

Comparative Analysis of Effectiveness of Clomiphene Citrate and Letrozole Combined with Low Dose Human Menopausal Gonadotropin for Controlled Ovarian Stimulation in Intrauterine Insemination Cycles

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ABSTRACT

Objective: To compare the efficacy of clomiphene citrate and letrozole in combination with low dose human menopausal gonadotropin for controlled ovarian stimulation in intrauterine insemination (IUI) cycles.

Methods: During January-2018 to December-2019 for intending 496 IUI cycles, controlled ovarian stimulation was performed with either clomiphene or letrozole combined with human menopausal gonadotropin (hMG), in two arms: subjects in one arm (Group A) were with clomiphene and hMG in 222 cycles; those in the second arm (Group B) were with letrozole and hMG in 274 cycles. Pregnancy rate and clinical pregnancy rate of both groups were considered as the primary outcomes.

Results: Patient characteristics like female age, indications for IUI, type of IUI (Artificial insemination with husband semen or donor sperm), endometrial thickness and total motile fraction (TMF) of spermatozoa of male partners were seen similar in both groups. The letrozole-hMG group (Group B) had significantly higher numbers of cycles with single dominant follicle ($P=0.01$) than the other one and human chorionic gonadotropin (hCG) was more frequently used as the ovulation trigger ($P=0.03$). Pregnancy rate (18.5% vs. 15.3%, $P=0.35$) and clinical pregnancy rate (18.5% vs. 15.3%, $P=0.35$) were similar in groups A and B, respectively.

Conclusion: Clomiphene citrate and letrozole combined with low dose human menopausal gonadotropin were equally effective for controlled ovarian stimulation in IUI cycles.

Keywords: Clomiphene; letrozole; human menopausal gonadotropin; intrauterine insemination; ovarian stimulation (Siriraj Med J 2021; 73: 198-203)

INTRODUCTION

Over the last four decades, clomiphene citrate was widely used for ovarian stimulation. Since 2001 the third generation aromatase inhibitor, letrozole become popular as its alternative agent. Several studies were performed

to compare underlying functional aspects of these two drugs; two randomized trials described higher pregnancy rate with letrozole for ovulation induction in anovulatory infertility patients with polycystic ovarian syndrome (PCOS).^{1,2} This was due to a better ovulation rate and

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the lack of anti-estrogenic effect on endometrium, which could be the major limiting factor of using clomiphene citrate.³ Hence, letrozole was recommended as the first-line agent for ovulation induction in PCOS leading to anovulatory infertility.⁴ Moreover, significantly higher ovulation rate with letrozole compared to clomiphene was reported in a subset of PCOS patients with clomiphene resistance.⁵ Furthermore, a randomized trial of infertility patients with minimal and mild endometriosis undergoing IUI after controlled ovarian stimulation had similar pregnancy rate for both clomiphene and letrozole.⁶ In a systematic review with meta-analysis of 8 randomized trials, similar pregnancy rate was seen with both letrozole and clomiphene in unexplained infertility.⁷

Clomiphene and letrozole are often used in combination with human menopausal gonadotropin (hMG) for controlled ovarian stimulation in IUI cycles. Ovarian stimulation with low dose gonadotropin is always preferred due to a low risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Published studies using low dose gonadotropin with clomiphene or letrozole in IUI cycles show conflicting results.⁸ Pregnancy rate was significantly higher with letrozole in another study.⁹ In contrast, clomiphene citrate was reported as better than letrozole in terms of pregnancy rate.¹⁰ The present study compares the efficacy of clomiphene and letrozole combined with low dose gonadotropin in overlapping regimen amidst confusing literature on the both ovarian stimulators. All categories of patients undergoing IUI including PCOS, minimal and mild endometriosis, unexplained infertility and male factor were included.

MATERIALS AND METHODS

Patient selection and controlled ovarian stimulation

The present retrospective cohort study was performed at a tertiary care infertility centre; 496 IUI cycles were performed in 2 years from January 2018 to December 2019; clomiphene or letrozole was used with human menopausal gonadotropin (hMG) for ovarian stimulation as a comparison. After routine infertility workup, fallopian tubal patency was evaluated by hysterosalpingography (HSG) or diagnostic laparoscopy with chromopertubation. IUI was advised to specific couples with unexplained infertility, ovulatory dysfunction, minimal or mild endometriosis and mild to moderate male-factor infertility. AID was advised for severely abnormal male factor, when the couple was unable to afford ART. In all cases written and informed consent was obtained from the couple.

Controlled ovarian stimulation was done with clomiphene citrate (Clofert, Svizera Healthcare, India) or letrozole (Letroz, Sun Pharma Laboratories, India) combined with urinary human menopausal gonadotropin (hMG) (GMH, Sun Pharma Laboratories, India). 'Group A' patients received clomiphene with hMG while, Group B constituted patients receiving letrozole with hMG. Clomiphene citrate 50 or 100 mg was given orally from 2nd or 3rd day of cycle for five days. Injection of human menopausal gonadotropin (75 IU) was administered intramuscularly on 5th and 7th day of cycle. Similarly letrozole 2.5 mg or 5 mg was started on 2nd or 3rd day of menstrual cycle combined with hMG (75IU) on 5th and 7th day. Follicular monitoring was conducted with transvaginal ultrasonography on 10th or 11th day of cycle. The patients were advised for further daily doses of hMG depending on the size of the dominant follicle(s). When the dominant follicle(s) reached at least 17 mm in diameter, ovulation trigger was administered and IUI was performed 38-40 hours later. Urinary human chorionic gonadotropin (hCG) 5,000 units were used as trigger for ovulation. If there were ≥ 4 follicles measuring more than 13 mm including one or two leading follicles measuring more than 16mm, injection Leuprolide acetate 1mg was administered subcutaneously as ovulation trigger to prevent ovarian hyper stimulation syndrome (OHSS). In the presence of more than 3 follicles measuring more than 16 mm, the cycle was cancelled in view of high risk for OHSS and multifetal gestation.

Semen preparation and IUI

Semen was collected by the male partner into a wide-mouth, sterile plastic container was kept at 37°C for liquefaction; semen samples were processed by using double layered density gradient method. An aliquot of 1ml 40% (v/v) density gradient medium was layered over 1 ml of 80% (v/v) density gradient medium (Nidacon International, Sweden) in a 15 ml conical polystyrene centrifuge tube (Falcon, USA). The liquefied semen sample was placed over the upper gradient layer and centrifuged at 300 g for 15 minutes. The supernatant was carefully removed without disturbing the pellet. The sperm pellet was transferred to another tube containing 3 ml of wash medium and centrifuged at 200 g for 10 minutes. The supernatant was discarded and 0.5 ml of wash medium was added to the pellet which was inseminated inside the uterine cavity using a disposable sterile IUI catheter. In AID cases, the prewashed frozen sample was thawed and inseminated.

All the patients received 200 mg of vaginal micronized progesterone twice daily for 15 days as the luteal support in the controlled ovarian stimulation cycles. The urine pregnancy test was performed 20 days post IUI. The patients with positive urine pregnancy test results were advised for trans vaginal ultrasonography after 2 weeks for confirmation of pregnancy. A clinical pregnancy was defined as the presence of gestational sac with or without fetal pole.

Sample size

A previous study reported clinical pregnancy rate of 23.3% and 13.3% with clomiphene and letrozole respectively combined with gonadotropin in IUI cycles.¹⁰ Taking this study into consideration with α -error of 5%, power of 80% and 95% confidence interval, the sample size was calculated to be 468 with 234 in each group. Sample size calculation was done using STATA version 13. As the present study included the IUI cycles within a specific time frame and the total sample size was more than the minimum required sample, the entire available data was considered for analysis. The minimal deficiency in the sample size in the group A was due to less frequent use of clomiphene after letrozole came into clinical use.

Statistical analysis

SPSS, Inc version 20.0 (IBM, USA) was used to check the normal distribution of age, endometrial thickness and total motile fraction (TMF) in the groups by Shapiro-Wilk test. Since the dataset did not follow the normal distribution, the Mann-Whitney U test was applied to compare age, endometrial thickness and TMF between the groups. Chi-square test and Fisher's exact test were applied for categorical data to find out the difference in the number of cycles, presence of ovulation and rate of pregnancy among the groups. P value ≤ 0.05 was considered statistically significant. Data had statistical calculations with SPSS version 20.0.

RESULTS

Comparison of patient characteristics

Four hundred ninety six intrauterine insemination cycles were analyzed including 325 AIH (Artificial insemination with husband semen) cycles and 171 AID (Artificial insemination with donor sperm) cycles. Both the groups were compared for baseline characteristics (Table 1). Mean age of females (29.5 ± 4.7 vs 28.9 ± 3.7 $p=0.16$), cycle distribution according to different indications of IUI ($p=0.11$) were similar in the compared groups. There was no difference in the number of AIH (64.4% vs 66.4%) and AID (35.6% vs 33.6%) cycles ($p=0.64$).

Mean endometrial thickness in mm (7.4 ± 1.7 vs 7.6 ± 1.8 $p=0.41$), and total motile fraction of spermatozoa in million (10.08 ± 2.5 vs 9.9 ± 4.4 $p=0.14$) were also similar. There was significant difference in number of dominant follicles ($p=0.011$). Letrozole-hMG group had more number of monofollicular cycles compared to clomiphene-hMG stimulated group (61.7% vs 54.5%). Conversely cycles with three dominant follicles were more in number in clomiphene-hMG group (13.1% vs 5.5%). Though human chorionic gonadotropin (hCG) was commonly used as ovulation trigger in both the groups (95.9% cycles in group A and 98.9% cycles in group B) compared to leuprolide, the difference in number of cycles assigned to different trigger agents reached statistical significance ($p=0.03$) (Table 2).

IUI cycle outcomes

Comparison of IUI cycle outcomes between the groups was done (Table 2). Pregnancy rate was found to be similar in group A and B (18.5% Vs 15.3% $p=0.35$). Similarly there was no difference in the clinical pregnancy rates for both the drugs (18.5% vs 15.3% $p=0.35$). There were three miscarriages in each group and Group B reported to have one twin pregnancy (Table 2).

DISCUSSION

The study groups were similar for major baseline characteristics which are likely to affect the cycle outcome. The effect of female age on IUI success rate was demonstrated in a retrospective study of frozen donor sperm cycles, where the pregnancy rate was 18.5% in women <35 years and 5.4% in women >40 years ($p<0.05$).¹¹ Similar results are obtained in the present study reflecting age having a deep impact on IUI success, especially due to oocyte quality issues. Pregnancy rate after IUI varies for different indications of IUI. In the study by Cabry-Goubet et al. clinical pregnancy rate was 17.6% for anovulation versus 8.6% in cases of endometriosis, 15% for male factor infertility and 10.7% in cases of unexplained infertility ($p=0.41$).¹² But, in the study by Soria et al., pregnancy rate was highest in patients with the polycystic ovarian syndrome (PCOS) and lowest in severe endometriosis (13.3% vs 6.4%. $p<0.05$).¹³

Association of endometrial thickness and success of IUI cycle has been demonstrated by many authors. Endometrial thickness on the day of hCG administration was significantly higher in the cycles where pregnancy was achieved.¹⁴ But a recent systematic review and meta-analysis showed no significant impact of endometrial thickness on pregnancy rate.¹⁵

TABLE 1. Comparison of patient characteristics.

Parameters	Group A (n=222)	Group B (n=274)	p value
Age (Mean \pm SD)	29.57 \pm 4.7	28.9 \pm 3.7	0.161*
Indication (n,%)			
Oligo anovulation	25 (11.3%)	48(17.5%)	
Unexplained	80(36.0%)	111(40.5%)	
Endometriosis	2(0.9%)	2(0.7%)	0.114‡
Male factor	98(44.1%)	94(34.3%)	
Combined	17(7.7%)	19(6.9%)	
IUI			
AIH	143 (64.4%)	182 (66.4%)	0.640†
AID	79 (35.6%)	92 (33.6%)	
No. of dominant follicles			
1	121 (54.5%)	169(61.7%)	
2	72(32.4%)	90(32.8%)	0.011†
3	29(13.1%)	15(5.5%)	
Trigger			
hCG	213(95.9%)	271(98.9%)	0.033†
Leuprolide	9(4.1%)	3(1.1%)	
Endometrial thickness	7.47 \pm 1.7	7.6 \pm 1.8	0.416*
TMF(AIH only)	10.08 \pm 2.5	9.9 \pm 4.4	0.147*

*, Mann Whitney U test was applied to compare age, endometrial thickness and TMF between group A and group B. †, Chi-square test and ‡, Fisher's exact test were applied for other categorical data. P value was significant at ≤ 0.05 .

Abbreviations: n, number of subjects; SD, Standard deviation; IUI, intrauterine insemination; AIH, artificial insemination with husband's sperm; AID, artificial insemination with donor sperm; CC, clomiphene citrate; hCG, human chorionic gonadotropin; TMF, total motile fraction

TABLE 2. Comparison of IUI cycle outcomes between the groups.

Outcome (n,%)	Group A (n= 222)	Group B (n=274)	P value
Pregnancy positive	41(18.5%)	42(15.3 %)	0.352*
Clinical pregnancy	41(18.5%)	42(15.3 %)	0.352*
Multiple pregnancies	0	1	
Miscarriage	3(7.3%)	3(7.14%)	

*, Chi-square test was applied to compare the parameters between Group A and Group B. P value was significant at ≤ 0.05 .

Abbreviation: n, number of subjects

Total motile fraction (TMF) of sperms in a washed semen sample is also an important parameter for IUI cycle outcome. In a study by Panda B et al. pregnancy rate was significantly lower when TMF was less than 5 million.¹⁶

Pregnancy rate after IUI has been positively correlated with the number of dominant follicles. But, simultaneously the risk of multiple pregnancies increases with the number of dominant follicles.¹⁷ Soria et al. in the previously mentioned study, demonstrated higher pregnancy rate in IUI cycles with 2 or more dominant follicles than single follicle (12.3% vs 8.1%, $P < 0.01$), where the upper limit for the number of follicles was not mentioned.¹³ But in the prospective study by Kamath et al. similar pregnancy rate was documented after IUI in patients with one dominant follicle compared to patients with two and three dominant follicles (8.52% vs 13.33% vs 21.4%, $P = 0.303$).¹⁸ Majority of the cycles in the present study had single dominant follicle probably because of the use of low dose gonadotropin.

The choice of ovulation trigger was determined based on the number of dominant and intermediate follicles. Though many individual studies have compared pregnancy rate in IUI cycles for different types of ovulation triggers, a systematic review and meta-analysis of these studies showed no difference in pregnancy rate for hCG and GnRH agonist trigger.¹⁹

The present study compares efficacy of letrozole and clomiphene combined with gonadotropin in IUI cycles. Both the drugs are found to be equally effective in terms of pregnancy rate and clinical pregnancy rate. The outcome of the current study agrees with the previous studies demonstrating a similar pregnancy rate with clomiphene and letrozole in IUI cycles but differs in some aspects. In the studies by AL fozan et al and Badway et al gonadotropin was not used with clomiphene or letrozole for super ovulation.^{20,21} In the study by Hembram et al¹⁰, though gonadotropin was used along with these two drugs, only unexplained infertility cases were included. Jee et al. in a prospective study also reported similar pregnancy rate after IUI when clomiphene and letrozole were used combined with hMG.²² The present study contradicts the only prospective study by Mitwally et al which demonstrates significantly higher pregnancy rate with letrozole combined with gonadotropin than clomiphene in unexplained infertility.²³ The above mentioned study was primarily designed to compare efficacy of clomiphene with hMG, letrozole with hMG and only hMG, where endometrial thickness was significantly higher in letrozole group than clomiphene group. In the current study, endometrial thickness was similar for

both the drugs. But in patients with persistently thin endometrium, letrozole should be the drug of choice.²⁴ Pregnancy rate was similar with both the drugs despite a significant difference in number of dominant follicles. The maximum acceptable number of dominant follicles was three in the current study and pregnancy rate was reported not to vary with the number of dominant follicles up to three follicles.¹⁸ Though there was difference in the type of ovulation trigger in the groups, it is unlikely to affect the pregnancy rate after IUI.¹⁹ In majority of the cycles in the present study, letrozole or clomiphene was used with low dose hMG (75 IU). Hence it will help to choose an alternative protocol for controlled ovarian stimulation with use of low dose gonadotropin and to achieve low multiple pregnancy rates. In the current study, only one twin pregnancy was documented (1/83, 1.2%).

Retrospective nature of the study warrants caution while interpreting its outcome and applicability in clinical practice. The other limitation is unavailability of data regarding IUI cycles which was cancelled due to suboptimal and hyper response during ovarian stimulation after use of these treatment regimens. As properly randomized trials in this context are scanty in existing literatures, a well-designed randomized trial will help to reach at an appropriate conclusion while taking care of the limitations.

CONCLUSION

Clomiphene citrate and letrozole combined with gonadotropins are equally effective for controlled ovarian stimulation in IUI cycles. Either of the drugs can be used with low dose gonadotropin making ovarian stimulation more flexible depending on the availability of the drugs and physician's preference.

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Ethics: As the present study involves only retrospective analysis of pre existing data, ethical committee approval was not obtained. However, all the couples had shared informed written consent for undertaking the IUI procedure.

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