

Role of spontaneous cycle follicular monitoring in unexplained infertility: an observational study

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ABSTRACT

Objective: The incidence of unexplained infertility is high and thus there is a need to assess usefulness of unexplored modalities i.e. ultrasonographic follicular monitoring and endometrial changes and its correlation with hormonal levels in spontaneous menstrual cycle. Our study aimed to detect subtle defects responsible for the same. **Methodology:** It was an observational study conducted in the infertility clinic of Lady Hardinge Medical College, New Delhi, India. Fifty women with unexplained infertility were included in the study. The subjects were called on day 2-3 of onset of menses for a baseline transvaginal ultrasound for antral follicular count, endometrial thickness and hormonal profile. Follow-up ultrasonographic monitoring was done till ovulation was documented. Hormonal profile was done on day of dominant follicle of 18-20 mm and on day 21, serum progesterone levels were done. **Results:** The mean age of study group was 26.48 ± 3.3 years. Primary infertility was seen in 66% cases and secondary infertility in 34%. Seventy eight percent women had at least one or a combination of subtle defects in their monitored cycles. The mean antral follicle count was 13.8 ± 5.17 . Only one woman had antral follicle count of less than 5. 30% of women had levels of FSH more than 10 IU/L and levels of serum estradiol more than 80 pg/ml. Luteal phase defect and premature LH surge was seen in 34 % women each. **Conclusion:** Spontaneous cycle follicular monitoring is a useful tool to provide insight into the subtle defects like premature depletion of ovarian reserve, defective folliculogenesis, poor oocyte quality, premature luteinization, luteinized unruptured follicular syndrome contributing to unexplained infertility. Transvaginal sonographic assessment of a spontaneous cycle thus, appeared to be a useful addition in the investigative work-up of women with unexplained infertility.

Keywords: Unexplained infertility, transvaginal ultrasound, follicular monitoring, endometrial changes, hormonal assessment.

Unexplained infertility is defined when standard infertility evaluation, namely husband's semen analysis, tests of ovulation and tubal patency fail to detect any gross abnormality.¹ The incidence of unexplained infertility varies in different studies and ranges between 10-20%.²

Various studies have suggested the role of various diagnostic modalities in the evaluation of infertile couples with unexplained infertility. This study is an attempt to assess the usefulness of unexplored modalities like sonographic monitoring of follicular growth and endometrial changes and its correlation with hormonal levels in spontaneous menstrual cycle in women with unexplained

infertility to detect subtle defects responsible for unexplained infertility.

Methods

This was an observational study conducted in the infertility clinic of Lady Hardinge Medical College and Smt. Sucheta Kriplani Hospital, New Delhi from November 2016 to March 2018. Ethical clearance was taken from institutional ethical committee. Fifty women with unexplained infertility, less than 35 years of age were recruited in the study after written and informed consent. Detailed history and clinical examination were followed by routine investigations for infertility including CBC with

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peripheral smear and ESR, Mantoux, chest Xray, husband semen analysis, premenstrual endometrial biopsy and post menstrual hysterosalpingography.

The subjects were called on day 2-3 of onset of menses for a baseline transvaginal ultrasound for antral follicular count, endometrial thickness and hormonal profile. Serial ultrasounds were then done from day 8-10 of menstrual cycle, on alternate days till ovulation was documented. Hormonal assessment included serum FSH, LH, estradiol and progesterone levels on day 2-3 of menstrual cycle; serum LH, estradiol and progesterone levels at a follicular size of 18-20 mm and serum progesterone levels on day 21.

The outcome variables were proportion of subjects with poor ovarian reserve (antral follicle count (AFC) <5, day 2 FSH > 10 IU/l), poor quality oocyte (serum estradiol levels <180 pg/ml at a follicle size of 18-20 mm), premature LH surge (serum progesterone >1.5 ng/ml, at a follicle size of 18-20 mm), size of follicle at the time of ovulation, unruptured luteinized follicle and luteal phase defect (day 21 progesterone levels < 10 ng/ml and or duration of luteal phase < 10 days). The results were compiled and analysed using latest version of SPSS software and results were expressed as mean ± SD.

Results

The demographic profile of the subjects recruited in the study is depicted in table 1. The mean age of the subjects was 26.48 ± 3.3 years. The mean duration of infertility was 4.39 ± 2.86 years. Seventy eight percent women had at least one or a combination of subtle defects in their monitored cycles as can be highlighted subsequently.

Day 2/3 mean serum FSH was 8.25±3.36 IU/L (4.02 to 21.58), LH 6.97±4.07 IU/L (3.1 to 26.29), estradiol 72.74±29.64 pg/ml (30.3 to 181.5) and progesterone levels were 0.69±0.39 ng/ml (0.2 to 2.46) as shown in table 2. The mean antral follicular count was 13.8 ± 5.17. AFC less than 5 was seen in 2% subjects, 5-10, 10-15 and more than 15 in 16%, 42% and 40 % subjects respectively.

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The mean serum progesterone, estradiol and LH levels on the day the size of dominant follicle reached 18-20mm was 1.93±1.47ng/ml (range 0.28 to 6.40 ng/ml), 264.25 ± 109.6

pg/ml (range 65- 598pg/ml) and 32.35 ± 21.1 IU/L (range 8-96 IU/L) respectively.

Table 1: Demographic profile

Parameters	N=50	Percentage (%)
Age (years)	<30 = 45	90
	>30 = 5	10
Body Mass Index (kg/m ²)	< 23 = 33	66
	23-24.9 = 12	24
	>25 = 5	10
Type of infertility	Primary = 33	66
	Secondary = 17	34
Duration of infertility (years)	< 5 = 34	68
	>5 = 16	32

On serial ultrasound examination, it was observed that in one woman, follicle failed to grow beyond 11 mm and was excluded from the study. The mean diameter of maximum size of the follicle achieved was 19.53±2.07mm. In 24 % subjects, dominant follicle ruptured on day 14 of cycle, 46 % on day 16, 14 % on day 18, 2 % on day 20 and 2 % on day 22 of cycle. In six women, the follicle had poor growth or did not rupture.

In our study, the minimum and maximum length of luteal

Table 2: Hormone levels on day 2/3 of menstrual cycle (N=50)

Hormones	Day 2/3 (Mean ± Std. deviation)	Day of follicle size (18-20 mm)	Day 21
LH (IU/L)	6.97 ± 4.07	32.35± 21.10	NA
FSH(IU/L)	8.25± 3.36	NA	NA
Estradiol (pg/ml)	72.74± 29.64	264.25±109.62	NA
Progesterone (ng/ml)	0.69± 0.39	1.93±1.47	16.89±12.36

LH – Luteinising hormone, FSH – Follicle stimulating hormone

phase was 6 and 15 days. One had length of 6 days while four women had length of 15 days. Majority of the women i.e. 40.5% had luteal phase length of 13 days. Mean serum progesterone levels on day 21 were 16.89± 12.36ng/ml (range 0.28-51.15 ng/ml).

Discussion

Ultrasound imaging is an effective, easy to use, safe and reliable non-invasive means to evaluate fertility potential. Ovarian reserve tests are day 3 FSH, LH, estradiol and anti-mullerian hormone (AMH) levels and AFC. Serum progesterone levels on day 21 of menstrual cycles or a week prior to expect menses in an irregular cycle is an important measure of ovulation. Value of more than 3ng/ml is suggestive of ovulation.

Out of 50 subjects recruited in the study, 22 % had normal cycles as per follicular growth, endometrial growth and differentiation, ovulation and hormonal levels. Remaining, 78 % had at least one or a combination of subtle defects in their monitored cycles. On follow up, it was found that two women conceived spontaneously in the monitored cycle, one conceived spontaneously but aborted. One woman was subjected to ovulation induction and she responded to the same and conceived.

According to American Society of Reproductive Medicine (ASRM), 2012, low antral follicular count is defined as a range of 3-10 antral follicles in both the ovaries.³ This is considered to be the single best predictor of ovarian response and thus pregnancy outcome.⁴ In our study, subject with antral follicle count less than 5, had day 2 FSH more than 10 IU/L, day 2 estradiol levels more than 80pg/ml and less than 180 pg/ml at a follicle size of 18-20 mm. Dominant follicle developed on day 14 and ruptured on day 18. This woman had short luteal phase of 6 days. On follow up, she was referred for in-vitro fertilization.

In this study, day 2/3 serum FSH levels was seen to be negatively related to antral follicular count with correlation coefficient of -0.33 and p value of 0.18. R Fanchin et al in their study also observed, that serum FSH levels on day 3 of menstrual cycle correlated with antral follicular count ($r = -0.29$, $p < 0.001$).⁵ Serum FSH levels between 10-20 IU/L were taken as an indicator of poor ovarian response with sensitivity of 80% and specificity of 100% according to ASRM.⁶ In our study, 70% subjects had serum FSH levels of less than 10 IU/L and 30% had levels of more than 10 IU/L.

Serum estradiol levels at a follicle size of 18-20 mm reflect the quality of maturing oocytes, its capacitation for fertilization and post-fertilization development.⁸ In our study, 9 subjects had serum estradiol levels less than 180pg/ml at dominant follicle of 18-20mm. Among these 7 had one or more markers of premature depletion of ovarian reserve and poor folliculogenesis. One had AFC <5 and three others has AFC < 10, four had day 2 FSH levels > 10IU/L and seven had day 2 estradiol levels > 80 pg/ml.

Luteal phase defect is a condition secondary to insufficient progesterone production by the corpus luteum and failure to maintain normal secretory endometrium required for embryo implantation with day 21 serum progesterone levels less than 10 IU/L.⁹ In our study, 17 women had serum progesterone levels less than 10 pg/ml. Only, one woman had short luteal phase of less than 10 days. Among these 17 women diagnosed with luteal phase defect, four had poor follicle growth and unruptured follicle each, two had premature depletion of ovarian reserve, premature luteinization and delayed ovulation in each and no cause was elicited in 3 women. Various studies even demonstrate that there is a higher incidence of small follicles in luteal phase defect cycles.^{10,11} Luteal phase defect has also been associated with luteinized unruptured follicular syndrome as depicted by our study as well. In our study, it was seen in

23.5% subjects as compared to a study by Ying Y K et al, it was seen in 15% cases.¹²

Women with early conversion of endometrium as seen on ultrasound and serum progesterone levels of more than 1.5 ng/ml at dominant follicle of 18-20 mm found to have premature luteinization. Premature luteinization adversely affects clinical pregnancy rate probably due to embryo-endometrial asynchrony. In our study, 34 % subjects had premature luteinization as compared to 10% in a study done by Check JH et al.¹³

Conclusion

Spontaneous cycle follicular monitoring is a useful tool to provide insight into the subtle defects like premature depletion of ovarian reserve, defective folliculogenesis, poor oocyte quality, premature luteinization, luteinized unruptured follicular syndrome contributing to unexplained infertility. Transvaginal sonographic assessment of a spontaneous cycle thus, appeared to be a useful addition in the investigative work-up of women with unexplained infertility.

The limitations of our study were that anti-mullerian hormone levels was not included as a measure of ovarian reserve. Also, we did only one cycle monitoring. In cases of premature luteinization, serum progesterone levels at dominant follicle size of 18-20mm could be randomly increased and we should have repeated it in subsequent cycle. Ultrasound for follicular monitoring was done only till day 22, if rupture was not documented till then, further follow up scans were not done.

Conflict of interest: None. **Disclaimer:** Nil.

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