

Comparitive Study of Various Tests for Estimation of Ovarian Reserve in Infertile Women

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Abstract

Objective:

1. To assess the ovarian reserve in infertile women by clinical, biochemical and radiological evaluation.
2. Controlled Ovarian Stimulation in female infertility by clomiphene citrate challenge test.
3. To monitor growth of ovarian follicle by ultrasonography and assess the response of ovarian stimulation in term of follicular development.

Study Design: It is a prospective interventional study of 170 cases with complain of infertility attending OPD of obstetrics and gynaecology Mahatma Gandhi Medical College and Hospital. After the enrolment, demographic data i.e. age, education, occupation were obtained and recorded. Detailed history including details of infertility and complaints other than infertility, duration of active married life, menstrual history, obstetric history, occupational and past history were documented and systemic examination followed by per speculum and per vaginal examination was performed. Clinical parameters including age, body mass index, menstrual cycle length was recorded. Local examination was done for breast and hirsutism. Grading of hirsutism was done according to Ferriman and Gallwey scoring system. Hysterosalpingography (HSG) or Video hysteroscopy (VHL)

was done to rule out tubal factor infertility. Blood Investigations including blood group, CBC, blood sugar, HIV, HBs antigen, VDRL were done. Husband semen analysis was done to rule out male factor infertility. Specific investigations like TSH, prolactin, day 3 FSH, LH, Estradiol, AFC were recorded. Patients who fulfilled the inclusion criteria were included in the study and all the details were recorded on preformed Proforma for all subjects. After preliminary interrogation and recording of details the subjects were called on second day of menstrual cycle, reviewed, baseline TVS done for AFC and tablet clomiphene citrate 100mg daily given from day 5 to day 9. Patients were instructed to take tablets daily and to inform immediately if any side effects like flushing, nausea, vomiting, visual disturbance, headache, diarrhoea, abdominal discomfort or pelvic mass or tenderness are seen. For the purpose of clomiphene citrate challenge test, Day 10 FSH level recorded and serial ultrasonography was done from day 7 to day 16 for monitoring of endometrial thickness, number and size of dominant follicle. All transvaginal sonography was done on the same machine. At each transvaginal ultrasonography (TVS) internal diameter of each visible follicle were measured in two dimensions. The endometrial thickness defined as the maximum distance between the echogenic interphase of myometrium which was measured in the plane through the central longitudinal axis of uterus. When at least one dominant follicle had a diameter of 18 mm. injection HCG 10,000 IU was given intra muscularly as ovulation triggering agent and women were instructed to have planned relation. TVS was repeated after 48 hours to look for evidence of ovulation i.e. disappearance of dominant follicle which was seen previously and presence of free fluid in cul-de-sac. If the follicle was

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unruptured then ultrasound was repeated after 48 hours and 72 hours of the HCG injection to observe luteinized unruptured follicle (LUF). Women were instructed to follow up on the second day of next cycle or immediately after missed period. If the patient is not pregnant, tablet clomiphene citrate 100mg was given in next cycle and similar protocol was followed. Before starting the ovulation induction in the next cycle all subjects were taken for transvaginal ultrasonography on day 2 of periods to rule out the presence of any ovarian cyst. All subjects were followed up for total three cycles. Women who missed their period urinary pregnancy test was done and pregnancy were recorded. All the recorded data of all subjects for 3 cycles were recorded in MS XL sheet. Outcome was measured in the form of utility of clomiphene citrate challenge test as a marker of ovarian reserve.

Result: The ovarian reserve testing helps us to give the estimation of pool of primordial follicles and it is a useful in detecting ovulation induction response. Any of the tests for ovarian reserve does not have sensitivity and specificity of 100% for the detection of poor ovarian reserve. So, clinical, biochemical and radiological parameters are combined in an effort to increase the prognostic reliability of each test. A scoring system is used by combination of age, BMI, AFC, FSH, estradiol, etc for the prediction of ovarian response more accurately than each of the parameters alone.

In our study, CCCT is found as the most reliable test followed by basal AFC and basal FSH.

Keywords: Ovarian Reserve; Ovulation; CCCT .

Introduction

Infertility is classically defined as one year of regular unprotected intercourse without conception [1] according to Speroff.

The American Society for Reproductive Medicine [2008], recently revised its infertility definition [2] – “Infertility is a disease, and it is defined by failure to achieve successful pregnancy after regular unprotected intercourse of 12 months or more. Earlier evaluation and treatment may be justified based on medical history and physical findings and is warranted after 6 months for women over 35 years”. Subfertility refers to couples who conceived after 12 months of attempted impregnation [3].

Fecundability is defined as probability of pregnancy per cycle, it is about 20% in fertile couples [3]. Fecundity is the probability that a cycle will result in live birth [1]. Approximately 90% of healthy

young couples conceive within 12 months of regular unprotected intercourse [3]. Infertility affects about 10-15% of couples [1].

World Health Organization (WHO) estimates that currently 60 to 80 million couples suffer from infertility worldwide [4]. Infertility varies from the region to region across the world and it is estimated that infertility affect 8 to 12 percent of couples worldwide [5,6].

Overall, major causes of Infertility include [1] –

1. Ovulatory dysfunction (20-40%)
2. Tubal and peritoneal pathology (30-40%)
3. Male factor (30-40%)
4. Uterine pathology is uncommon, and rest is largely unexplained.

In couples, ovulatory dysfunction accounts for about 15% of overall factors and 40% of total female factor infertility. Usually ovulation can be affected by multiple factors like excessive weight gain or weight loss, excessive exercise, extreme emotional stress and lifestyle changes. In anovulatory women, menstrual cycles are infrequent and exhibits no consistent pattern whereas in normal ovulating women menstrual cycles are generally regular, predictable, consistent in volume and duration and typically accompanied by premenstrual and menstrual symptoms.

To treat infertility, in today's era there are various new and advanced technologies like Assisted Reproductive Techniques (ART) and In Vitro Fertilization (IVF) are used. But the success rate is still low and cost is high. So, the need to predict the ovarian follicular inducibility is always felt in an effort to optimise the response and outcome in infertile couples prior to infertility treatment or IVF and counsel them regarding the success rate.

Ovarian Reserve Testing (ORT) denotes the size of resting primordial follicle population in the ovaries. In turn ORT determines the number of growing follicles, quality and reproductive potential for oocytes [7].

The various tests which are used to predict the ovarian reserve prior to controlled ovarian stimulation for the success rate of Assisted Reproductive Techniques are – serum Follicle Stimulating Hormone, Luteinizing Hormone, Estradiol, Antral Follicle Count and Clomiphene Citrate Challenge Test.

Objective

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Material & Methods

Study Design

Prospective interventional study

Period of Study

Nov 2015 – Oct 2017

Study group – 170 cases with complain of infertility attending OPD of obstetrics and gynaecology Mahatma Gandhi Medical College and Hospital during a period of 2015-2017. Institutional ethics committee and scientific committee approval was obtained. The patients were informed in detail about the nature of the study and a written informed consent was obtained.

Inclusion Criteria

All women seeking treatment for infertility in reproductive age group 18-40 years.

Exclusion Criteria

1. Male factor infertility
2. Infertility due to tubal factor
3. Ovarian cyst
4. Irregular or undiagnosed vaginal bleeding

After the enrolment, demographic data i.e. age, education, occupation were obtained and recorded. Detailed history including details of infertility and complaints other than infertility, duration of active married life, menstrual history, obstetric history, occupational and past history were documented and systemic examination followed by per speculum and per vaginal examination was performed. Clinical parameters including age, body mass index, menstrual cycle length was recorded. Local examination was done for breast and hirsutism. Grading of hirsutism was done according to Ferriman and Gallwey scoring system. Hysterosalpingography (HSG) or Video hysteroscopy (VHL) was done to rule out tubal factor infertility. Blood Investigations including blood group, CBC, blood sugar, HIV, HBs antigen, VDRL were done. Husband semen analysis

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Patients who fulfilled the inclusion criteria were included in the study and all the details were recorded on preformed Proforma for all subjects. After preliminary interrogation and recording of details the subjects were called on second day of menstrual cycle, reviewed, baseline TVS done for AFC and tablet clomiphene citrate 100mg daily given from day 5 to day 9. Patients were instructed to take tablets daily and to inform immediately if any side effects like flushing, nausea, vomiting, visual disturbance, headache, diarrhoea, abdominal discomfort or pelvic mass or tenderness are seen. For the purpose of clomiphene citrate challenge test, Day 10 FSH level recorded and serial ultrasonography was done from day 7 to day 16 for monitoring of endometrial thickness, number and size of dominant follicle. All transvaginal sonography was done on the same machine.

At each transvaginal ultrasonography (TVS) internal diameter of each visible follicle were measured in two dimensions.

The endometrial thickness was noted. When at least one dominant follicle had a diameter of 18 mm. injection HCG 10,000 IU was given intra muscularly as ovulation triggering agent and women were instructed to have planned relation. TVS was repeated after 48 hours to look for evidence of ovulation i.e. disappearance of dominant follicle which was seen previously and presence of free fluid in cul-de-sac.

If the follicle was unruptured then ultrasound was repeated after 48 hours and 72 hours of the HCG injection to observe luteinized unruptured follicle (LUF).

Women were instructed to follow up on the second day of next cycle or immediately after missed period. If the patient is not pregnant, tablet clomiphene citrate 100mg was given in next cycle and similar protocol was followed. Before starting the ovulation induction in the next cycle all subjects were taken for transvaginal ultrasonography on day 2 of periods to rule out the presence of any ovarian cyst. All subjects were followed up for total three cycles. Women who missed their period urinary pregnancy test was done and pregnancy were recorded. All the recorded data of all subjects for 3 cycles were recorded in MS XL sheet.

Outcome was measured in the form of utility of clomiphene citrate challenge test as a marker of ovarian reserve.

Observation

Subsequent table 3 showing 72.94% of patient underwent 3rd cycles of ovulation induction of clomiphene citrate where as 14.12% of patients conceived in 2nd cycles of clomiphene citrate ovulation induction 12.94% of patient conceived in 1st cycle of ovulation induction by clomiphene citrate.

Table 1: Distribution of the cases according to age

Age Group	Number	Percentage
21 to 25	48	28.24
26 to 30	79	46.47
31 to 35	38	22.35
>36	5	2.94
Total	170	100.00

Table 2: Distribution of the cases according to BMI

	Number	Percentage
Low	7	4.12
High	45	26.47
Normal	118	69.41
Total	170	100.00

Table 3: Distribution of the cases according to No. of treatment cycle (N=)

No. of treatment cycle (N=)	Number	Percentage
1	22	12.94
2	24	14.12
3	124	72.94

Table 4: Distribution of the cases according to Prolactin

N= no of cases =170		
Prolactin	Number	Percentage
Normal	138	81.18
Abnormal	32	18.82
Total	170	100.00

Table 5: Association of duration of Menstrual Cycle with ovulation

Days	No ovulation		Ovulation		Total
	Number	%	Number	%	
1 to 2	9	5.39	2	66.67	11
2-3	13	7.78	0	0.00	13
3-4	60	35.93	1	33.33	61
5 to7	85	50.90	0	0.00	85
Total	167	100.00	3	100.00	170

Chi-square = 18.868 with 3 degrees of freedom; p <0.0015

Table 6: Distribution of the cases according to D3 FSH Levels

FSH D3	N= no of cases =170		N=No of cycle =391	
	Number	Percentage	Number	Percentage
Abnormal	9	5.29	37	9.46
Normal	161	94.71	354	90.54
Total	170	100.00	391	100.00

Table 7: Distribution of the cases according to Antral Follicle Count

Antral Follicle Count	No. of patients	Percentage	No. of cycles	Percentage
Abnormal	30	17.65	51	13.04
Normal	140	82.35	340	86.95
Total	170	100.00	391	100.00

Table 8: Distribution of the cases according to FSH D10

FSH D10	N= no of cases =170		N=No of cycle =391	
	Number	Percentage	Number	Percentage
Abnormal	28	16.47	62	15.85
Normal	142	83.53	329	84.14
Total	170	100.00	391	100.00

Table 9: Distribution of the cases according to LH

LH	N= no of cases =170		N=No of cycle =391	
	Number	Percentage	Number	Percentage
High	3	1.76	20	5.11
Low	5	2.94	11	2.81
Normal	162	95.29	360	92.07
Total	170	100.00	391	100.00

Table 10: Distribution of the cases according to Estradiol D3

Estradiol D3	N= no of cases =170		N=No of cycle =391	
	Number	Percentage	Number	Percentage
Abnormal	14	8.24	43	11.00
Normal	156	91.76	348	89.00
Total	170	100.00	391	100.00

Table 11: Distribution of the cases according to PCOS

	Number	Percentage
PCOS	16	9.41

In our study, out of 170 patients, 16 (9.41%) were diagnosed as cases of PCOS (according to ESHRE/ASRM)

Table 12: Distribution of the cases according to hypothyroidism

	Number	Percentage
Hypothyroid	20	11.76
Normal	150	88.24
Total	170	100.00

In our study 20 patients (11.76%) of infertile women were diagnosed as hypothyroidism

Table 13: Distribution of the cases according to Number of dominant follicles (≥ 18)

No. of dominant follicles (≥ 18)	Number	Percentage
0	52	13.29
1	208	53.19
2	120	30.69
3	11	02.81
Total	391	100.00

Subsequent table 13 showing distribution of patients according to the number of dominant follicle in 391 cycles of ovulation induction. 53.19% of cycles shows one number of dominant follicle whereas 30.69% of cycles shows two number of dominant follicles. 2.81% shows three dominant follicles and 13.29% doesn't form significant dominant follicle.

Subsequent table 14 showing distribution of patients according to the number of dominant follicle in each cycle of ovulation induction. Maximum patients had 1 to 2 dominant follicles in each treatment cycle. But no significant difference was observed in no of dominant follicles in relation with each cycle of ovulation induction. Although it was increase in second cycle. $p=0.91NS$.

Table 14: Number of Dominant follicle $\geq 18mm$ in 3 subsequent cycles

	1		2		3		Total
	No	%	No	%	No	%	
0	36	21.18	12	11.88	4	3.33	52
1	92	54.12	55	54.46	61	50.83	208
2	40	23.53	31	30.69	49	40.83	120
3	2	1.18	3	2.97	6	5.00	11
Total	170	100.00	101	100.00	120	100.00	391
Mean (No. of dominant follicles) with S.D.	1.218 \pm 0.765		1.257 \pm 0.6878		1.225 \pm 0.6412		1.23 \pm 0.707

Table 15: ET on day of ovulation trigger with Number of sequencing of cycle

ET on the day of ovulation trigger (mm)			
No. of treatment cycle actual	N	Mean	Std. Deviation
1	170	7.55	1.462
2	101	7.48	1.389
3	120	7.54	1.248
Total	391	7.53	1.377

Maximum cycles had endometrial thickness < 8 mm.
Mean endometrial thickness \pm SD = 7.5 ± 1.3 mm in 391 cycles (range 3.9 to 12mm).

Table 16: Ovulation rate per cycle

Ovulation	No. of Cycles	Percentage
Yes	287	73.40
No	104	26.60
Total	391	100.00

Table 17: Pregnancy rate per cycle

Pregnancy	No. of Cycles	Percentage
Yes	162	41.43
No	229	58.57
Total	391	100.00

Discussion

Ovarian reserve testing may prove an important marker in assessing the ovarian reserve.

Clinical parameters including age, BMI and menstrual cycle length. Hormonal assays consist of FSH D3 and D10, estradiol, LH, prolactin, thyroid levels. USG was used to measure AFC.

In present practice Clomiphene citrate challenge test is most commonly used method and it is cheapest method in all dynamic test. Clomiphene citrate is considered as a first line of treatment because of its low cost, relative ease of use and the most commonly prescribed drug for ovulation induction in oligo-ovulatory and anovulatory women with minimal side effects. In our study, maximum patients were in 26-30 years of age group. 4.12% were having low BMI where as 26.47% of infertile women belongs to high BMI group. 18.82% of infertile women were having hyperprolactinemia. In our study 170 patients were included, in which 161 patients (94.71%) showing normal basal day 3 FSH and 9 patients (5.29%) showing abnormal value. Total 170 subjects underwent 391 cycles of dynamic test (CCCT) and 354 cycles (90.54%) were having normal basal day 3 FSH level and 37 cycles (9.46%) were having abnormal value. Mean value \pm SD = 6.59 ± 2.80 .

In our study, 140 patients (82.35%) were having normal day 3 AFC and 30 patients (17.65%) were having abnormal day 3 AFC value. After the application of dynamic test total 391 cycles, in 340 cycles (86.95%) were having normal AFC value and 51 cycles (13.04%) were having abnormal AFC value. Mean value \pm SD = 5.76 ± 2.42 Out of 170 patients, 83.53% of infertile women had normal post ovulation FSH level and 16.47% women had abnormal post ovulation FSH levels. In total 391 cycles of ovulation induction by clomiphene, 84.14% were having normal post exposure FSH level whereas 15.85% where having abnormal post exposure FSH level. Mean value \pm SD = 5.76 ± 2.42 .

Out of 170 patients total 8 patients (4.70%) showing abnormal LH value and in 391 cycles of post exposure by clomiphene citrate abnormal value of LH seen in 31 patients (7.92%).

Out of 170 patients D3 Estradiol shows normal value in 156 patients (91.76%) and abnormal value in 14 patients (8.24%). Whereas out of 391 cycles abnormal value of estradiol are in 11% and normal value are seen in 89%.

In our study it has been observed that out of 391 cycles 73.40% (287 cycles) achieved ovulation. In this study, pregnancy rate achieved per cycle is 41.43% (162 cycles).

Conclusion

The ovarian reserve testing helps us to give the estimation of pool of primordial follicles and it is useful in detecting ovulation induction response. But there is insufficient evidence present which recommend that any of the ovarian reserve tests now available should be used as a sole criterion for the purpose of ART.

So, clinical, biochemical and radiological parameters are combined in an effort to increase the prognostic reliability of each tests. A scoring system is used by combination of age, BMI, AFC, FSH, estradiol, etc for the prediction of ovarian response more accurately than each of the parameters alone.

In our study, CCCT is found as the most reliable test followed by basal AFC and basal FSH.

Referance

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