

Sub Endometrial Doppler Flow in Spontaneous and Clomiphene Citrate Stimulated Cycle.

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Abstract

Background: Anovulation is the most common etiological factor responsible for female infertility. Clomiphene citrate is one of the widely practiced induction method for ovulation induction, however the efficacy of treatment is limited. One of the hypothesis is that with Clomiphene citrate treatment, endometrial receptivity may be decreased despite good follicle count. There is a major difference in uterine perfusion between spontaneous and clomiphene citrate induced cycle of women with infertility.

Objectives: Primary objective of the research is the assessment of the sub endometrial doppler flow changes in spontaneous cycle and sub endometrial flow in clomiphene citrate induced cycle.

Methodology: There will be two groups of women, one group stimulated by clomiphene citrate and there will be no stimulation for other group of women. For ovulation induction 50 women will be administered placebo multivitamin tablet and for other 50 women will be given 100mg of clomiphene citrate given from day 3 of LMP to day 7. Folliculometry is performed on day 10 of the cycle, when follicles are greater than 17 mm, human chorionic gonadotropin 5000 IU intramuscular injection will be given, after this endometrial doppler will be done to see zonal flow in different zones of endometrium, and also endometrial perfusion, pulsatility index and resistance index, and vascular impedance in uterine arteries.

Results: Sub-endometrial flow changes will be observed in four endometrial areas.

Conclusion: The purpose of this research is to evaluate subendometrial doppler flow changes of endometrium in spontaneous cycle as compared to endometrium of clomiphene citrate stimulated cycle.

Keywords: Sub-endometrial flow, clomiphene citrate stimulated cycle, Applebaum scoring

INTRODUCTION:

Amongst the causes of infertility, anovulation is the most frequently involved factor of female infertility (1). One of the most widely used ovulation induction methods is clomiphene citrate, but efficacy of this treatment is limited. One of the hypothesis is that there could be reduced endometrial receptivity with Clomiphene citrate despite good follicle generation. Transvaginal doppler sonography is most commonly used in modern gynaecology as an indirect method to assess the vascularity of endometrium.

Both anatomical and physiological reproductive system will be assessed using transvaginal doppler, vascular impedance will be studied in uterine artery of infertile women. (2)

Attempts will be made to study uterine receptivity in sub endometrial zone by doppler ultrasound and also of uterine arteries at various menstrual cycle stages. High resistance of uterine blood flow in the luteal phase be the cause of unexplained infertility.

In our study we will be using transvaginal color doppler to assess endometrial perfusion in different zones of endometrium in both spontaneous and clomiphene citrate stimulated cycle in infertile women. (3) Better the endometrial perfusion, better the chances of success of treatment.

The maximum endometrial thickness would imply the uterus's longitudinal plane and the measurement offered an echogenic interface between the endometrium and myometrium. Transvaginal sonography describes ovulation as a reduction in follicular size by 5 mm. Doppler transvaginal colour will be used for the visualisation of sub endometrial flow. To decrease the potential effects of exertion on the uterine blood flow, patient seeking counselling will be required to wait for at least 20 minutes before treatment in the resting room. After explaining the procedure to the patient, patient will be placed in the knee flexed position for examination. We will use colour Doppler sonography that has a 7.5-MegaHz transvaginal imaging probe and a 6-MegaHz pulsed blood flow analysis Doppler system (Hitachi-Medico EUB-515 A, Hitachi, Tokyo, Japan).

By using Apple Baum scoring criteria endometrial perfusion will be seen in different zones of endometrium. (4)

1. Zone 1: The area of myometrium surrounding the endometrium.
2. Zone 2: The hyperechoic area of endometrial edge.
- 3., Zone 3: The inner endometrial hypoechoic zone.
4. Zone 4: The area of endometrial cavity.

Variations in the depth of vascular penetration can be seen before, after and after the mid-menstrual period.

By using transvaginal doppler sonography pulsatility index and resistance index also seen and compared in both spontaneous and stimulated group. It is seen in stimulated group pulsatility index increases and resistance index decreases.

RATIONALE

The most common use of clomiphene citrate is for mild to moderate cases of infertility. (5)

It is for ovulation induction and is speculated to decrease blood flow and uterine receptivity despite strong follicular generation. Using transvaginal colour doppler sonography,

assessment of vascularity of endometrium can be done as an indirect predictor of endometrial receptivity. Hence successful outcome of treatment in spontaneous compared to stimulated cycle can be observed in this study.

AIM: The Aim of this study is to evaluate the sub endometrial doppler flow in spontaneous cycle while comparing it with clomiphene citrate stimulated cycle.

OBJECTIVES- The primary objective of this study is:

1. To Assess The Subendometrial Flow In Spontaneous Cycle Of Infertility
2. To Assess The Subendometrial Flow In Clomiphene Citrate Induced Cycle
3. To Compare The Subendometrial Flow In Spontaneous And Clomiphene Citrate Induced Cycle.

RESEARCH QUESTION :What is the effect of clomiphene citrate on sub endometrial vascularity in comparison to spontaneous cycle?

POPULATION –

Patients with mild to moderate cases of infertility

INTERVENTION-

Colour Doppler transvaginal ultrasound.

COMPARISON – Observing the endometrial perfusion by transvaginal color doppler in spontaneous cycle compared to clomiphene citrate stimulated cycle.

OUTCOME-Sub endometrial flow in four zones of endometrium

i.e zone 1 :the area of myometrium surrounding the endometrium, zone 2 : the hyperechoic area of the endometrial edge, zone 3 :the internal area of endometrial hypoechoic zone, zone 4 cavity of endometrium.

METHODS

STUDY DESIGN- cross sectional study.

Setting: JNMC, Wardha, IVF Unit

Duration of study- 2 years after IEC approval

Place of Study- Datta Meghe Institute of medical sciences, Acharya Vinod Bhave Hospital, Wardha.

Sample size:

FORMULA

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{d^2}$$

d(sq)

out of which

$Z_{\alpha/2}$ is the level of significance at 5 %

I.e. 95% confidence interval=1.96

P=prevalence of infertility=15%=0.15

D= desired error of margin=7%=0.07

$n = 1.96(sq) \times 0.15 \times (1-0.15)$

0.07(sq)

=99.96

N=100 patients needed in study

N=100 patients needed in study

Participants: By computerised system randomization two groups of women are taken.

FIRST GROUP: Spontaneous ovulation group-50 women without medication for ovulation induction, multivitamin tablets will be as placebo from the Day 3 of LMPTO Day 7.

SECOND GROUP: 50 women will be administered Clomiphene Citrate 100 mg from Day 3 of LMP to day 7. Clomiphene citrate is taken orally from day 3 until day 7, The follicular scan will be done from day 10th onwards.

Once the follicle size is greater than 17 mm, the HCG trigger will be in the form of 5000 IU IM to the participants of both the groups.

After 48 hours by transvaginal color doppler, subendometrial flow will be studied in both groups to see zonal flow in endometrium, and also the endometrial perfusion.(6)

INCLUSION CRITERIA: -

- 1.The age 19 years -40 years
- 2.The length of their menstrual cycle should be 28+-7 Days
- 3.One of the fallopian tubes should be Patent on Hysterosalpingography or Laparoscopic Photoperturbation.
- 4.Ant Mullerian Hormone within normal range 2-8 nanogram/mm.
- 5.Antral Follicle count 4-6 in each ovary on day 2 of menses.
- 6.Husband sonogram should be normal fulfilling the criteria of WHO Seminogram
- 7.Women willing for Timed intercourse or IUI.

EXCLUSION CRITERIA

1. Previous hysteroscopic surgeries
2. Chronic PID
3. Women age for than 40 years.

4.Ovulatory disorders

5.Hormonal abnormalities.

6.Previous history of Tb (Extrapulmonary /Pulmonary TB)

7.Already diagnosed adenomyosis or submucousmyomas.

Variables: Sub endometrial doppler, Applebaum scoring, Induced cycle of clomiphene citrate

Statistical analysis

DATA will be included in the SPSS VERSION 20 package.

Sub endometrial flow using T test and Chi-square test seen in all four regions .It is considered that the measured significance test and a P value of less than 0.05 are important.

Bias: The two separate groups, the research and control group, blinded by radiologist.

METHODOLOGY

After taking consent, every woman will be explained the type and nature of the study,informed consent will be taken. A total of 100 patients full-filling the exclusion and inclusion criteria will be admitted and monitored in the ward. Detailed menstrual history, obstetric history, past history, personal & family history will be taken. General, systemic and obstetric examination to be done. All blood & urine investigation will be done

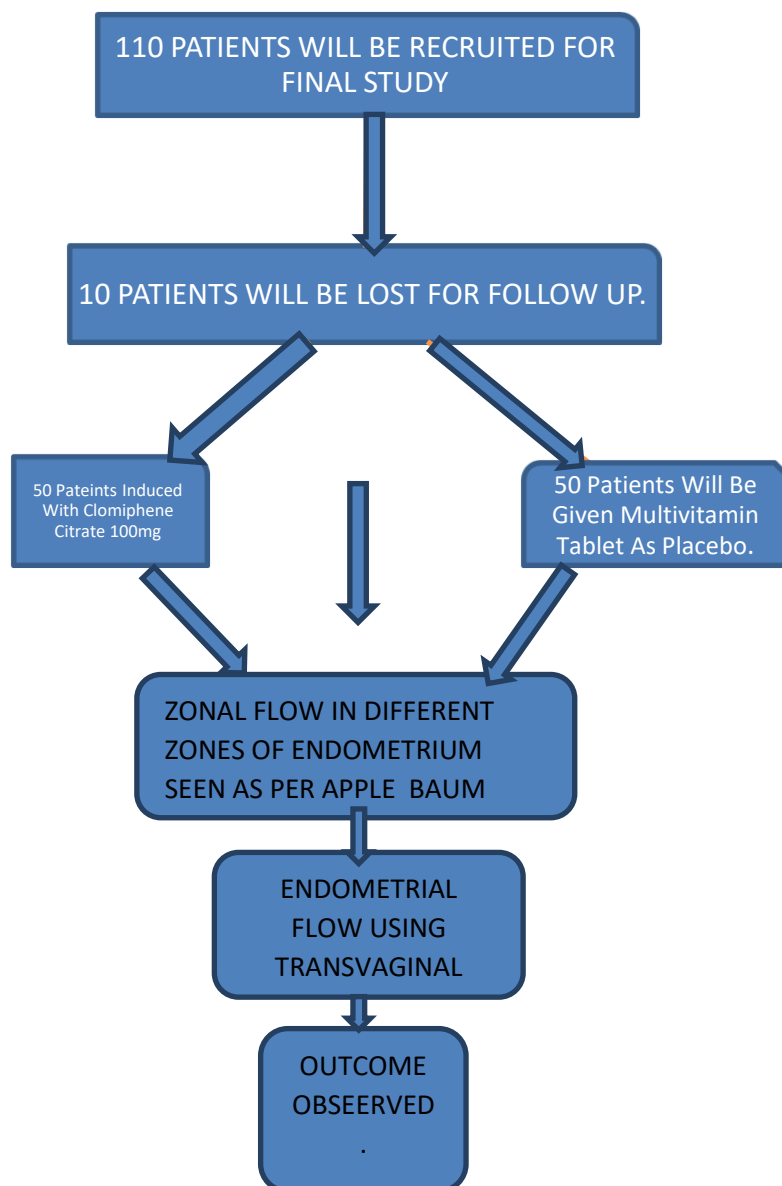
There will be two groups of women:

One group of 50 women, spontaneousgroup, multivitamin tablet will be given as placebo to these women from day 3 o LMP to day 7.

Other group of 50 women will be stimulated with clomiphene citrate 100mg from day 3 of LMP to day7. follicular study will be done in these group of women on day 10th .Once the follicle size is 17mm,Human chorionic gonadotropin 5000IU intramuscularly will be given as trigger. After 48 hours of HCG trigger ,endometrial doppler flow seen using transvaginal colour doppler to see endometrial perfusion in different zones of endometrium,and also to see the effect of clomiphene citrate on the pulsatility index and resistance index.

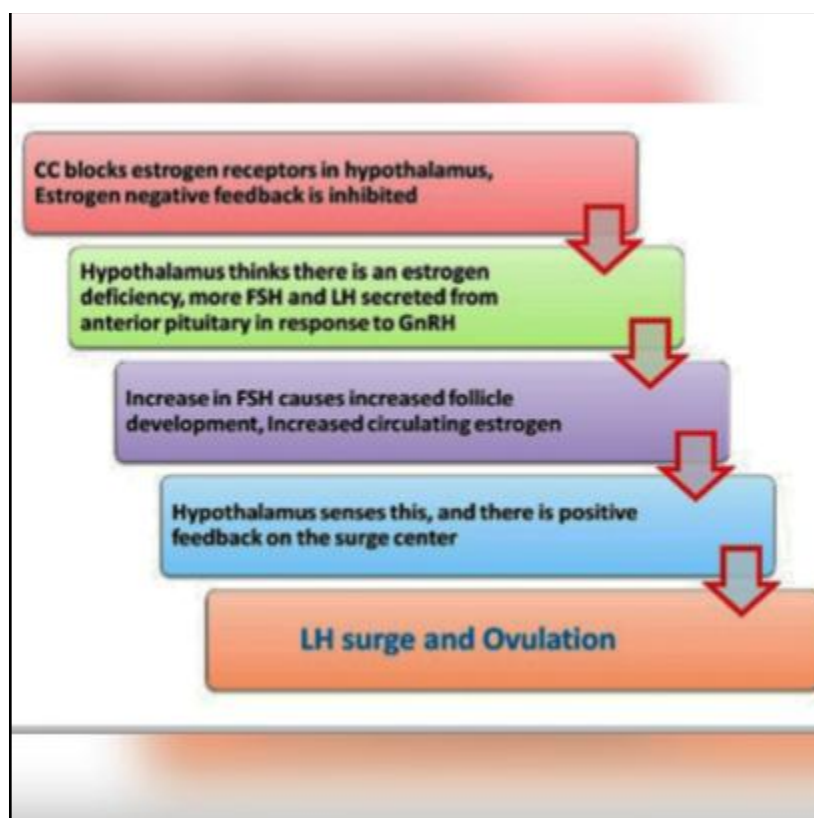
FLOW CHART-

110 patients will be recruited for final study,out of which 10 patients will be lost to follow up.Out of 100 patients,50 patients i will be induced with clomiphene citrate 100mg ,while other 50 patients will be given multivitamin tablet as placebo from day 3 of LMP to day 7 Endometrial flow using transvaginalcolordoppler will be checked on day 10.HCG trigger of 5000 IU intramuscularly will be given to participants of both groups.When follicles size is greater than 17mm,endometrial flow in different zones of endometrium will be seen.



Outcomes/Predicted /results

Sub endometrial doppler flow changes in the spontaneous and clomiphene citrate stimulated cycle with transvaginal ultrasonography seen in different zones of endometrium.



DISCUSSION

The most widely used drug in cases of infertility is clomiphene citrate. However, the efficacy is limited. Since anovulation is considered the most common cause of female infertility, Clomiphene citrate is known to block oestrogen receptors in the hypothalamus, oestrogen negative feedback is inhibited, hypothalamus perceives that there is oestrogen deficiency so from the anterior pituitary in response to GnRH, more of the FSH and LH are secreted. Increase in FSH causes increase in follicle development, increasing circulating oestrogen. Hypothalamus senses this and there is positive feedback on the surge centre which results in LH surge and Ovulation.

For success of ART procedures assessment of endometrial receptivity is most important. USG has many advantages as it is non-invasive real time monitoring and it is predictable. The different markers of sonography are endometrial thickness echogenicity and colour doppler study of uterine and endometrial blood flow by using power and colour doppler vascular changes which are reflection of biochemical change. The colour doppler visualises blood flow with the energy of moving reflectors. High sensitivity to slow blood flow is another benefit. Pulsatility index and resistance index are commonly used indices of uterine artery but they cannot reflect endometrial blood flow. The pulse doppler gives objective while colour mapping gives subjective assessment of endometrial vascularity. Some authors have found that vascular resistance in the sub-endometrial arteries was substantially lower in patients who were pregnant on the day of oocyte retrieval or embryo transfer.

It has recently been suggested that the best day to test uterine receptivity is HCG trigger day. This is based on reports of increased Uterine artery impedance after HCG injection. This is why day of HCG is selected for colour doppler. Absence of colour mapping in endometrial and sub endometrial areas means absolute failure of implantation or decrease of rate of

implantation. Conversely, as vessels enter the sub-endometrial halo and endometrium, the pregnancy rate increases. When the vessels are present in endometrium it means endometrium is thicker. When pregnancy is completed, but sub-endometrial and endometrial flow cannot be seen on the day of ET, most of half of these pregnancies will result in spontaneous miscarriage, which means that the development of the endometrial vascular network is essential for supporting the first stage of pregnancy. On the day of follicle aspiration, in one of the study uterine arteries checked to which it was concluded that in patients who became pregnant after embryo transfer, the resistance index of the uterine arteries was significantly lower than in those who did not become pregnant. Another research showed that patients with a low PI in the uterine artery on the day of embryo transfer were more likely to conceive than those with a high PI. No one with a $PI > 3.0$ conceived of this sequence. A gradual decrease in the uterine artery pulsatility index is seen in active IVF pregnancies during the second half of the menstrual cycle. Inadequate vascular penetration of endometrial blood flow (not within Zone 3) prior to transfer was associated with an adverse outcome using the Color Doppler technique. Successful pregnancies have been reported with demonstrable blood flow in Zone 4, indicating the presence of intracavitary mass. Few of the related studies were reported (7-9). Wankhade et. al. reported on transvaginal sonography findings in the endometrium of patients with abnormal uterine bleeding (10). Jain and Phatak reported on ultrasound and color Doppler imaging of live ectopic pregnancy (11).

CONCLUSION

We concluded in our study that endometrial perfusion is significantly lower in clomiphene citrate stimulated group of infertile women as compared to the endometrial perfusion in spontaneous group of women.

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