group 25- 40 years. About 78% were in the same group with only 6% as 2° infertility.42.3% of women presented with BMI in the range 26-30 predominantly 1° infertility.61.5% women were seen to have proliferative phase with 93.75% in 1° infertility.63.46% women had a raised LH levels more than 12 IU/ml, with 90% as 1°infertility. About 55.76 % had an endometrial thickness > 10 mm, amongst which 93.10% with 1° infertility.61.53% presented with anovulation suggestive of cystic changes with 93.75% women with 1° infertility. About 44.23% patients on Ultrasonography had an endometrial thickness less than 10mm, which on biopsy were found to be in proliferative phase. On correlating, LH with endometrial Ultrasonography findings, we found a positive correlation of 0.634, with a p value of 0.000 which is extremely significant. Also on correlating, BMI with endometrial ultrasonography findings, we found a positive correlation 0.506, with a p value of 0.000, that is extremely significant. On correlating, LH with BMI we found a positive correlation with a Pearson's coefficient of 0.542, with a p value of 0.000 which is extremely significant.

DISCUSSION

In our prospective study of healthy women with no reported menstrual problems the prevalence of obesity in our study was 61.75 %. The frequency of obesity in women with anovulation and polycystic ovaries have been reported to be from 35%- 60%. Obesity is associated with three alterations that interfere with normal ovulation, and weight loss improves all three:

1) increased peripheral aromatization of androgens into estrogens.
2) Decreased levels of sex hormone binding globulin, resulting in increased levels of free estradiol and testosterone.
3) Increased insulin levels that can stimulate ovarian stromal tissue production of androgens. [13]

The maximum infertility rates were in the age group 25- 40 years with 78% of the cases. Data from studies in the Hutterites and other natural populations suggest that fertility in women peaks between the ages 20 and 24, decreases relatively until approximately age 30 to 32, then declines progressively, more rapidly after age 40. [13]

In our study 63.46% women presented with raised LH levels more than 12IU/ml with anovulatory findings on ultrasonography. Ecchord et al reported that ultrasonography and luteinizing hormone-expected date of ovulation might be considered as equivalent in average. [10] Patients with persistent anovulation have higher mean concentrations of LH. The elevated levels are partly due to an increased sensitivity of the pituitary to releasing hormone stimulation, manifested by an increase in LH pulse and amplitude but mainly amplitude. [13] Severe forms of polycystic ovarian disease is associated with higher luteinizing hormone concentrations. [5] In our study we found about 61.75% females presenting with ovarian cystic changes on ultrasonography. Thus the presentation of raised serum luteinizing hormone levels in patients with polycystic ovarian disease is similar to the findings by Hendricks et al. [5] Earlier studies have reported that follicle number and ovarian volume are positively correlated with to luteinizing hormone and an increased infertility rate were found with increasing luteinizing hormone concentrations in polycystic ovarian disease patients. [5]

Biopsies performed on luteal phase days 6 and 10 according to the luteinizing hormone peak have provided the best correlation of histological dating. [11] We found a positive correlation between LH and endometrial biopsy with Pearsons coefficient 0.634. Earlier Cheung et al had suggested a cut off of endometrial thickness less than 7mm in polycystic ovarian disease with 100% sensitivity for proliferative endometrium. [11] Later McCormick et al reported a higher cutoff of 9.35mm in patients with polycystic ovarian disease with comparable sensitivity (100%), but superior specificity. [14] Accordingly we set a cut off of 10 mm as endometrial thickness in patients. We found 44.24% women had endometrial thickness less than 10 mm. The remaining 55.76% patients who had endometrial thickness more than 10 mm also underwent biopsies. Cheung et al had reported that the incidence of endometrial hyperplasia on endometrial biopsy rises by 34.6% when the endometrial thickness is in the range 9-10 mm. [11] In our prospective study we found 44.5% in proliferative phase (fig1), 49.2 % in secretory phase and 6.3% cases as hyperplastic (fig 2). The results, with respect to the usefulness of endometrial biopsy as a screening tool for all couples presenting with infertility are conclusive. [12] The prevalence of "out of phase" biopsy findings in our study was 63.46% with hyperplasia contributing to 6.3% of the cases. The women with hyperplasia and proliferative endometrium had cystic findings on ovarian ultrasonography (fig 4), thus contributing to 61.5% polycystic ovarian disease cases in the study. Despite the presence of other methods to detect endometrial maturity, their cost/benefit ratios become prohibitive. Histological analysis remains the most useful tool for evaluating endometrial maturation and the morphometric process. [11]

The hyperplastic women in our study had a higher BMI (>30) presenting with polycystic ovarian syndrome. The findings are similar to the one reported by McCormick et al. [14]

LH and BMI were positively correlated with Pearsons coefficient 0.542 in our study. Increased levels of serum luteinizing hormone were seen in 63.4% patients. Around 42.3 %patients were under overweight (BMI in the range 26-30) and 20.4 % were seen in obese category (BMI >30). Studies have reported that abnormalities of hypothalamic gonadotrophin releasing hormone and pituitary gonadotrophin secretion are relatively common in overweight and obese. [13]

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