Observational study of changes in dominant ovarian follicle with and without administration of clomiphene citrate in primary infertility cases among eastern Indian women

Susmita Ghosh[1], Kausiki Ray Sarkar[2], Oyndrila Sengupta[3], Subhajit Halder[4]

[1,4]Assistant Professor, Department of Anatomy, Calcutta National Medical College, Kolkata, [2]In-charge, Fertility Unit, AMRI Medical Centre, Kolkata, [3]Assistant Professor, Department of Anatomy, Murshidabad Medical College, Murshidabad, West Bengal, India

A B S T R A C T

Background: Changing environment and social factors with advancement of science bestowed the human population with both curse and boon. Increasing number of primary infertility is one of the many challenges which is encountered among 3.59% of Indian women population in reproductive age group. Use of clomiphene citrate (CC) is one of the commonly practiced way to find out a ray of hope. **Aims and Objectives**: The aim of the study was to measure the changes of morphometric parameters of dominant ovarian follicles and their numbers and compare the same before and after administration of CC was the aim of the present study. **Materials and Methods**: This interventional and cohort study performed on 50 women coming to the infertility clinic at Institute of Post Graduate Medical Education and Research, Kolkata, with primary infertility of reproductive age group. Informed consent was taken from all. Transvaginal ultrasonography was utilized to measure the parameters related with dominant follicles. Comparison among them with respect to number, size, and time to rupture both before and after ovulation induction with CC was made. **Results:** Statistically significant increase in number and average size of dominant follicle is seen from 1.08 to 2 and 12.26 to 15.31 mm following CC in the third cycle. In polycystic ovarian disease, cases also ovulation has increased significantly. **Conclusion**: Transvaginal ultrasonography proves to be regarded as a gold standard procedure to measure and monitor the ovarian follicle status. Undoubtedly CC can be regarded as an easy and dependable solution toward treatment of primary infertility.

Key words: Clomiphene citrate; Dominant follicle; Dominant follicle rupture; Ovulation induction; Primary infertility

INTRODUCTION

Advancing female age is one of the "greatest enemies" of fertility. This contrasts the most males who can father children for their entire lives, if one graphs female age against declining fertility, there is a direct relationship which cultivates in the menopause.[1,2] The human ovaries become unresponsive to gonadotropins[3] with advancing age and their function declines, so that sexual cycles disappear (menopause). This unresponsiveness is associated with and probably caused by a decline in the number of primordial follicles, which becomes precipitous at the time of menopause. Story of ovulation starts long back when oogonia multiply by dividing mitotically; this proliferation ends when the oogonia enter meiosis. The amount of time that oogonia multiply by mitosis is species specific. In the

Address for Correspondence:
Dr. Subhajit Halder, Assistant Professor, Department of Anatomy, Calcutta National Medical College, Kolkata, West Bengal, India.
Mobile: +91-6296414419. **E-mail:** baptu.subhajit@gmail.com

human fetus, cells undergoing mitosis are seen until the second and third trimester of pregnancy. After beginning the meiotic process, the oogonia (now called primary oocytes) can no longer replicate. Therefore, the total number of gametes is established at this time. In humans, therefore, oocytes are established in the ovary before birth and may by dormant awaiting initiation for up to 50 years.[4]

Clomiphene citrate (CC) is a synthetic and non-steroidal estrogen antagonist drug which has been in use since the 1970's. It binds to both estrogen receptor α and β. It induces gonadotropin secretion in women by blocking estrogenic feedback inhibition of pituitary.[5] In the present study, clomiphene alone was used with similar results. Even in polycystic ovarian disease (PCOD) cases, clomiphene has proved to be an effective first line therapy without major side effects. Similar findings have been reported by Nasrullah et al.[6] The patients likely to benefit from CC are patients diagnosed with polycystic ovarian syndrome.[7]

Aims and objectives

Infertility is a relatively common problem, it is estimated that at any time, approximately 10% of those who wish to have children can be considered infertile and about 30–40% of them present with ovarian dysfunction.

Some of the causes of infertility lie with the male partner, some with the female and some are undetermined. Among the female causes of infertility, the problem of anovulation is important. Initially, the patient is examined for the basic tests of ovulation as mentioned in the introduction. However, ovulation can only be reliably monitored by ultrasonography. In the present study, the dominant ovarian follicles in each ovary in a particular cycle with and without the use of CC were monitored in patients with primary infertility, by transvaginal sonography.
a. The present study was conducted to find out ovarian height in women having primary infertility with transvaginal ultrasonography
b. The presence and number of dominant follicles will be determined before and after the use of CC
c. The study also aims at exploring the efficacy of ovulation induction by CC alone by the difference of day of rupture of dominant follicle.

MATERIALS AND METHODS

In this observational study, cases were selected from the month of March 2008 to August 2009, from the Infertility Clinic of Department of Gynaecology and Obstetrics of SSKM and IPGMER, Kolkata. Bilateral ovaries of 50 subjects were seen.

Inclusion criteria
The following criteria were included in the study:
1. Female patients
2. Age group of 19–36 years
3. Primary infertility
4. Defect in ovulation
5. Patients with PCOD.

Exclusion criteria
The following criteria were excluded from the study:
1. Patients with comorbidities
2. Patients with secondary infertility
3. Debilitated or moribund patients
4. Patients under Contraceptives
5. Primary infertility due to other causes
6. Menopause
7. Unmarried subject
8. Post-obstetric surgery subjects
9. Patients with positive history of Tuberculosis
10. Patients with positive history of exposure to tuberculosis.

The main outcome measures were changes in the parameters of dominant follicle status before and following clomiphene therapy.

Tab CC is provided to the patients free of cost from the government supply.

Methodology
The patients were examined thoroughly in the infertility clinic of the same hospital. General survey was done with special reference to body weight, excess body hair, hirsuitism, any pelvic mass, etc. Before Application of Clomiphene: The study started from Day 9 of menstrual cycle with proper history taking and folliculometry by transvaginal ultrasonography.

Application of Clomiphene: 50 mg orally daily from Day 3 to Day 7 for each cycle.

After Application of Clomiphene: Similar alternate day transvaginal sonography was done to observe changes, if any, in the same parameters, starting from Day 9 of the same cycle in which clomiphene was given. The changes in the third cycle are noted and documented for analysis on day 16.

Parameters seen
1. Ovarian height (Right and Left)
2. Number of dominant follicle (Right and Left)
3. Size of dominant follicle (Right and Left)
4. Number of ruptured follicle and day of rupture (Right and Left).

Operational definitions

a. Dominant follicles are determined by size. They are supposed to be the largest of the growing follicles. They are clear, anechoic, and thin-walled cystic structures with a regular margin. The mass of cells within it is not visible and size is the only criterion. As this is a three-dimensional structure, ideally three measurements of length should be taken at right angles to each other. Practically however, only two dimensions are measured at right angles to each other, as they are easier to follow up. Dominant follicles have to be distinguished from ovarian cysts (follicular or luteal cysts) and parovarian cysts arising from para ovarian tissue, remaining adherent to the ovary. By convention, follicles >25 mm in maximum dimension are called cysts. Cysts may be simple or complex in appearance. Simple cysts are usually follicular or luteal. They are anechoic, clear, and have a regular margin. Their walls are not visible. Complex cysts are thick walled, heterogeneous, and irregular with septation.[8]

b. Transvaginal ultrasonography
   Toshiba Xario Model ultrasonography machine was used. It is performed by a registered medical sonologist. Preparation of the patient: Patients need to empty the urinary bladder before transvaginal sonography. During the sonogram, the patient lies on the examination table in supine position with knees flexed. Gel is applied on the vaginal probe and then covered by a condom. The gel helps elimination of the formation of air pockets between the transducer and the organ examined. The sonographer introduces a probe into the patient's vagina and presses the transducer against the part examined, moving from one area to another as necessary to view the uterus and ovaries. Sonography usually is a painless procedure. However, one may experience some discomfort as the sonographer guides the transducer. Transvaginal imaging utilizes a higher frequency imaging achieving better resolution of the ovaries.[9-11]

Ethical clearance

It is obtained from the Institutional Ethical Committee of IPGME and R, registered under memo no. Inst./IEC/320, 31/03/2008.

Statistical analysis

Analysis of the data was done using Statistica version 6 (Tulsa, Oklahoma; STATSOFT. Inc; 2001).

RESULTS

From Table 1, average height of ovaries before Clomiphene found to be 2.04 cm and that following Clomiphene was 2.05 cm. P=0.061 showed that there was no significant change with Clomiphene therapy.

In Table 2, the number of dominant follicles was counted by transvaginal ultrasonography. Usually, one or two follicles dominate in a particular cycle. Under the influence of Clomiphene, up to three follicles may mature. Size and progressive growth are the criteria for dominance. If several follicles grow at a rapid rate, they are followed to see which one of them ruptures first. In some cases, no dominant follicles were seen even after Clomiphene therapy. It was seen that the number of dominant follicles before Clomiphene on an average was 1.08, and the same after Clomiphene were 2.00, the range being 0–3.

Table 3 was showing the size of dominant follicle. Normally, the optimum size of Graafian follicles before rupture is 20 mm. All the follicles in the present study ruptured between 12 and 21 days. Therefore, the size of the dominant follicle was measured on the 11th day for comparison. However, the size of the follicle continued to increase till rupture. The average Size before Clomiphene was found as 12.26 mm whereas that after Clomiphene = 15.31 mm.

Table 4 showed the number of follicles ruptured before and after Clomiphene therapy in the third cycle and the day of rupture. For best results, three consecutive cycles should be examined. The results were before Clomiphene 0.08 and after Clomiphene 0.68, Range = 0–1. Normally, the dominant follicle ruptures between 14 and 18 days after the 1st day of the last menstrual period. In the present study (Figure 1), the day of rupture before Clomiphene was on

Table 1: Height of the ovary before and after Clomiphene therapy (average of rt. and lt. ovaries)					
Height of ovary (cm)					
Before clomiphene	Range	Mean	Median	Standard deviation	P-value for average height before and after clomiphene=0.061875
Right	1.20–2.60	2.03	2.10	0.37	
Left	1.20–2.60	2.05	2.15	0.36	
Average	1.20–2.50	2.04	2.15	0.35	
After clomiphene	Range	Mean	Median	Standard deviation	
Right	1.20–2.60	2.04	2.10	0.39	
Left	1.40–2.60	2.06	2.10	0.35	
Average	1.30–2.50	2.05	2.15	0.36	

Table 2: Number of dominant follicles (total of rt. and lt. ovaries)

Number of dominant follicles					
Before clomiphene	Range	Mean	Median	Standard deviation	P-value for no. of dominant follicles before and after clomiphene=0.001437
Right	0.00–3.00	0.52	0.00	0.84	
Left	0.00–4.00	0.56	0.00	0.91	
Total	0.00–7.00	1.08	0.00	1.55	
After clomiphene	Range	Mean	Median	Standard deviation	
Right	0.00–3.00	1.00	1.00	0.88	
Left	0.00–3.00	1.00	1.00	0.93	
Total	0.00–5.00	2.00	2.00	1.47	

Table 3: Size of dominant follicle before and after clomiphene administration

Size of dominant follicles in both ovaries on day 11 (mm)					
Drug effect	Range	Mean	Median	Standard deviation	P-value for size of dominant follicle before and after clomiphene=0.001718
Before clomiphene	0.00–22.00	12.26	12.00	4.94	
After clomiphene	6.00–32.00	15.31	13.00	5.86	

Table 4: Day of rupture of the dominant follicle before and after clomiphene administration

| Number of follicles ruptured and day of rupture					
Before clomiphene	Range	Mean	Median	Standard deviation	P-value for no. of follicles ruptured=0.000000
Number of follicles ruptured	0.00–1.00	0.08	0.00	0.27	
Day of rupture	15–19	16.25	15.50	1.89	
After clomiphene	Range	Mean	Median	Standard deviation	P-value for day of rupture=0.030466
Number of follicles ruptured	0.00–1.00	0.68	1.00	0.47	
Day of rupture	12–21	14.97	15.00	2.14	
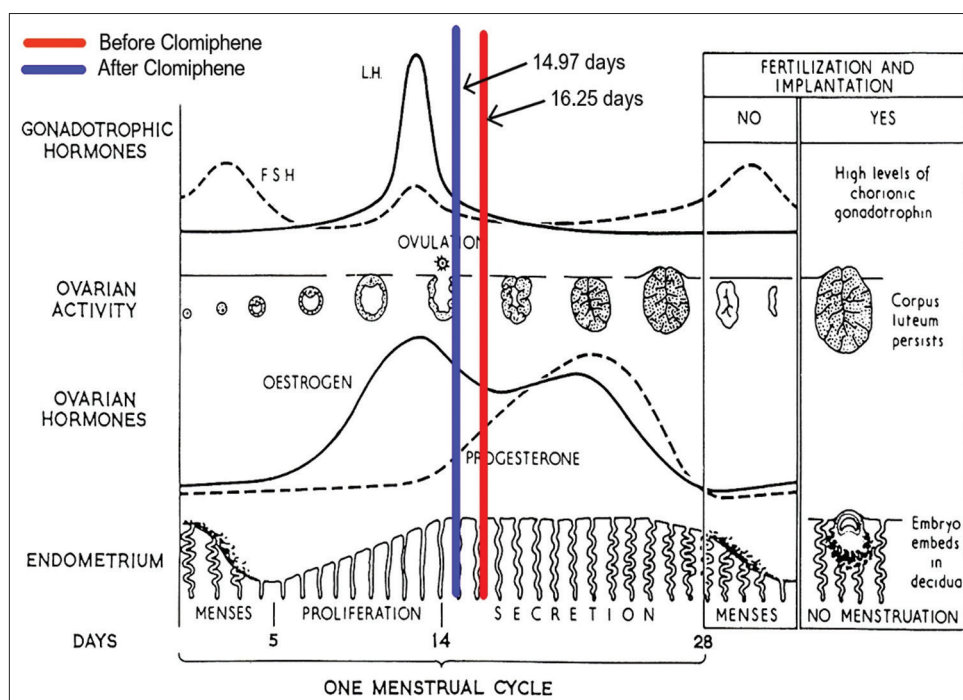
Figure 1: Day of rupture of dominant follicle before and after use of clomiphene

average 16.25 days. The day of rupture after Clomiphene was 14.97 days. Therefore, the range before Clomiphene was 15–19 days and after Clomiphene 12–21 days.

In PCOD cases, many follicles are small and immature and may be ≥10 in number. In the present study, significant follicles were taken to be >10 mm in maximum dimension.

DISCUSSION

The height of ovary increased from 2.04 cm to 2.05 cm. Although not significantly increased, the change was noticeable.

Of all the follicles recruited in a particular cycle, one assumes the dominant status and grows more than the rest. The criteria for determination of dominant follicles had been discussed under materials and methods. Usually, only one follicle dominates in an ovarian cycle. Rarely two or more follicles may mature together. However, under the influence of clomiphene, frequently two or more follicles may dominate in a cycle. This brings about a greater chance of twin, triplet, and higher order pregnancies as a result of drug induced stimulation of ovulation. This situation brings about all the hazards of multiple pregnancies such as premature onset of labor, preterm deliveries, abnormal lie and presentation, abnormal implantation of placenta, placental insufficiency, hypertensive disorders of pregnancy, and fetal hypoxia.[12,13]

In the present study, the number of dominant follicles significantly increased from 1.08 before clomiphene therapy to 2.00 after clomiphene. This shows the high incidence of multiple pregnancy with clomiphene use. However, there is one sobering conclusion. In some cases, there were no dominant follicles even with clomiphene use. Changes in follicle counts with the use of clomiphene were also studied by Johnson et al., in 1966 and Chang et al., in 1998.[14,15] The findings of all these and other workers are in agreement with the findings of the present study.

The average size of the dominant follicles in this study was 12.26 mm before clomiphene therapy and 15.31 mm after therapy. This significantly appreciable increase in size is important as it has a greater chance of rupture. This should be contrasted with the number of small and ineffective follicles seen in cases of PCOD. However, even the cases of PCOD showed a modest increase in the size of dominant follicles and thus had a greater chance of survival. The change in the size and number of follicles in both normal and PCOD cases was extensively studied by MacDouquall et al., in 1994.[16] The study was carried out both before and after the use of clomiphene. Serial monitoring of follicles was also carried out by Das et al., in 1995.[17]

Change in the day of rupture of dominant follicles after the treatment with Clomiphene was measured by Trans Vaginal Sonography. Another interesting feature of clomiphene therapy obtained was the shortening of the preovulatory phase of the ovarian cycle. As a result of rapid growth and maturation of the follicles, ovulation occurred earlier than usual. Usually, the dominant follicle rupture between the 14th and 18th day of the cycle. In the present study, the dominant follicle ruptured at 16.25 days of the cycle before clomiphene therapy and at 14.97 days after therapy. This shortening of the cycle should be kept in mind for planning of treatments, particularly oocyte retrieval for *in vitro* fertilization.

Effect of Clomiphene on Poly Cystic Ovarian Disease cases is seen by transvaginal sonography in 20 cases in the present study who presented with PCOD. They were subjected to transvaginal sonography before and after the treatment with CC. All cases had recruitment of ovarian follicles some of which enlarged to an acceptable size. All of the patients ovulated over one to three cycles. This confirmed the efficacy of clomiphene in PCOD cases. Similar results were obtained by Chohan et al.[18] In a few patients, in addition to ovarian stimulation, a wedge resection of the ovary may be necessary to facilitate ovulation.[1,19]

Solutions after failure of treatment of infertility
Despite all efforts, some couples are not benefited by infertility therapy. For these cases, there remain the options of assisted reproduction measures, surrogate pregnancy, and adoption.

Limitations of the study
In the present study we have assessed the parameters in the third cycle of Clomiphene Citrate taking it the optimum situation but a human may also response better in other cycles of CC which is not considered here. There are multiple causes of infertility which are not within the scope of this study.

CONCLUSION

As rising infertility poses a threat to the lives of human being with advancement in lifestyle, some easy and acceptable solution must be in hand. CC is that miracle which may convert utter despair into sparkling ray of hope with overall significant rise in number, size, and rate of rupture of dominant follicles in ovary as well as reduction in day of rupture of them. This held true even in cases of poly cystic ovarian disease. Although risk of multiple pregnancy and ectopic pregnancy could not be fully ruled out in case of Clomiphene administration, regular monitoring and counseling can bring down the risks to a good extent.

The future of reproductive technology is opening up at a rapid rate. Selective culture of embryos, screening for birth defects

at the primodrial stage, and genetically engineered fetuses are looming on the horizon. The womb and the mother's lap may be replaced by culture solutions and incubators. The role of the individual parents in conceiving a child is likely to decrease with unknown consequences for the child. A compromise will have to be made between conception of perfect children and parental and child bonding. The challenges of the future are likely to bring forth unique solutions to the age-old process of natural childbirth and rearing.

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Authors Contributions:
JSG- Concept and design of the study, prepared first draft of manuscript; **KRS-** Interpreted the results; reviewed the literature and manuscript preparation; **OS-** Concept, coordination, statistical analysis and interpretation; **SH-** Preparation of manuscript and revision of the manuscript.

Work attributed to:
Department of Gynaecology and Obstetrics and Department of Anatomy, IPGME and R and SSKM Hospital, Department of Anatomy, Calcutta National Medical College.

Orcid ID:
Dr. Susmita Ghosh - https://orcid.org/0000-0002-7841-8939
Dr. Kausiki Ray Sarkar - https://orcid.org/0000-0003-3169-2196
Dr. Oyndrila Sengupta - https://orcid.org/0000-0002-5715-4380
Dr. Subhajit Halder - https://orcid.org/0000-0002-0696-7007

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