

# Use of clomiphene-based stimulation protocol in oocyte donors: A comparative study

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#### ABSTRACT

**INTRODUCTION:** This study was undertaken to compare between clomiphene citrate (CC) and gonadotropin-releasing hormone antagonist-based protocols in donor-recipient cycles in terms of parameters of ovarian stimulation and obstetric outcome.

**MATERIALS AND METHODS:** Two hundred and three fertile oocyte donors were stimulated using two different protocols: Clomiphene based ( $n = 103$ ) and antagonist based ( $n = 100$ ). Donors in the one group were stimulated from day 1 or 2 of spontaneous or withdrawal bleeds with CC (50 mg/day) and recombinant follicle-stimulating hormone (FSH) till the day of trigger while donors in the other group were stimulated using recombinant FSH from day 1 or 2, and the antagonist was added as per flexible antagonist protocol. When  $>3$  follicles were  $>17$  mm in diameter, trigger was given with 2 mg leuprolide intramuscular. Transvaginal oocyte retrieval was done after 34 h of trigger.

**RESULTS:** There was no significant difference in between the two groups in terms of age, antral follicle count, starting dose of gonadotropins, total dose required, duration of stimulation, number of follicles retrieved, mature follicles, and fertilization rate. The serum estradiol levels were significantly raised in the clomiphene group ( $P < 0.001$ ). Pregnancy rate was similar in both the groups. The clinical pregnancy rate was 65.94% in the clomiphene group and 57.46% in the antagonist group. The live birth rate per cycle started was 47.8% in the clomiphene group and 39.55% in the antagonist group. There was one case of ectopic pregnancy in the antagonist group. **CONCLUSION:** Controlled ovarian stimulation using clomiphene and gonadotropin is a viable option for donor oocyte cycles. The cost and number of injections used per cycle can be reduced by using the clomiphene-based protocols.

**KEY WORDS:** Antagonist, clomiphene citrate, oocyte donor

#### INTRODUCTION

The first live birth with *in vitro* fertilization (IVF) was after a natural cycle IVF. Later on, gonadotropin-releasing hormone (GnRH) analogs and gonadotropins were added to prevent luteinizing hormone (LH) surge and to increase the number of recruited follicles which, in turn, led to an increase in the number of oocytes retrieved and fertilized embryos. Multiple embryos give the choice of selection and hence, transfer of better quality embryos which improves the success rate; at the same time, the surplus good quality embryos can be vitrified for future transfers. Thus, the primary aim of controlled ovarian hyperstimulation (COH) is multi-follicular development while inhibiting spontaneous ovulation at the same time. Gonadotropins have long been used for increasing the

oocyte yield.<sup>[1]</sup> GnRH analogs (agonist and antagonist) have been used to prevent LH surge.<sup>[2,3]</sup> An ideal IVF protocol should aim for a high chance of embryo transfer with a low cancellation rate, a high pregnancy rate, less side effects, low cost, fewer hospital visits, and minimal intervention. Clomiphene citrate (CC), a novel molecule

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which was approved by the Food and Drug Administration in 1961 for ovarian stimulation, has been used successfully throughout the world. CC has been used in IVF as a part of minimal stimulation protocols which is also known as mild or soft stimulation in patients with poor ovarian response along with gonadotropins and GnRH antagonist.<sup>[2-5]</sup> CC with its antiestrogenic action on the pituitary causes the release of follicle-stimulating hormone (FSH), thus complementing the action of externally administered gonadotropins. It has also been hypothesized that if clomiphene is continued till the day of ovulation trigger, antiestrogenic action on the pituitary in the presence of rising estradiol (E2) prevents LH surge.<sup>[6-8]</sup> Thus, clomiphene-based stimulation protocol in IVF is expected to be cheaper first because of the reduction in the dose requirement of gonadotropins, and second because there will be no need to add GnRH analogs to prevent LH surge. The main concern with such prolonged use of CC is its antiestrogenic effect on the uterus which may adversely affect the implantation. However, if this clomiphene-based protocols are used in oocyte donors, its antiestrogenic effect on uterus will no longer be a matter of concern. This study was undertaken to compare between the protocols using CC and GnRH antagonist in oocyte donors.

## MATERIALS AND METHODS

This retrospective study was conducted at a tertiary care center in the Department of Reproductive Medicine. The case records between January 2013 and December 2014 were studied. During this period, donors were stimulated using two different protocols: antagonist-based and clomiphene-based. The procedure and analysis of records were done in accordance with the Institutional Review Committee.

### Inclusion criteria

Donors who were fertile females with previous at least 1 live birth, aged 21–35 years, undergoing COH for the first time, total antral follicle count in both the ovaries  $\geq 10$ , with no known or family history of heritable medical disorder, infection screen negative (human immunodeficiency virus [HIV], and hepatitis B surface antigen [HBsAg]) were included in the study.

### Exclusion criteria

The exclusion criteria were no previous live birth, age  $< 21$  or  $> 35$  years, history of oocyte donation, poor ovarian reserve (total antral follicle count  $< 10$ ), known case of or family history of heritable diseases, HIV or HBsAg positive.

The oocyte donors were given oral contraceptive pills for a maximum of 14 days if required to synchronize the cycle with the recipient. Stimulation was started from day 1 or 2 of the menses. Baseline evaluation including E2 and

transvaginal sonography (for ovarian morphology and endometrial thickness) was done. Stimulation was started with recombinant FSH (Gonal-F, Merck Serono, Switzerland) if the E2 value was  $< 50$  pg/ml, and endometrial thickness was  $< 5$  mm. The dose of recombinant FSH (150–275 IU) was individualized based on age, antral follicle count, and body mass index. As CC was used for the sole purpose of preventing LH surge, the starting dose of gonadotropins used was similar in both the protocols.

### Clomiphene-based protocol

CC 50 mg once a day orally was started from the 1<sup>st</sup> day of stimulation daily till the day of trigger.

### Antagonist protocol

GnRH antagonist (Ganirelix 0.25 mg S.C., MSD Ltd., India) was added according to the flexible protocol, i.e., when the leading follicle was  $> 13$  mm in mean diameter and continued till the day of trigger.

Follicular study was done on day 5, and thereafter every 2–3 days, dose adjustment was done accordingly. Ovulation trigger was done using 2 mg leuprolide acetate, when at least three follicles were more than 17 mm in diameter. Serum E2 and P4 were done on the day of the trigger. Blood samples were centrifuged within 2 h and stored at  $-20$  C until tested, P4 and E2 levels were assessed by radioimmunoassay.

Transvaginal oocyte retrieval was done 34 h after trigger under local anesthesia and sedation using ovum aspiration needle single lumen (Cook Medical) in all cases. Intracytoplasmic sperm injection was performed in all cases. Number of follicle seen, number of oocytes retrieved, number of metaphase II (MII) oocytes, embryo fertilized, and pregnancy rates were calculated. Fertilization and embryo quality were assessed from day 2 onward. Embryo grading was done according to the consensus scoring system.<sup>[9]</sup> Grade 1:  $< 10\%$  fragmentation, stage-specific cell size, no multinucleation, Grade 2: 10–25% fragmentation, stage-specific cell size, and Grade 3: Severe fragmentation, cell size not specific, evidence of multinucleation. Each recipient was transferred a maximum of three embryos on day 3, and the remaining were cryopreserved for subsequent frozen thaw transfer. Endometrium of the recipients was prepared with E2 valerate and P4 according to the day of transfer. On day 14, post-transfer, pregnancy test was done by urine pregnancy test kit and serum beta human chorionic gonadotropin.

Pregnancy rate was defined as the presence of a positive urine pregnancy test and positive serum beta human chorionic gonadotropin expressed per cycle started. Live birth rate was based on delivery after 20 weeks of gestation

and expressed per IVF cycle started. Pregnancies from both fresh and frozen embryo transfer were included in the analysis.

Primary outcomes measured were number of mature oocytes retrieved, while the secondary outcomes measured were serum E2 and P4 levels on the day of trigger, fertilization rate, number of good quality embryos per cycle, and other outcomes such as CPR and live birth rate (LBR) were calculated in the recipients.

Statistical analysis was done using MedCalc Statistical Software (trial version) (MedCalc Software bvba, Ostend, Belgium). Student's *t*-test was performed to calculate the significant difference in the mean of quantitative data in two groups. Chi-square test was applied to see the difference in the frequency of discrete variables in two groups.  $P < 0.05$  was considered significant.

## RESULTS

Two hundred and twenty-five fertile females were stimulated during this time period, of which 203 met the inclusion criteria and were included in the study. One hundred and three donors were stimulated using CC and gonadotropins while 100 were stimulated with the antagonist protocol. The mean age was similar in both the groups (27.64 and 28.45). There was no significant difference in the starting dose, duration of stimulation, the total dose requirement, and pretrigger P4 values [Table 1].

Donors in the clomiphene-based protocol had a significantly higher level of pretrigger E2 ( $P < 0.0001$ ). None of the donors had failed retrieval of oocytes.

Although the number of retrieved follicles were nonsignificantly higher in the antagonist protocol (17.46) compared to the clomiphene-based cycles (16.93), the number of MII oocytes were similar in both the groups (12.96

and 13.04), respectively. None of the donors required admission for moderate-to-severe OHSS; there were ten cases of mild OHSS in the clomiphene group and nine cases in the antagonist group. The number of fertilized embryos and good quality Grade 1 embryos were similar in both the groups.

In 203 recipients, 63/103 patients conceived during the fresh cycle in clomiphene group (pregnancy rate 61.16%) whereas 57/100 conceived in the antagonist group (pregnancy rate - 57%). A total of 272 transfers were done including the frozen thaw cycle, 91/138 (65.94%) cycles resulted in pregnancy (positive beta human chorionic gonadotropin) in the clomiphene group whereas 77/134 (57.46%) cycles in the antagonist group were positive, the difference was not statistically significant ( $P = 0.15$ ). The LBR per cycle started was 47.8% in the clomiphene group and 39.55% in the antagonist group, respectively ( $P = 0.18$ ). There was one case of ectopic pregnancy in the antagonist group [Table 2].

The serum E2 levels per follicle were below the average when more than 15 follicles were retrieved (antagonist protocol: average - 218.91 pg/ml, >15 follicles - 192.17 pg/ml; clomiphene protocol: average - 327.32 pg/ml, >15 follicles - 309.85 pg/ml) [Table 3].

## DISCUSSION

Newer stimulation protocols have been tried repeatedly to find alternatives to the currently used regimens in IVF. Clomiphene has long been studied for milder ovarian stimulations. Clomiphene is a cheaper, orally active, and easily available drug. Clomiphene by its antiestrogen effect on the pituitary helps ovarian stimulation by releasing FSH; at the same time, it prevents the release of LH and thus prevents premature LH surge which can cause premature ovulation. This property of clomiphene can be used in IVF stimulation protocol. GnRH analogs have been conventionally used to prevent LH surge in ovarian

**Table 1: Parameters associated with stimulation in both groups**

Parameters	Clomiphene/ FSH protocol	Antagonist/FSH protocol	<i>P</i>
Age	27.64±3.25	28.45±4.3	0.135
Starting dose	181.2±52.16	189±55.42	0.303
Total dose required	1913.31±814.1	2007.4±749.09	0.393
Duration of stimulation (days)	9.49±1.37	9.64±1.44	0.436
Pretrigger E2 (pg/ml)	5452.76±3368.14	3622.26±3105.09	<0.0001*
Pretrigger P4 (ng/ml)	1.52±1.27	1.32±1.54	0.47
Retrieved follicles	16.93±7.44	17.46±8.44	0.637
Mature oocytes (MII)	13.04±5.73	12.96±6.08	0.924
Fertilized embryos	11.18±5.16	11.21±5.76	0.973
Grade 1 embryos	8.32±5.09	7.95±4.77	0.594
OHSS	10	9	0.862

FSH=Follicle-stimulating hormone, OHSS=Ovarian hyperstimulation syndrome, MII=Metaphase II, E2=Estradiol, P4=Progesterone, \*Significant

**Table 2: Obstetric outcomes**

Parameters	Clomiphene/ FSH protocol	Antagonist/ FSH protocol	P
Fresh cycle CPR	63/103	57/100	0.645
CPR (fresh+freeze thaw)	91/138	77/134	0.15
LBR	66/138	53/134	0.18

CPR=Clinical pregnancy rate, LBR=Live birth rate, FSH=Follicle stimulating hormone

**Table 3: Average serum E2 levels based on the number of follicles**

Parameters	Clomiphene/ FSH protocol	Antagonist/ FSH protocol
Average E2 level per cycle	327.32	218.91
Average E2 level when >15 follicles retrieved	309.85	192.17

FSH=Follicle-stimulating hormone, E2=Estradiol

stimulation protocol, but these drugs are costly and require proper storage facility. Clomiphene-based protocol has been studied in the past for milder stimulation mainly in poor responders to reduce the amount of gonadotropin used.<sup>[4,6,10]</sup> Extended use of clomiphene till the day of trigger prevents LH surge and thus avoids the use of GnRH analogs, reducing the cost. However, its antiestrogen effect on the genital tract is a matter of concern as its prolonged use might alter the endometrial receptivity, hence this protocol can be used in oocyte donors and when sequential transfer is planned.

This clomiphene-based protocol is a modification of the original protocol used at the Kato Clinic in Japan where minimal ovarian stimulation was done with an aim to recruit 1–4 follicles.<sup>[10]</sup> It was a retrospective study of 43433 cycles, in which the mean number of oocytes retrieved were 2.2, premature LH surge was noted in 5.1%, with a LBR of 11.1%. GnRH agonist was used for ovulation trigger with the benefit of avoiding OHSS.

Clomiphene has a half-life of 24 h or less, and discontinuation of clomiphene as in soft stimulation can cause the initiation of LH surge. This problem was solved by continuing clomiphene till the day of trigger. The use of GnRH agonist for ovulation trigger not only helps to prevent OHSS but also helps in sustaining normal LH dynamics and normal luteal function.

Clomiphene has also been used in natural cycle IVF to prevent premature ovulation.<sup>[11]</sup> Two hundred and eleven natural cycle IVF were studied, of which 103 patients were started on 25 mg clomiphene daily from day 7 till the day of trigger; there was a significant decline in the premature ovulation rate (6.8% vs. 27.8%) with improvement in the transfer rate (54.4% vs. 39.8%).

Thus, the role of clomiphene in preventing premature ovulation has been studied earlier, but most of these studies were conducted in natural or minimal ovarian stimulation cycles. However, whether clomiphene can prevent premature ovulation in COH needs to be studied. At our center, we have used CC from the beginning of stimulation till the day of trigger in oocyte donors and compared it with the other donors, in whom antagonist was used as per the flexible protocol. The age in both the groups was comparable (27.64 and 28.45). There was no significant difference in the starting dose (181.2 vs. 189), total dose of gonadotropins (1913.31 vs. 2007.4), and the duration of stimulation (9.49 vs. 9.64). The average number of follicles retrieved (16.93 vs. 17.46), the number of mature oocytes, fertilized embryos, and good quality Grade 1 embryos were similar in both the groups. The pregnancy rate was also similar in both the groups. Pretrigger E2 ( $P < 0.0001$ ) was significantly higher in the clomiphene-based protocol group; this could be the result of the synergistic action of gonadotropins and clomiphene. The pretrigger P4 values were similar.

Total E2 levels on the day of trigger are dependent on the number of follicles developed. However, E2 levels per follicle were below the average when more than 15 follicles were retrieved (antagonist protocol: average 218.91, >15 follicles 192.17; clomiphene protocol: average 327.32, >15 follicles 309.85), similar to the findings of Teramoto and Kato.<sup>[10]</sup> This probably indicates the decrease in oocyte quality with an increase in the number of oocytes recruited during stimulation, in turn making the case for softer or milder stimulation stronger.

Clomiphene causes endogenous release of FSH which acts synergistically with the exogenous gonadotropins. As the starting dose of gonadotropins was similar in both the protocols, it could be the possible reason behind the slight increase (10 vs. 9) in the incidence of OHSS in the clomiphene group. There were ten cases of OHSS in the clomiphene group while nine cases in the antagonist group were the cases of mild OHSS, and none needed admission were managed symptomatically on an outpatient basis. In the present series, no case of moderate or severe OHSS was noted. This could be attributed to agonist trigger as well as the fact that no transfer was done in these women.

There continue to be concerns regarding the continued use of clomiphene and its effect on endometrium and ovarian tumor pathogenesis.<sup>[12]</sup> In the present study, no embryo transfer was done, as the sample population comprised oocyte donors. Therefore, concerns regarding the effect of prolonged use of clomiphene on endometrium remain unaddressed. Prolonged use of CC may be associated with an increased risk of breast carcinoma.<sup>[13]</sup> As women may donate oocytes multiple times at different centers following

the same clomiphene-based protocols, there is an increased risk of breast carcinoma in these women. Therefore, screening of breast carcinoma is advisable, though it is expensive. Transfers in the recipients of clomiphene-based protocol have shown an implantation rate of 65.94%, which is comparable to that seen in antagonist donor cycle (57.46%). The relatively young age and known fertile potential of the population could be the reason of relatively high pregnancy rates in the series. The LBR was 47.8% in clomiphene group while it was 39.55% in the antagonist group, the difference was not significant statistically.

This study was done to evaluate the feasibility of using the same approach in patients with good ovarian reserve and get oocyte yield comparable to the standard agonist or antagonist protocol. The duplication of similar protocol for all infertile patients will have to be validated by larger studies. Even though this study was not designed to evaluate the cost-effectiveness and convenience of patients, we have found this protocol to be patient-friendly and less expensive as fewer injections were used. The major limitation of this study is the retrospective nature, and further, properly designed prospective study is required to draw a firm conclusion.

## CONCLUSION

Use of clomiphene has been studied in multiple protocols where it is generally administered from day 2 to day 7, and antagonist is added later to reduce the propensity of LH surge. The current study has demonstrated that it is possible to obtain reasonable results using an IVF protocol with a combination of gonadotropin-CC for ovarian stimulation without concurrent use of antagonist or agonist. In this study, the role of clomiphene is not just to act as anti-estrogen and facilitate an increase in endogenous FSH but also to inhibit premature LH surge while maintaining endogenous pituitary function. The concomitant administration of CC did not influence oocyte maturity in terms of MII oocytes. The extended use of clomiphene associated with FSH without GnRH analogs in young donors yielded a reasonable number of oocytes while reducing the total cost and number of injection.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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