ORIGINAL ARTICLE

Outcome of Co-administration of Clomiphene Citrate in Gonadotropins Stimulated *In vitro* Fertilisation Cycles

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ABSTRACT

Introduction: Co-administration of clomiphene citrate during the early phase in in vitro fertilisation (IVF) cycles has been used by clinicians for several years to improve outcomes. **Materials and methods:** This is a single centre retrospective study involving 222 patients with 81 patients underwent IVF using co-administration of Clomiphene Citrate (CC) with gonadotropins (study group - group A) and 141 patients used only gonadotropins (control group - group B) were compared. The outcome data of the IVF cycles were focused on four major aspects of which are the total gonadotropin usage, number of oocytes obtained, fertilisation rate and top-quality embryos produced. **Results:** The Mean ± Standard Deviation (SD) of patients age is (34.72 ± 3.25) and (34.39 ± 3.39) years old from group A and B respectively. Mean ± (SD) of total usage of gonadotropins (1834.57 ± 477.34iu) vs (2645.04 ± 669.66iu), (p< 0.0001), mean number of oocytes ± (SD) retrieved (8.54 ± 5.95) vs (10.32 ± 6.67), (p = 0.048), mean ± (SD) fertilisation rate (39.82 ± 30.46) vs (49.04 ± 27.62), (p=0.027) and mean percentage ± (SD) of the top-quality embryos are (17.96 ± 25.68) vs (28.02 ± 32.19), (p=0.02) from group A and B respectively. **Conclusion:** Co-administration of CC with gonadotropins in IVF reduces the total amount of gonadotropins required in the IVF cycle however it also reduces the number of oocytes retrieved, fertilization rate and top quality embryos.
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Keywords: Clomiphene citrate, Gonadotropins, In vitro fertilisation, Oocyte, Embryo

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INTRODUCTION

The outcome of in vitro fertilisation (IVF) depends on the effectiveness of controlled ovarian hyperstimulation (COH) (1). Many regimes for COH in IVF have been reported, however the current standard of care still utilises stimulation with gonadotropins exclusively. In the recent years, there has been an upsurge in interest in the administration of clomiphene citrate (CC) for IVF treatment during gonadotropin-induced ovarian hyperstimulation(2). The use of a low effective pharmaceutical dosing for ovarian stimulation reduces patient discomfort and the risk of ovarian hyperstimulation syndrome (OHSS), as well as expenses for patients and society(3,4). CC derives from the selective oestrogen receptor modulator group that exhibits estrogenic and antiestrogenic actions (2). The pulse frequency of LH increases two to three days after beginning CC therapy in the follicular phase of the ovarian cycle, indicating that the drug's primary activity is to increase in the hypothalamus' pulsatile release of gonadotropin-releasing hormone (GnRH). A direct oestrogenic impact of CC on the gonadotrophs may increase their receptivity to GnRH. The aforementioned actions lead to an increase in the plasma concentration of gonadotropins and the quantity of follicles recruited (5,6).

Previous research demonstrated that IVF regime with CC and low gonadotropin dosages was equivalent to or not inferior in terms of pregnancy outcomes with usual gonadotropin dosages (7). The purpose of this study was to evaluate the IVF outcomes in patients receiving co-administration of CC with a flexible amount of gonadotropins against patients taking only gonadotropins. The outcomes will primarily focus on the amount of gonadotropin used in IVF cycles, the quantity of oocytes obtained, the rate of fertilization, and the production of top-quality embryos.

MATERIALS AND METHODS

This retrospective study was conducted at the Reproductive Centre in a tertiary teaching hospital, Malaysia. The study was approved by the local research and ethics commission board committee with the ethics authorization reference code project FF-2020-375. The

data on patients undergoing IVF treatment from 1st January 2020 till 31st December 2020 were analysed. The inclusion criteria were patients undergoing single IVF treatment throughout the year of 2020, age ranging from of 18 to 40 years old, co-administration of gonadotrophin and CC or gonadotrophin only in IVF cycle stimulation. The cause of infertility includes anovulation, polycystic ovarian syndrome (PCOS) endometriosis, adenomyosis, premature ovarian insufficiency, tubal factor, male factor and unexplained infertility. The exclusion criteria were male with azoospermia requiring surgical sperm retrieval in the IVF cycles.

Two groups of patients were compared in this retrospective analysis. They were divided into group A (study group), who had co-administration of CC with gonadotropins and group B (control group) who received only gonadotropins in the IVF treatment. In group A controlled ovarian hyperstimulation was started with introduction of CC 100 mg for 5 to 10 days from day 2 of period and followed by gonadotropin administration while in group B patients were started on gonadotropin from day two of their period for approximately ten to twelve days. Follicular tracking was done on alternate days beginning day seven of menstrual cycle and flexible antagonist protocol was used.

Throughout the cycle, gonadotropin dosages were modified based on size of the follicles using 2D ultrasound monitoring. Once the size of the leading follicle had reached 18mm and above then final oocyte maturation was induced and the patients were prepared for transvaginal oocyte retrieval 36 hours later. Double lumen aspirator with the pressure of 140mmHg was used during the oocyte retrieval procedure.

The collected oocytes were then fertilised using intracytoplasmic sperm injection method. Fertilisation rate was calculated by dividing total oocytes that had undergone normal fertilisation with total MII oocytes obtained. Embryo quality was categorised using previously published assessment criteria; a top quality embryo was defined as Grade 1 as blastomeres of equal size with no cytoplasmic fragments for the day three embryos (8,9) while for day five blastocyst grading was according to Gardner et al. with 4AA, 4AB, 5 AA, 5AB, 6AA and 6AB considered as top quality ones (10).

SPSS version 26 was used to carry out the statistical analysis presented as descriptive and inferential statistics. Mean and standard deviation were used to present normally distributed data with Independent T test was used to evaluate significant mean differences between compared data.

RESULTS

A total of 222 out of 403 patients who had undergone IVF treatment fulfilled the inclusion and exclusion criteria. Group A consisted of 81 patients, while Group B consisted of 141 patients. The demographic data including the age, years, types and causes of subfertility of the patients in both groups is shown in Table I while Figure 1 illustrates causes of subfertility. Figure 1 displays large number of male factor infertility cause for both groups: 29 (35.80%) of Group A and 42 (29.80%) of Group B. Endometriosis is the second leading cause of infertility, which is in group A with 14 (17.30%) and group B with 27 (19.10%) cases. These infertility causes were followed by Unexplained causes with 15 (18.50%) for group A and 27 (19.1%) for group B. Next, multiple factors recorded 7 (8.6%) for group A while group B is 8 (5.7%). This occurs as a result of a patient having more than one cause of infertility. Infertility causes with the lowest prevalence are Adenomyosis, group A 0(0%) and group B 1(0.7%) and Premature ovarian insufficiency, group A 1(1.2%) and group B 5(3.5%) respectively.

Table I : Demographic data.

GROUPS	A(STUDY) (n = 81)	B(CONTROL) (n = 141)	P VALUE
Age (Mean ± [a]SD)	34.72±3.25	34.39±3.39	0.48
Years of subfertility (Mean ± [a]SD)	6.23±3.07	6.31±3.13	0.87
Causes, n (%)			
Anovulation	3(3.7)	6(4.3)	-
Endometriosis	14(17.3)	27(19.1)	-
Male	29(35.8)	42(29.8)	-
Unexplained	15(18.5)	27(19.1)	-
PCOS	9(11.1)	14(9.9)	-
Tubal	10(12.3)	19(13.5)	-
Adenomyosis	0	1(0.7)	-
POI	1(1.2)	5(3.5)	-
Multiple factors	7(8.6)	8(5.7)	-
Types of Infertility, n (%)			
Primary	66(81.5)	118(83.7)	-
Secondary	15(18.5)	23(16.3)	-

Notes : [a]SD = Standards Deviation ; Group A = (co-administration of Clomiphene Citrate and gonadotropins - study group); Group B = (gonadotropins - control group)

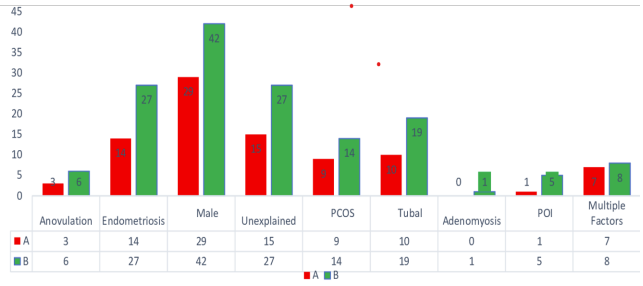

Figure 1: Causes of Infertility between Group A and B. Notes: Group A = (co-administration of Clomiphene Citrate and gonadotropins - study group); Group B = (gonadotropins - control group); PCOS = polycystic ovarian syndrome, POI = premature ovarian insufficiency.

Table II shows the IVF outcomes in co-administration of CC with gonadotropins compared to gonadotrophins only. It elaborates on total usage of gonadotropins,

total number of oocytes retrieved, fertilisation rate and percentage of top quality embryos between Group A (Clomiphene Citrate + Gonadotropin) and Group B (Gonadotropin only). There were significant differences in all groups with mean total usage of gonadotropins between Group A (1834.57±477.34) and Group B (2645.04 ± 699.66) with p< 0.0001. Besides that, the mean number of oocytes in Group A were (8.54±5.95) and Group B was (10.32±6.67) with p=0.048. Adding to that the mean fertilisation rate for Group A was (39.80 ± 30.46) and Group B was (49.0 ± 27.62) with p=0.027. The mean percentage of top quality of embryos in Group A was (17.96 ± 25.68) and Group B was (28.02 ± 32.19) with $p = 0.02$.

Table II: IVF outcomes in co-administration of Clomiphene Citrate with gonadotropins compared to gonadotropins only.

OUTCOMES	A (study group)	B (control group)	p value
Total Usage Of Gonadotropins, [a]IU (Mean±[b]SD)	1834.57 ±477.34	2645.04 ±669.66	< 0.0001
Number Of Oocytes (Mean±[b]SD)	8.54 ±5.95	10.32 ±6.67	0.048
Fertilization Rate (Mean±[b]SD)	39.80 ±30.46	49.0 ±27.62	0.027
Percentage of Top Quality Embryo (Mean±[b]SD)	17.96 ±25.68	28.02 ±32.19	0.020

Notes : [a]IU = International unit; [b]SD = Standards Deviation; Group A = (co-administration of Clomiphene Citrate and gonadotropins - study group); Group B = (gonadotropins - control group)

DISCUSSION

The most used drug for controlled ovarian stimulation in IVF is gonadotropin (11). They carry a risk of OHSS, are costly and invasive(11). There has been interest in using CC with or without gonadotropins to lessen the strain of injections in light of recent calls for patient-friendly IVF. In the present study, we compare the IVF outcomes of co-administration of CC with gonadotropins versus gonadotropins alone.

CC binds competitively to oestrogen receptors which encourages the release of endogenic FSH to support the growth of follicles by inhibiting the negative feedback of oestrogen to the hypothalamus and pituitary (12,13). Our study result was consistent with Gibreel et al., which demonstrated reduction in total amount of gonadotropin used in the co-administration of (CC) with gonadotropins in comparison when only gonadotropins were used in GnRH antagonist regimens(12). Similar findings quoted by Ilhan Sanverdi et al., in a retrospective study involving 77 individuals with the supplementary use of CC in IVF resulted in good efficacy for reducing the gonadotropin level (14). This study supports the previous evidence that co-administration of CC in IVF cycles reduces the gonadotropin usage which can lead to monetary savings.

Each woman's oocytes have a different developmental potential after ovarian stimulation, the overall results of IVF are unpredictable (15). CC causes an increase in the number of follicles by elevating the secretion of endogenous gonadotropins during the time when dominant follicles are drawn from the follicular pool. Exogenous gonadotropins administered on cycle day five to ten would therefore not be anticipated to have an impact on the quantity of recruited follicles (16). In this study, the mean number of oocytes were significantly lower in the co-administration group (p= 0.048). Similar to our study, Siristatidis et al., has reported less oocytes retrieved in the group with co-administration of CC with gonadotropin compared to the control group which only uses gonadotropins using GnRH antagonist protocol (17). Although we did not anticipate co-administration to result in higher oocyte quantity, we were surprised that it resulted in lower oocyte yield. This is possible as Houmard et al., showed that commencement of exogenous gonadotropins administered during the late follicular phase would not be anticipated to affect the number of follicles recruited given the known effects of CC (18).

There are many confounding factors which lead to the fertilisation factor of the oocyte such as the age of the women, quality of the oocytes, consanguinity history, genetic abnormalities and sperm quality (19). The mean fertilisation rate in our study was 39.82 ± 30.46 and 49.0 ± 27.62 in Group A and B respectively with p value of 0.027. Similar to our study Zhang et al., had demonstrated there was significance in terms of fertilisation rate in which the mean value was lesser in the co-administration group compared to the conventional IVF group using only gonadotropins (20). On the contrary, Jovanovic et al., concluded that co-administration of CC with high dose of gonadotropins in comparison with only gonadotropins did not have difference in terms of fertilisation rate (21). This may be due to the differences of the total dosage of gonadotropins used in cycles stimulation done by Jovanovic et al. Therefore, other confounding factors leading to better fertilisation has to be looked into and analysed further for better outcome.

There are many factors involved in creating good quality embryos such as age of the patient, quality of the gametes and the type of protocol used in IVF(22). Elevating endogenous FSH and LH secretion and by promoting ovarian aromatase activity, CC promotes follicular recruitment and maturation (23). Once the maturation of the oocytes is achieved then there would be better possibilities of fertilisation and forming top quality embryos. In our research there was significant difference with less quantity of top quality embryos in the study group with the mean percentage of top quality embryos between the study group (17.96 ± 25.68) and control group (28.02 ± 32.19) with p value of less than 0.02. To our knowledge there is no molecular evidence published regarding the quality of embryos obtained

with co-administration of CC in IVF cycles and this study shows that it may be detrimental in obtaining top quality embryos.

CONCLUSION

The present studies amongst patients attending a tertiary teaching reproductive centre suggest that co-administration of CC can lower the utilization of gonadotropins in IVF cycles. This is important in a setting, where monetary resources are limited. However, this may be at a cost of a lower oocyte quantity, fertilisation rate and top quality embryos.

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