ROLE OF GRANULOCYTE COLONY STIMULATING FACTOR EFFECTS ON UNRESPONSIVE THIN ENDOMETRIUM IN WOMEN UNDERGOING CONTROLLED OVARIAN STIMULATED INTRAUTERINE INSEMINATION CYCLES

ABSTRACT

Back ground: It is a prospective observational study that included unexplained infertility cycles with COS –IUI protocols. Thin endometrium of less than 7mm has a negative effect on pregnancy rate. Endometrial function and receptivity factors are important in the success of implantation.

AIM: To monitor the effects of G-CSF on thin endometrium in improving endometrium thickness and pregnancy rates in G-CSF administered COS-IUI cycles.

METHODS: This study was conducted in the Laxmi Narasimha hospital IVF center under the guidence Dr. Adaboina Anitha,Hanamkonda, warangal. Thin endometrium was defined less than 7mm on Trans vaginal ultrasound. Letrozole 5mg (2.5mg, BD) is used for ovulation induction on day 2 or day 3 of their cycle, G CSF 300units was added with significantly improved pregnancy rates in ART cycles. Trigger used was injection 10,000IU urinary hCG & 300 units G-CSF was instilled into the uterus. After 48hours ET was measured & IUI was performed under aseptic precautions. After 16 days β -hCG levels were monitored to determine whether there is a pregnancy.

RESULTS: In present study, we concluded that, 250 COS-IUI cycles were analysed and 50 cycles showed a thin endometrium and in them G-CSF was used. The pregnancy rate (28%) was evaluated, 14 members are positive for pregnancy.

CONCLUSION: In our study we concluded that G-CSF and letrozole will improve increases endometrium thickness and conceiving rates in COS-IUI cycles.

KEY WORDS: Granulocyte colony stimulating factor, Controlled ovarian stimulation, Intra uterine insemination.

INTRODUCTION

Infertility is a failure to conceive within one year of regular unprotected intercourse.[1] The average percentage for unexplained infertility is around 10 to 15%.Un explained infertility diagnosed when a couple fails to conceive without any cause.[2]

The unexplained infertility, the conventional approach starts with expectant management, moving to ovulation induction combined with intrauterine inseminations.[2] Ovulation induction sets a goal to develop a minimum of one follicle in an otherwise an ovulatory cycle. Controlled ovarian stimulation (COS) involves recruiting a multiple number of follicles.[4] Most commonly used protocol is five days of letrozole (2.5-5mg) from day 2 to day 6 of menstrual cycle. The target is to get a 1-3 follicles (18-20mm).The aim of controlled ovarian stimulation is to achieve a sufficient number of fertilizable oocytes of good quality and improve pregnancy rates. It also helps in preventing ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. In controlled ovarian stimulation cycles a normal responder may give 8-15 follicles.

"letrozole" is a non-steroidal Aromatase inhibitor and is a potential ovulation induction (OI) drug. This competitive inhibition prevents the change of androgens to estrogens in the ovarian follicles, peripheral tissues, and in the brain. This action leads to a reduction in uterine weigh and elevated luteinizing hormone. Thus, there is a surge in follicle stimulating hormone (FSH) release, which results in follicular development.[5]

Intrauterine insemination (IUI) is sensible first line therapy for patients with typical sperm morphology more than 4%. Sperm count, motility and morphology are important factors for success. A significant rise in pregnancy rate is seen with post wash complete motile sperms of in excess of five million per ml. The trigger for ovulation is given when the dominant follicle is 18-20mm in diameter. hCG is the most commonly used drug for inducing the final maturation of the follicle and ovulation. Urinary derived hCG in the dose of 5000 IU or 10,000 IU IM is given and IUI is done 34-36 hours after the trigger in case of single insemination.[2] To achieve an effective pregnancy outcome when the follicle reaches the size of 18-20mm, an endometrium thickness of 7mm is justified. A triple line endometrium with

thickness of more than 9-14mm is best conducive for implantation and pregnancy rates. Endometrial Function and receptivity are major factors in establishment of pregnancy.[4]

G-CSF is a glycoprotein growth factor and cytokine.[6] G-CSF is produced by endothelial cells, monocytes, macrophages, and fibroblasts. In the reproductive tract, G-CSF is secreted in follicular, granulosa cells, endometrial cells and cells from decidual, placental and various fetal tissues.[7]G-CSF promotes neutrophil proliferation and maturation.[8]G-CSF involved in a wide variety of reproductive functions implicated in normal ovulation.[10] Maintaining healthy endometrium for successful implantation and further development of embryo.[11] Useful biomarker of oocyte competence before fertilization improve implantation rate and pregnancy outcome in infertility, IUI and IVF procedures. Sharky et al. shows that immunological mechanism in endometrium are very important for implantation process. G-CSF has been proposed as a treatment for implantation failure and repeated miscarriages and thin endometrium. A growth spurt in endometrial thickness can be observed within 48hrs of G-CSF administration. In endometrium G-CSF is secreted epically in polarized epithelial cells.[12]

MATERIALS AND METHODS:

A Prospective observational study conducted over a period six months. Total of 250 COS IUI cycles were analyzed and 50 people had unresponsive thin endometrium less than 7mm on Trans vaginal ultrasound. For ovulation induction drug Letrozole of 2.5mg or 5mg and G-CSF is used. Urinary HCG 10,000 is used as trigger injection when the follicle is >/=to 18mm. G - CSF of 300mcg is instilled into the uterus on day 11, day 13 using IUI catheter. IUI is done 34-36 hrs after giving trigger injection.

INCLUSION CRITERIA:

People with clomiphene resistance, Unexplained infertility, Thin endometrium, previous IUI failed cycles, past history of D&C and miscarriages.

EXCLUSION CRITERIA:

Known causes of infertility, surgical disorders.

Procedure:

All the people underwent a baseline trans vaginal ultrasound to determine their antral follicle count on day 2 of their cycle. Letrozole (2.5mg or 5mg) was used for ovulation induction on

day 2 or 3 of their cycle based on the antral follicle count. Endometrial thickness is monitored trans vaginally. People with thin endometrium of 2mm to 5mm on day 11 were taken for day 11 intrauterine G-CSF 300mcg 0.5ml instillation. If the endometrial had not reached at least 7mm within 48hrs, a second infusion was given i.e. on day13. Injection urinary HCG 10,000 units is used as ovulation trigger. IUI is performed under strict aseptic precautions 34-36hrs following trigger. All people were followed up after 15days for serum beta HCG to determine whether they are pregnant.

RESULTS:

In all, 250 people participated in this study and COS –IUI cycles were analyzed. Total 50 people are showed thin endometrium and in them 300 units of GCSF is given. The people age group was 19 to 40 years. Table 1 and figure1 exhibits age distribution of the people included in this study. Out of the 50 people, 14 people were under the age of 24years, 18 people were between 24 to 29 years, 12 people were between 29 to 34 years, 6 people were more than 34 years. The mean age of people included in this study was 27.52years

Age distribution of people	No.of people
<24 years	14
24 to 29 years	18
29 to 30 years	12
>34 years	6

TABLE 1: Age distribution of people

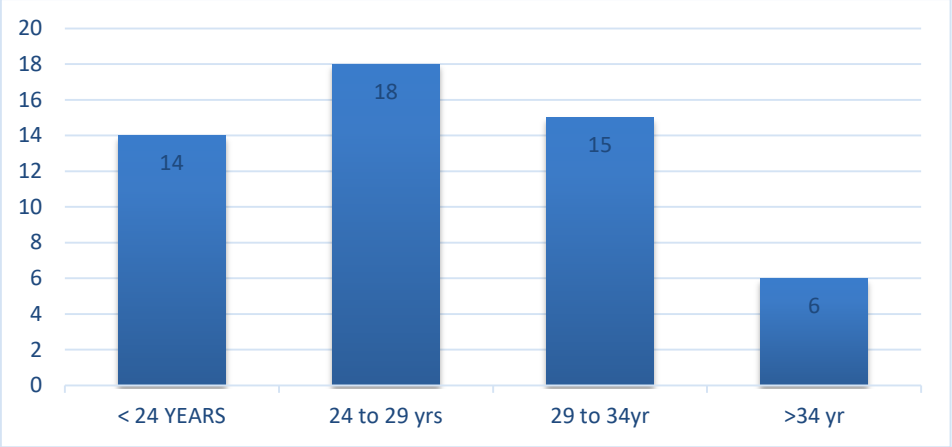

FIGURE 1: Age distribution of people

Table 1 and figure 2 shows the types of infertility people in this study. Primary infertility people are 36 and percentage was 72%, secondary infertility people are 14 and percentage was 28%.

Parameter	No. of people	Percentage
Primary infertility	36	**72%**
Secondary infertility	**14**	**28%**

TABLE 2: Infertility type

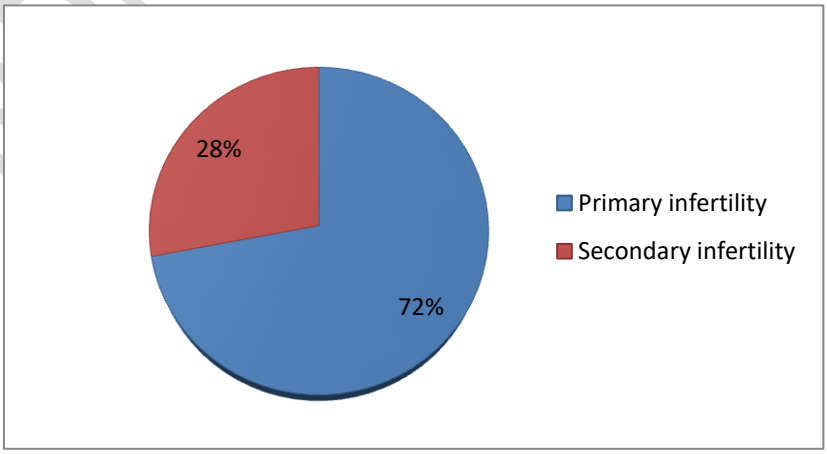

FIGURE 2: Infertility type

The people received Letrozole within the strength of 2.5mg or 5mg consistent with their antral follicle count. Out of the 50 people 32(64%) had took 5mg of Letrozole and rest 18(36%) took 2.5 mg of letrozole. Table 3 and Figure 3 show the distribution of letrozole among the people.

Parameter	No. of people	Percentage
2.5 mg of Letrozole	32	64%
5mg of Letrozole	18	36%

TABLE 3: Distribution of Letrozole

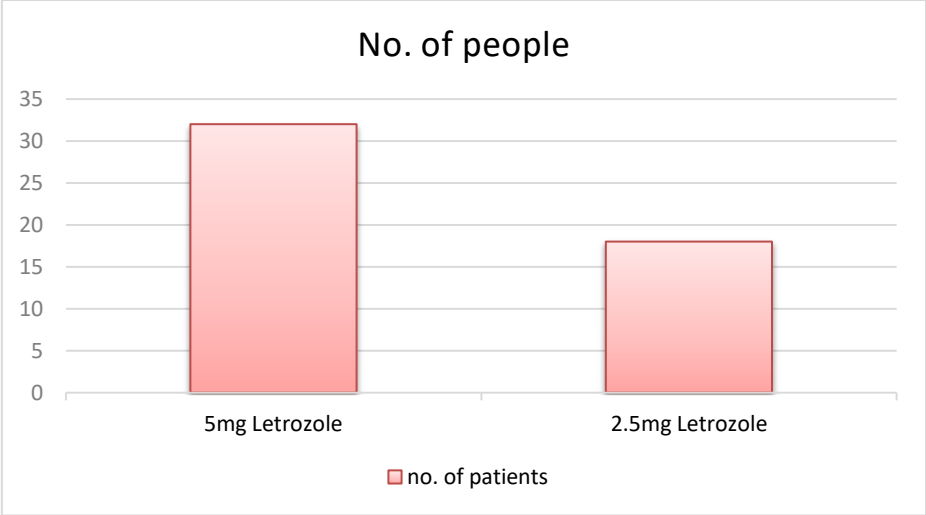

Figure 3; Distribution of letrozole

When the endometrial thickness was analyzed before and after the instillation of G-CSF, the average increase in endometrial thickness after instillation of G-CSF was 7.8mm was highest increased was 8.5mm and the lowest increased was 1mm and the one patient unresponsive to the G CSF.

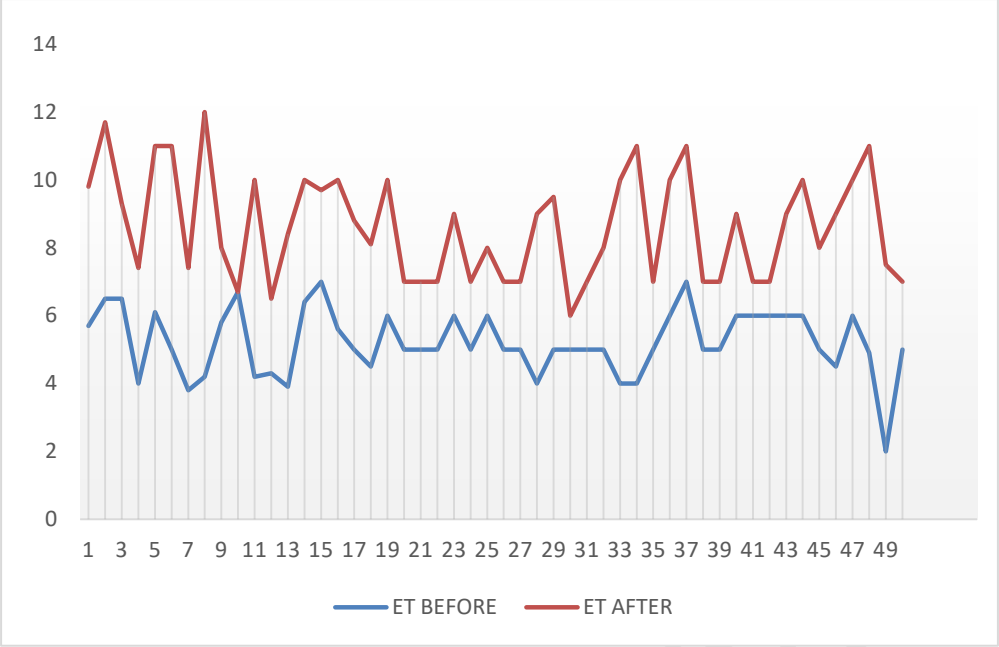

Figure 4: The difference between endometrial thickness before and after infusion of G-CSF

The baseline parameters of people are shown in table 4 and table 5 presents endometrial thickness in people before and after infusion of G CSF who had people undergoing COS - IUI cycles.

S.No	Parameter	Total no of people n = 50	People who conceived n = 14	People who did not conceived n =36
1	Age	27.52 ± 4.69	26.07 ± 3.75	28.08± 4.94
2	Primary infertility Secondary infertility	36(72%) 14(28%)	12 2	24 12
3	Duration of infertility in years	1.28 ± 0.456	3.28 ± 1.8	6.45 ± 4.88

4	**No. of ovulation induction cycles**	4.36 ± 2.15	4.14 ± 2.56	4.278 ± 1.966
5	**No. of developing follicles at time of trigger**	2.4 ± 1.74	1.76 ± 0.699	1.729 ± 0.87

TABLE 4: The Baseline Parameters of people

In all the subjects at the time of G CSF administration Endometrial thickness was 5.71 ± 1.55mm after G CSF administration endometrial thickness was 8.91 ± 1.77mm.

When we divided the group into two subgroups according to whether they conceived and not conceived. The conceived people, we showed that the endometrium increased from 5.82 ± 1.2mm to 8.5 ± 2.4mm and the not conceived patients the endometrium increased from 5.66 ± 1.66mm to 9.01 ± 2.03mm.

S.No	**Characteristic**	**Total no of people n=50**	**People who conceived n =14**	**People who did not conceive n =36**
1	ET before GCSF administration	5.71± 1.55	5.82 ± 1.2	5.66± 1.66
2	ET after GCSF administration	8.91±1.77	8.5±2.4	9.01±2.03

TABLE 5 : The difference between Endometrial thickness before and after infusion of G-CSF

The increased endometrium thickness in all the subjects at the time of G CSF administration Endometrial thickness was 5.71 ± 1.55mm after G CSF administration endometrial thickness was 8.91 ± 1.77mm. The statistical analysis of endometrial thickness is P value is 0.11.

Finally, when the pregnancy rate was evaluated, there were 14 pregnancies with beta HCG levels are positive and the pregnancy rate was 28%.

DISCUSSION:

Endometrial infusion with G-CSF is effective in expanding chronically unresponsive thin endometrium and pregnancy rates in COS IUI cycles. Our study on G -CSF on Un responsive thin endometrium COS IUI cycles using Letrozole as ovulation induction drug in showing increase in endometrium thickness and pregnancy rates.

Many studies were done in recent years on G - CSF in IVF or ICSI cycles. Ours is the only study done in COS IUI cycles using Letrozole as ovulation induction drug in unexplained infertility with unresponsive thin endometrium. Till now only one study by Shetty DP et.al in 2019 done on 200 patients in COS IUI cycle using Clomiphine citrate as ovulation induction drug, they concludes GCSF increases significantly in COS IUI cycles in the thin endometrium.

A pilot cohort study by N.Gleicher et al on G - CSF in the treatment of unresponsive thin endometrium resistance to standard therapies. They analyzed 21 women and 19.1% ongoing clinical pregnancy rates was observed.

In our study, we analyzed total 50 patients showed a thin endometrium and in then 300 units of GCSF is given. Our results give a positive inclination towards the use of G-CSF in IUI cycles. All patients are responding to GCSF treatment and endometrium thickness is significantly improved in all patients. Finally, when the pregnancy rate was evaluated, there were 14 pregnancies with beta hCG levels are positive and the pregnancy rate was 28%.

CONCLUSION:

G-CSF increases endometrial thickness and clinical pregnancy rate significantly after administration of intrauterine instillation of COS-IUI cycles in unresponsive thin endometrium.

At this present study is done on a small group of patients in COS-IUI cycles, large randomized controlled trials are necessary for better pregnancy rate. This study showed that G CSF and letrozole might be associated with increased endometrial thickness in women and also, it can lead to higher implantation rate.

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