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Comparison *In Vitro* Fertilization Outcomes between DouStim and Minimal Stimulation Protocols in Poor Ovarian Responders: A Randomized Clinical Trial

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Abstract

Background: Various protocols have been approved to improve the response rate leading to successful fertilization in poor ovarian responders (PORs). The application of double ovarian stimulation (DuoStim) in the follicular and luteal phases of the same ovarian cycle has been shown as an intriguing option to achieve more oocyte retrievals in the shortest time. The aim of the current study is to compare the outcomes of different protocols, minimal stimulation (MS) and Duostim.

Materials and Methods: This randomized clinical trial was performed on 42 *in vitro* fertilization (IVF) candidates with POR diagnosis. Patients were classified into two equal groups and treated with the DuoStim protocol and MS protocol. The IVF outcomes, including retrieved follicles, oocytes, metaphase II (MII) oocytes and embryos, were compared between these groups.

Results: The patients' characteristics including age, anti-mullerian hormone (AMH), follicle stimulating hormone (FSH), luteinizing hormone (LH), and antral follicle count (AFC) were collected and compared. It showed there was no significant difference between the two groups baseline characteristics ($P>0.05$). We observed that the DuoStim protocol resulted in a significantly higher score in comparison with the MS protocols , including the number of follicles (6.23 ± 2.93 vs. 1.77 ± 1.66, $P<0.001$), retrieved oocytes (3.86 ± 2.57 vs. 1.68 ± 1.58, $P=0.002$), MII oocytes (3.36 ± 2.42 vs. 1.27 ± 1.27, $P=0.001$) and obtained embryos (2.04 ± 1.64 vs. 0.77 ± 0.86, $P=0.003$).

Conclusion: The DuoStim protocol is a favourable and time saving plan that is associated with more oocytes in a single stimulation cycle. The DuoStim protocol significantly can result in more frequent MII oocytes and embryos. We figured that the higher number of oocytes and embryos might have led to a higher rate of pregnancy (registration number: IRCT20200804048303N1).

Keywords: Clinical Protocol, *In Vitro* Fertilization, Oocyte Retrieval, Ovarian Follicle, Ovulation Induction

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Introduction

Please add at least two sentences that show why a reader must be attention to your "introduction" such as infertility and *in vitro* fertilization (IVF) and their challenges. A substantial increase in the daily dose of gonadotropins was presented for IVF for the first time in the 1980s. It seems that increasing the dose of gonadotropin leads to an increase in the number of oocytes in both groups of poor and good responders and also more embryos in number (1, 2). Studies have shown higher doses of gonadotropins results in the introduction of agonists and antagonists of gonadotropin-releasing hormone (GnRH) as luteinizing hormone (LH) suppressor markers (3, 4). Although, there is no doubt about the benefits of the conventional method for oocyte maturation triggering which lead to higher maturation response, but this approach has some limitations. First, this method is expensive. Second, it increases the incidence of multiple pregnancies when it is transferred to more than one embryo. Third, the danger of certain threats, such as ovarian hyperstimulation syndrome (OHSS), will increase in cases who human chorionic gonadotropin used to finalize the oocyte maturation (4-7). The usual method of long-term stimulation protocol using GnRH agonists prevents anterior pituitary inhibition, thereby preventing an increase in the LH (4).

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In many countries, long-term stimulation protocols by GnRH agonists are approved as a standard method. The GnRH agonist usually begins in the middle part of the luteal phase before the onset of the cycle, followed by high-dose gonadotropin stimulation, which results in multiple follicle production (8). However, the GnRH agonist protocol has potential side effects such as ovarian cysts and estrogen deprivation symptoms, such as mood changes and headaches (9). In addition, some of the side effects of the usual IVF procedure include the need for several daily injections that cause pain and local skin reactions in patients. These side effects have led to attention to minimally reintroduced protocols for better results and fewer potential complications (10-12). Minimally stimulation involves a mild and controlled final oocyte triggering that produces a maximum of 5 to 6 oocytes (13). The use of the mini-IVF method has caused eliminated the problems associated with conventional IVF.

Kuang et al. (14) developed Shanghai protocol, to retrieve more oocytes over time. Using letrozole or clomiphene citrate with human menopausal gonadotropin (hMG) or only GnRH antagonists to inhibit the ovarian LH elevation and stimulate GnRH agonists, ultimately leads to more embryos production. The protocol called dual ovarian stimulation, produced the maximum number of oocytes in the minimum time (15). In contrast to this study, Ubaldi et al. (16) used recumbent gonadotropins (FSH and LH), and reported an increase in embryo production rate ranging from 41.9 to 69.8%. After five days of oocyte recovery, they initiated stimulation of the luteal phase, similar to the previous stimulation; hence, DuoStim for IVF, was successfully used in patients with a time constraint from 2016.

Since poor responders represent more than a third of women undergoing assisted reproductive technology, it remains a notable challenge. Therefore, we designed the present study to compare outcomes between DouStim and Minimal Stimulation (MS) protocols in poor ovarian responders (PORs).

Materials and Methods

This study was registered in the Iranian Registry of Clinical Trials (IRCT20200804048303N1). The study protocol was approved by the Shahid Beheshti University of Medical Sciences' Local Medical Ethics Committee under the reference number IR.SBMU.RETECH.REC.1398.480.

We investigated the number of follicles > 14 mm and MII oocytes. Secondary outcome was the number of embryos obtained.

Study population

The samples were chosen among volunteers in Shahid Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran. In this study, women with low functional ovarian reserve candidates for IVF with a history of poor ovarian response were included. A total of 42 women, who met our criteria, gave their informed consent and entered in our study.

The inclusion criteria were age ≥35 years, antral follicle count (AFC) level <5, and anti-mullerian hormone (AMH) level <1.2 ng/ml). The exclusion criteria include cycles with the only dominant follicle formation, it means produce one dominant follicle during menstrual cycle, uterus malformation and/or abnormalities , intrauterine adhesions, endometriosis, and history of tuberculosis or pelvic surgery.

In this study, all patients underwent a specific procedure called transvaginal ultrasonography. This procedure was used to measure and count the antral follicles on the second day of the menstrual cycle. Blood samples of the patients were collected in citrated or EDTA-containing tubes for the serum extraction by centrifugation at 3000 g for 20 minutes. The levels of LH and follicle-stimulating hormone (FSH) were measured by the immune radiometric assay (9800496 for LH and 9900196 for FSH, Pishtaz Teb, Iran). An Enzyme-linked Immunosorbent Assay (ELISA) kit (9900696, Pishtaz Teb, Iran) was used to measure the serum AMH concentrations.

Randomization and blinding

The statistician of the study prepared a computer-generated randomization schedule in blocks of four. A third party randomly assigned participants to one of two treatment arms. The clinician, embryologist, and data analyzer were blinded to the allocated treatments.

Interventions

Minimal stimulation protocol

Letrozole (Femati, AtiPharmed Pharmaceuticals, Iran), was given at a dose of 5.0 mg for five days, starting on the second day of the menstruation cycle. On the fourth day of treatment with Letrozole the Menotropins (Menopur, Ferring Pharmaceuticals, Copenhagen, Denmark), began with 150 units per day of gonadotropins (PDPreg, Pooyesh Darou Pharmaceuticals, Iran). Three days after Menopur initiation, the patients were evaluated with the conventional ultrasound sonography, and the Menopur dose was increased if the initial response rate was not satisfying and continued if response rate was good. By achieving the follicles with a size higher than 14 mm, a GnRH antagonist, Cetrotide, 0.25 mg) was administrated to prevent the LH level elevation. Then, 10,000 units of human chorionic gonadotropin (hCG) were prescribed to achieve at least one follicle with a size of 18 mm to stimulate the follicle final maturation. The gonadotropin dose in the MS group is 900 units for each patient during the study.

DuoStim protocol

The applying protocol for the DuoStim group was similar to the MS group, except that the final oocyte triggering was performed by using a GnRH agonist (triptorelin 0.2 mg daily SQ under Decapeptyl™ brand). The ovarian stimulation was repeated using the same protocol five days after

release the first oocyte. Continuing the second stimulation was similar to the first stimulation period with the onset of the GnRH antagonist by reaching a size of 14 mm follicles, re-stimulation with the GnRH agonist from three follicles with a minimum size of 16 mm. The number of retrieved oocytes, the number of metaphase II (MII) oocytes, and the embryo that was obtained from the final oocyte stimulation were then compared between the two groups. In the DouStim group, the gonadotropin dose for each patient was 900 units in the follicular phase and 750 units in the luteal phase during the entire study period.

Sample size

Assuming a reliability coefficient of 0.05 with a power of 90%, and considering a drop-out rate of 10%, the minimum sample size for each group was 21. This was calculated in accordance with previous studies based on our primary outcomes (17, 18).

Statistical analysis

Variables are represented as mean ± standard deviation (SD). Student's t test was applied to compare the groups using the statistical software SPSS version 28 (SPSS Inc., Chicago, IL) for the statistical analysis. A statistically significant level was considered to be less than 0.05 ($P<0.05$).

Results

Totally, 21 patients received the DuoStim protocol (DS group), while 21 patients were under our MS protocol (MS group) (Fig.1).

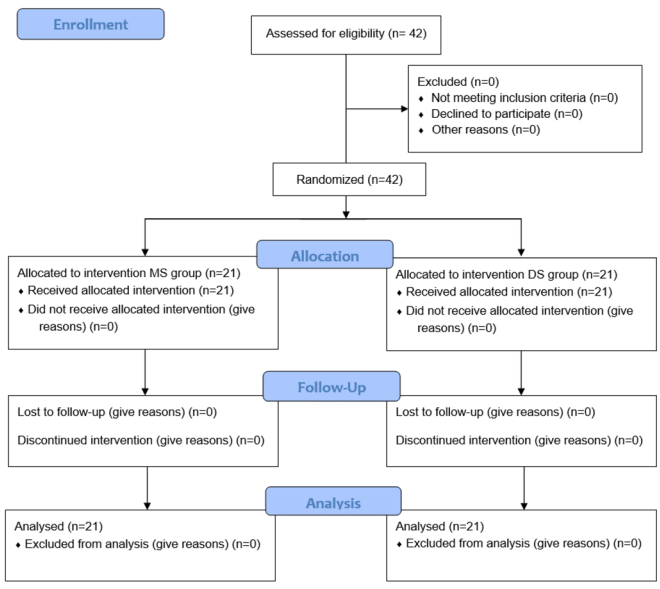

Fig.1: The flow diagram of the study. MS; Minimal stimulation and DuoStim (DS); Double ovarian stimulation.

We did not observe any significant differences between our group members in age and body mass index (BMI). There was also no difference in baseline hormone levels, including AMH, FSH, and LH (Table 1).

Table 1: Demographic data of our participants

Item	DuoStim group (n=21)	MS group (n=21)	P value
Age (Y)	39.19 ± 2.42	38.52 ± 3.03	0.44
BMI (kg/m^2)	26.41 ± 3.58	28.55 ± 4.95	0.12
AMH (ng/mL)	0.74 ± 0.39	0.65 ± 0.35	0.41
FSH (IU/L)	9.26 ± 1.84	10.32 ± 2.10	0.10
LH (IU/L)	7.48 ± 1.27	7.48 ± 2.09	0.51
AFC	5.36 ± 1.67	4.77 ± 1.77	0.26

Data are presented as mean ± SD. DuoStim; Double ovarian stimulation, MS; Minimal stimulation, BMI; Body mass index, AMH; Anti-mullerian hormone, FSH; Follicle-stimulating hormone, LH; Luteinizing hormone, and AFC; Antral follicle count.

All findings of the DuoStim treated group were significantly higher than the MS treated group (Table 2).

Table 2: IVF outcome of our participants

Item	DuoStim group (n=21)		MS group (n=21)	P value
Number of follicles retrieved > 14 mm	6.23 ± 2.93	FP: 3.45 ± 1.71 LP: 2.77 ± 2.02	1.77 ± 1.66	<0.001
Number of oocytes retrieved	3.86 ± 2.57	FP: 1.91 ± 1.57 LP: 1.95 ± 1.84	1.68 ± 1.58	0.002
Number of MII oocytes retrieved	3.36 ± 2.42	FP: 1.63 ± 1.40 LP: 1.72 ± 1.72	1.27 ± 1.27	0.001
Number of embryos obtained	2.04 ± 1.64		0.77 ± 0.86	0.003

Data are presented as mean ± SD. DuoStim; Double ovarian stimulation, MS; Minimal stimulation, MII; Metaphase II, FP; Follicular phase, and LP; Luteal phase.

Discussion

Several protocols have been adopted to improve response rates leading to successful fertilization. This study aimed to compare IVF outcomes including retrieved MII oocytes and consequent embryos between DuoStim and MS protocols in PORs. The chance for a successful pregnancy is related to different baseline parameters, such as the woman's age, the number of aspirated oocytes, and the protocol employed (19). The included patients already had preliminary results with the MS protocol, and the opportunity to dual ovarian stimulation in the same cycle desiring to increase the number of oocytes and embryos was the debate for the new treatment plan.

The present study findings showed that the number of both MII oocytes and aspirated oocytes increased significantly following the DuoStim protocol, which resulted in embryos number. The number of obtained oocytes is one of the factors that impact the positive outcome rate of ART. Our study's critical and highlighted issue is a partially low fertilization rate following both DuoStim and MS protocols. It is not uncommon for an embryo quality and its inadequate response. Recently, it was reported that a total fertilization failure occurs in 5 to 10 % of IVF cycles (20). It usually does not make it past the blastocyst stage or only comes in small amounts to become a euploid embryo status, so cycles and transfers are canceled (21). In a study by Vaiarelli et al. (22), The use of DuoStim

increased the probability of obtaining at least one euploid blastocyst in a single ovarian cycle by 40 to 70 percent. Contrarily, Cecchino et al. (23) showed no difference in aspirated oocytes, MII oocytes and fertilization rate between the DuoStim and standard protocol (24). Cecchino et al. (23) also reported that higher doses of gonadotrophins would never balance the absence of follicles. No pharmacological co-treatments, such as growth hormone, Dehydroepiandrosterone (DHEA), or testosterone administration, have significantly improved the ovarian reserve. In the DuoStim protocol, co-treatment with maximal gonadotropins and GnRH antagonists was mainly considered to discourage ovulation in both follicular and luteal phases and improve the recruitment and development of the follicles (24). Administrating a dose of FSH and LH in an antagonist protocol instead of a minimized stimulation can reduce the likelihood of cycle cancelation and even shorten the time to pregnancy by increasing the number of oocytes per stimulation (25).

In several studies, the DuoStim protocol, which uses dual stimulation during the follicular and luteal phases of the same ovarian cycle, has been shown to be an intriguing method for retrieving two oocytes quickly (26, 27). However, its related advantages and limitations have been questioned, particularly compared to standard protocols, such as MS protocols. As revealed in our trial, the DuoStim protocol is superior to the MS protocol concerning the number of retrieved follicles, oocytes, and obtained embryos, therefore the DuoStim protocol is preferred to obtain a proper response in IVF. Also, more parameters and larger study groups are required for more generalize results.

Various alternative stimulation protocols and ovulation triggers have likewise been assessed and can be utilized to address patients' issues. Physicians should consider the patient's requirements while deciding the best treatment choices. Pregnancy outcomes may determine this study's validity; therefore, lost to follow-up with the patient who underwent embryo transfer is the most important limitations of this work. Furthermore, a future research model associated with male sub-fertility is recommended in order to consider probable differences of cure.

Conclusion

The most significant advantage of the DuoStim protocol is that it collects more oocytes in a single stimulation cycle, thereby reducing time required for its execution. The DuoStim protocol can lead to significantly more frequently MII oocytes and embryos in comparison with Duostim. We figured that the higher number of oocytes and embryos might have led to a higher rate of pregnancy due to two times ovulation induction in one menstrual cycle.

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Authors' Contributions

S.S., T.D., S.Sh.; Study conception and Design. T.D., L.N.; All experimental work, Statistical analysis, and Data interpretation. N.S., S.S.; Supervision, Conception, and Data analysis. T.D. L.N., S.H.; Drafted the manuscript, Data acquisition, and Data analysis. All authors read and approved the final manuscript.

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