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Investigating Ovulation Induction Outcomes in Patients with Decreased Ovarian Reserve Treated with Double Stimulation during The Follicular and Luteal Phases Compared to The Conventional Antagonist Cycle: A Randomized Clinical Trial

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Abstract

Background: It is difficult to obtain healthy oocytes in poor ovarian responders with conventional treatment methods. Thus, the need to investigate new methods is essential. This study aims to investigate ovulation induction outcomes in patients with decreased ovarian reserve (DOR) in two groups treated with double stimulation (DuoStim) during the follicular and luteal phases in comparison with the antagonist cycle.

Materials and Methods: This was a randomised clinical trial that enrolled the patients with reduced ovarian reserve. The patients referred for *in vitro* fertilization (IVF) at Molud Infertility Clinic, Ali Ebn Abitalib (AS) Hospital, Zahedan, Iran from 2020 to 2021. Participants were randomly divided into two groups, those who underwent treatment with DuoStim during the follicular and luteal phase (case group) and those who received the conventional antagonist cycle (control group).

Results: The mean number of metaphase II (MII) eggs was 7.7 ± 3.1 in the case group and 6.1 ± 3.9 in the control group (P=0.063). The mean total number of retrieved eggs in the case group was 9.2 ± 3.7 and in the control group, it was 6.9 ± 4.4 (P=0.023). The mean number of embryos obtained in the case group was 6.5 ± 3.9; in the control group, it was 4.7 ± 2.8 (P=0.016).

Conclusion: The DuoStim method can effectively play a role in increasing the total number of retrieved eggs and embryos (registration number: IRCT20120817010617N8).

Keywords: Antagonist, Double Stimulation, Follicular, *In Vitro* Fertilization, Luteal

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Introduction

The global prevalence of infertility in women is between 2.5 and 10.5% (1). Unfortunately, decreased ovarian reserve (DOR) occurs in 10-40% of these women, in which the ovary loses its normal reproductive potential, resulting in conception and menstrual cycle disorders (2). Some should undergo assisted reproductive techniques (ART) such as *in vitro* fertilisation (IVF). The poor prognosis group consists of people who are older and have a poor ovarian response (3, 4). In women with DOR, the quantity and quality of eggs produced by the ovaries are reduced, which leads to low-quality embryos. Ovarian stimulation improves the results of ART treatments by increasing the number of oocytes and embryos (5). Although various treatment regimens and many interventions have been performed to improve IVF results (3), there is no single protocol to treat people with poor ovarian response. Treatment of this population is based on the protocols

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of the treatment centre and the doctor's preference. In general, there are three common protocols: long-cycle agonist, short-term gonadotropin hormone-releasing hormone (GnRH) agonist with the flare-up method, and GnRH antagonist. Poor ovarian response to external gonadotropins is one problem of ART in 9-26% of cycles and can cause the cycle to stop, access fewer oocytes and embryos, and eventually reduce the pregnancy rate (6, 7). Factors related to poor ovarian response include advanced age, previous ovarian surgery, pelvic adhesions, and high body mass index (BMI); in some cases, a weak response is also observed in young women (8).

A new method called double stimulation (DuoStim) in one ovarian cycle has been proposed for controlled ovarian stimulation. This protocol is particularly suitable for women who have a poor prognosis and need to maximise ovarian reserve during a limited period of time. The double ovarian cycle method is performed by combining two stimulation methods in the follicular and luteal phases, and can be a valuable option for patients with a reduced ovarian reserve and for older women (9-11). For the first time, Kuang et al. (12) showed that DuoStim of the ovarian cycle in the combined method of follicular and luteal phases led to the development of eggs with appropriate growth ability. DuoStim during the follicular and luteal phases provides a promising alternative or a rescue approach for patients with poor ovarian response. The number of antral follicles (AFC) after first oocyte retrieval was similar to the counts in the early follicular phase, and this offers an exciting potential target for extending ovarian stimulation and additional oocyte retrieval (13). Liu et al. (14), in a retrospective case-control study, aimed to investigate the efficacy of double ovarian stimulation in older women. Their results showed that double ovarian stimulation could increase the chances of achieving pregnancy by accumulating more oocytes/embryos over a short time, and this might serve as a useful strategy for older women. Moreover, Li et al. (15) compared pregnancy outcomes between DuoStim and two consecutive mild stimulations in poor ovarian responders. They observed that the DuoStim protocol was inferior to the two consecutive mild stimulations protocol in terms of the number of frozen embryos, which mainly occurs in older patients. However, there was no difference in pregnancy outcomes between the two protocols. Vaiarelli et al. (16) concluded that during preimplantation-genetic-testing-for-aneuploidies (PGT-A) treatments in advanced-maternal-age and/or poor-ovarian-reserve (AMA/POR) women, DuoStim could be proposed to rescue poor blastocyst yields after conventional-stimulation.Another study by the same author indicated that DuoStim is a promising strategy to manage poor responder patients, especially to avoid discontinuation after a first failed attempt (17).

Poor ovarian response, having diversity in specific treatment protocols and regimens, and the use of regimens other than antagonists are among the current problems of infertility centres in different regions. For this reason, the current research aims to investigate the results of DuoStim during the follicular and luteal phases in comparison with the conventional antagonist cycle in patients with DOR.

Materials and Methods

Ethics statement

This research was approved by the Research Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.REC.1399.447) and the Iranian Registry of Clinical Trials (IRCT20120817010617N8). This study was conducted in accordance with the Declaration of Helsinki and its subsequent revisions.

Study design

This randomised clinical trial study evaluated all women diagnosed with reduced ovarian reserve who underwent IVF treatment at the Infertility Centre of Ali-Ebn-Abitaleb (AS) Hospital from 2020 to 2021. The inclusion criteria of the study comprised: presence of reduced ovarian reserve including anti-müllerian hormone (AMH) ≤1.2 ng/ml, antral follice counts (AFC) ≤6 on the third day of the menstrual cycle, less than five oocytes harvested in the previous cycle, serum FSH concentrations between 10 and 19 IU/L, and the absence of evidence of primary ovarian insufficiency, which included follicle-stimulating hormone (FSH)<20 IU/L. Also, the cases with endometriosis higher than grade 3, a contraindication for the use of gonadotropins, and couples without severe male factor infertility were excluded from the study. The number of metaphase II (MII) oocytes was used to estimate the sample size (12). The effect size (ε) was considered to be 1.3. We employed the superiority formula of the mean for sample size computations. Therefore, 54 patients were included in the study by using the convenience non-probability sampling method, according to the study of Kuang et al. (12) and by taking into consideration the sample size formula (18) and 20% possibility of exclusion of patients during the research. After enrolment, the patients were randomly assigned to either the case or control group (Fig.1).

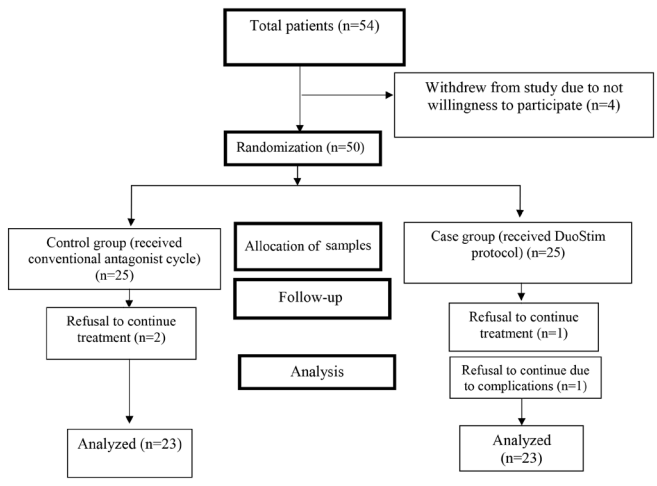

Fig.1: Flowchart for patient selection. DuoStim: Double stimulation

Procedure

The current research was performed in a randomised and single-blind method so that the patients were classified into the case (DuoStim) and control (conventional antagonist) groups using the permuted block stratified randomisation method (19). Initially, the objectives of the study were explained to the patients and written consent was obtained. The eligible patients were classified according to the order of entry, age, and BMI. Subsequently, they were assigned to one of the two groups based on blocks of four (consisting of two A and B groups and two repetitions for each) that were randomly selected from all the possible states of permutations (2). These blocks were created using statistical software R version 4.0.2. Finally, participants were assured that their information would remain confidential, and they were reminded that the research results would be provided to them if they wished.

The primary outcome was the total number of oocytes retrieved and the secondary outcomes were the number of MII oocytes and the number of embryos obtained.

Participants were assured that they could withdraw during any stage of the research if they did not want to continue. Two participants from each group withdrew from the study. In the case group, one patient refused to continue treatment and one was excluded due to complications; in the control group, two patients withdrew their consent to continue treatment.

Both groups of patients underwent transvaginal sonography (TVS) on menstrual cycle days 1-3. All patients daily received 225 units of Gonal-F (FSH, Merck, Serono, Italy) and 150 units of human menopausal gonadotropin (HMG, Karma Pharmatech, Germany). Patients had another TVS five to six days later to assess follicular growth, followed by TVS once every two days. When the cases had a dominant follicle greater than or equal to 14 mm, the GnRH antagonist (0.25 mg, Merck, Serono, Italy) was administered daily and continued until the presence of 2 to 3 follicles that were 18 mm in size, as observed by TVS. Then, in the DuoStim group, the final oocyte trigger was performed by two injections of Decapeptyl (0.1 mg); the control group in addition to Decapeptyl received 10,000 IU of human chorionic gonadotropin (hCG). Oocyte retrieval was done 36 hours after the injection, and all follicles above 12 mm were drained. In the DuoStim group, patients received a GnRH antagonist daily for 4 days from the day after the puncture, and five days after oocyte retrieval, regardless of the number of oocytes observed on ultrasound, similar to the previous cycle, ovarian stimulation was done using the GnRH antagonist protocol, and when at least two follicles reached 17-18 mm diameter, 10 000 IU HCG was administered. After 36 hours, oocyte retrieval was performed under TVS guidance.

The retrieved oocytes in both groups and each stage were incubated for 2-4 hours, after which the cumulus and corona rad iata cells were removed from the oocytes. The oocytes were subsequently evaluated and the MII oocytes were subjected to intracytoplasmic sperm injection. Then, the embryos were cultured in culture medium and placed in an incubator at 37°C with 6% CO_2 and 5% O_2. Three days later, the embryos were evaluated and scored, and finally, five days later, they were re-evaluated for blast formation. Subsequently, all of the embryos were frozen. The obtained data were recorded in information forms and analysed by SPSS software SPSS software (version 22, IBM Corp., Armonk, N.Y., USA).

Statistical analysis

The collected, raw data were entered into SPSS version 22 (IBM Corp., Armonk, N.Y., USA). The per-protocol approach was employed for data analysis. Frequency and percentage indicators were used to describe qualitative data. Common central indices (mean and median) and dispersion indices (standard deviation and interquartile range) were used to describe quantitative data. In order to compare the average variables between the two groups, the t test was used for two independent groups if the assumptions of the parametric tests were met. If the assumptions were not met we used alternative non-parametric tests, such as the Mann-Whitney U test. The relationship between categorical characteristics was assessed by the chi-square test. In all analyses, $P<0.05$ indicated statistical significance.

Results

A total of 57 infertile women with reduced ovarian reserve were selected for participation in the study that 4 patients were withdrawn due to not willingness to participate in the study; therefore 50 patients enrolled in this study and randomly assigned to DuoStim protocol (case group, n=25) or the conventional antagonist cycle (control group, n=25). After withdrawal of two participants from each group (case group: one patient refused to continue treatment and one patient had complications; control group: two patients refused to continue treatment), we assessed the treatment results in 46 patients (Fig.1). The participants had a mean age of 35 ± 4 years and a mean BMI of 26 ± 5 kg/m^2. There was no significant relationship between demographic factors and the duration of infertility in these women (P=0.508, Table 1).

Table 1: Comparison of demographic information and the duration of infertility in the two study groups

Group/Variable		Case (n=27)	Control (n=27)	Total (n=54)	P value*
Age (Y)	≤35	11 (40.7)	12 (42.9)	23 (41.8)	0.546
	>35	16 (59.3)	15 (57.1)	31 (58.2)	
BMI (kg/m^2)	≤25	10 (37)	9 (32.1)	19 (34.5)	0.461
	>25	17 (63)	18 (67.9)	35 (65.5)	
Duration of infertility		8.5 ± 1.3	7.4 ± 2.9	7.5 ± 6.1	0.508

Data are presented as n (%) or mean ± SD. BMI; Body mass index and *; Chi-square and t tests.

According to Table 2, the number of AFC and AMH were investigated in the two groups. No significant difference was found between the groups (P=0.335, P=0.973, respectively, Table 2).

Table 2: Comparison of the mean laboratory indices in the two study groups

Group/Variable	Case (n=27)	Control (n=27)	P value*
AFC	3.2 ± 9.4	4.3 ± 8.9	0.335
AMH	0.85 ± 0.6	1.3 ± 0.86	0.973

Data are presented as mean ± SD. AFC; Antral follicles, AMH; Anti-müllerian hormone, and *; t test and Mann-Whitney U test.

The mean number of MII oocytes in the case group was 7.7 ± 3.1; in the control group, it was 6.1 ± 3.9, which was not statistically significant (P=0.063, Table 3). Also, the mean total number of retrieved eggs in the case group was 9.2 ± 3.7 and in the control group, it was 6.9 ± 4.4 (P=0.023). The mean number of embryos obtained in the case group was 6.5 ± 3.9 and in the control group, it was 4.7 ± 2.8 (P=0.016). Also, the mean number of retrieved eggs and embryos obtained in the case group in the second round was significantly higher than in the first round (P=0.0001, Table 3).

Table 3: The frequency of MII eggs, retrieved eggs, and embryos obtained in the two studied groups

Group/Variable	Case (n=23)	Control (n=23)	P value*
MII oocytes	7.7 ± 3.1	6.1 ± 3.9	0.063
Retrieved oocytes			
Follicular phase	3.0 ± 1.5	6.9 ± 4.4	0.0001
Luteal phase	6.2 ± 3.3	-	-
Total	9.2 ± 3.8	6.9 ± 4.4	0.023
Total embryos			
Follicular phase	2.1 ± 1.4	4.7 ± 2.8	0.0001
Luteal phase	4.4 ± 2.5	-	-
Total	6.5 ± 3.9	4.7 ± 2.8	0.016

Data are presented as mean ± SD. MII; Metaphase II and *; t test and Mann-Whitney U test.

In this study, the number of days for medication administration and the number of doses used in the case group were higher than in the control group (P=0.001, Table 4).

Table 4: Frequency of the doses of medication in the two studied groups

Group/Variable	Case (n=23)	Control (n=23)	P value*
Medication administration (days)	19.4 ± 1.5	10.1 ± 5.9	0.001
HMG	3030.9 ± 14	1672.11 ± 5.1	0.001
Cetrotide	9.3 ± 4.3	6.4 ± 3.8	0.009
GONAL-F	3660.4 ± 25	1972.11 ± 5.8	0.001

Data are presented as mean ± SD. HMG; Human menopausal gonadotropin, GONAL-F; A brand name for a medication called gonadotropin, and *; t test and Mann-Whitney U test.

Discussion

Clinical knowledge and technological progress in recent years have greatly contributed to the success of ART methods, especially IVF. However, one of the most important success factors in this field is the number of oocytes produced by the ovaries following hormonal stimulation (20). Therefore, the main goal of the performed protocols is to stimulate the production of more oocytes and embryos, and to increase the probability of pregnancy (21). But this issue is more important in patients with risk factors that threaten their fertility over time. For example, cancer patients who need treatment with gonadotoxic drugs or surgery to remove their ovaries, or older people who have reduced ovarian reserves. Therefore, the implementation of methods that can induce good results over a shorter time is useful and satisfactory for many patients (22, 23).

Various studies, including a study by Kuang et al. (12), have shown that double ovarian stimulation in the same menstrual cycle provides more opportunities for egg retrieval in poor ovulatory responders. This stimulation can start in the luteal phase, and result in the retrieval of more oocytes in a short period of time. This is a new solution for women with a poor ovarian response who need to preserve their fertility. Therefore, in this study, the DuoStim method was used during the luteal and follicular phases in people with poor responses during IVF.

In the present study, the case and control groups were not statistically different in terms of participants' age, BMI, the number of AFC on the third day, and laboratory variables [AMH, FSH, thyroid stimulating hormone (TSH)]. The findings of the current study indicated that the mean numbers of retrieved oocytes and embryos obtained in the case group was higher than the control group. Also, the total number of oocytes and embryos obtained in the case group patients in the luteal phase was more than in the follicular phase. However, the total number of MII oocytes in the two groups did not show a statistically significant difference. The number of MII oocytes in the case group in the luteal phase showed a significantly better result than in the follicular phase.

DuoStim in one ovarian cycle is a new protocol developed for patients who undergo IVF that can maximise the number of retrieved oocytes in the shortest possible time. Unlike conventional IVF protocols in which patients undergo one round of stimulation with exogenous gonadotropins and egg retrieval in one menstrual cycle, patients who receive the DuoStim protocol undergo two rounds of gonadotropin treatment and two egg retrievals in the same menstrual cycle (1, 24, 25). Zhang et al. (26) showed that ovarian DuoStim in the luteal phase may be a promising protocol for the treatment of women with poor ovarian response, especially for patients who are not able to tolerate enough live embryos through follicular phase ovarian stimulation or other protocols. The results of their study were consistent with our study and it was observed that the percentage of eggs obtained was higher. Similarly, de Almeida Cardoso et al. (27) conducted a study on women who had a history of unsuccessful IVF and underwent DuoStim; they concluded that the number of eggs obtained increased from 6.7 to 11.7 compared to stimulation in the follicular phase. This

finding was consistent with our study. Vaiarelli et al. (28) reported that both stages of stimulation produced eggs of equal quality (based on fertilisation, blastocyst, euploidy rate, and clinical outcomes after euploid single embryo transfer). The second stimulation (luteal phase) considerably helped the patients who had at least one euploid blastocyst (from 42 to 65%). Finally, the DuoStim method was mentioned as the best method for fertility in patients with reduced ovarian reserve. However, de Almeida Cardoso et al. (27) conducted a study on 54 patients who underwent ovarian stimulation cycles, from which 13 patients underwent DuoStim. Although the results showed a higher number of extracted oocytes and mature oocytes in the patients that underwent DuoStim, there was no significant difference in terms of fertility and blastocysts (28). Similarly, Ubaldi et al. (9) reported no significant difference in the number of eggs and blastocytes or euploid blastocytes.

Therefore, according to the results of the conducted studies and the present study, it can be said that a greater number of MII oocytes are developed in this method in comparison with the conventional methods. In general, the data analysis showed that in patients with a weak ovarian response and the general infertile population, unconventional protocols such as DuoStim can be effective. This method can be a quick solution to recover more eggs and embryos in a shorter time, especially in older people who have reduced ovarian reserves or people with cancer. However, further studies with higher accuracy are needed to confirm these findings.

The efficacy of the DuoStim protocol was previously supported by the possibility to increase the oocyte yield and, more importantly, the number of euploid blastocysts. One of the limitations of the present study is that the clinical relevance of the finding in this study is limited by the lack of application of PGT-A. Another limitation is that since the study is a resident thesis and time was limited, so embryological and reproductive outcomes are missing. It is recommended that further studies in this field be performed in the future.

Conclusion

The findings of the current study indicate that the DuoStim method is significantly effective in increasing the total number of retrieved eggs and the number of embryos obtained in a short period of time. Therefore, this method can be useful in patients who, for various reasons such as increasing age or having underlying diseases, require a shorter treatment period and better results.

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Authors' Contributions

F.Gh.; Conceptualisation, Investigation, and Wrote the original draft. A.P.; Methodology, Validation, and Data collection. A.N.; Formal analysis and Software visualisation. M.Gh.; Review and editing, Resources, Supervision, Project administration, and Funding acquisition. A.J.; Data management and Data analysis. All authors read and approved the final manuscript.

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