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Comparison of ovarian stimulation in follicular and luteal phases in an ovarian cycle (DuoStim) in patients with poor ovarian response

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Abstract

Background Infertility is a global problem that refers to the absence of pregnancy after 12 months of intercourse without using contraception. Assisted reproductive techniques (ART) are a treatment technique for infertile patients. Poor ovarian response to stimulation (POR) is one of the challenges in the field of ART. POR refers to people in whom, despite appropriate ovarian stimulation, the number of oocytes retrieved is less than expected. Several ovarian stimulation protocols are applied in POR patients, but the best protocol is not defined. One of the protocols used in POR patients is the "double stimulation in the same ovarian cycle" (DuoStim) protocol. During the DuoStim protocol, both follicular and luteal phases of an ovarian cycle are stimulated. It allows us to retrieve oocytes twice in one ovarian cycle. In the current study, ovarian stimulation in follicular and luteal phases in an ovarian cycle in patients with the poor ovarian response is compared.

Results Twenty-six infertile patients with poor ovarian response were enrolled. They had a mean \pm standard deviation age of 36.3 ± 3.15 years. The duration of follicular phase stimulation (FPS) was shorter than luteal phase stimulation (LPS) ($p_{\text{value}} = 0.003$). The total dose of gonadotropin was significantly lower in FPS than in LPS ($p_{\text{value}} = 0.013$). Significantly fewer total oocytes were retrieved after FPS than after LPS ($p_{\text{value}} = 0.001$).

Conclusion This study supports the putative benefits of LPS in infertile women with POR. Utilizing the DuoStim strategy in people with poor ovarian response caused more oocytes to be retrieved.

Trial registration: irct.ir identifier: IRCT20221001056068N1, 11/22/2022, <https://www.irct.ir/trial/66187>.

Keywords Infertility, Follicular phase, Luteal phase, Ovarian stimulation, Poor ovarian response

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1 Background

Infertility is a global problem that affects about 80 million couples worldwide [1]. Infertility refers to the absence of pregnancy after 12 months of intercourse without using contraception [2]. Infertility can be primary or secondary, with a prevalence of 2% and 10.5%, respectively [3].

Infertility due to female factors has various causes, including ovarian, anatomical, endocrine, and genetic causes. Assisted reproductive techniques (ART) are a treatment technique for infertile patients [2]. Poor ovarian response to stimulation (POR) is one of the challenges in the field of ART [4].

POR refers to people in whom, despite appropriate ovarian stimulation, the number of oocytes retrieved is less than expected. The prevalence of POR is between 9 and 25% is variable [5]. Accordingly, POR is defined as at least two of the following three characteristics must be present: advanced maternal age (≥ 40 years), a previously characterized POR cycle, or an abnormal ovarian reserve test [5, 6]. The physiology of POR is unknown, and probably oxidative stress and mitochondrial function disorders could be the main reasons [4].

Patient-oriented strategies encompassing individualized oocyte number (POSEIDON) criteria is a new approach to defining POR patients more precisely. It provides the best outlook for diagnosing and treating POR patients. The POSEIDON groups are as follows: The first group includes patients younger than 35 who had a weak response in the previous IVF cycle despite receiving the standard medications. The second group includes patients older than 35 who had a weak response in the previous IVF cycle despite receiving the standard medications. The third group includes patients younger than 35 with abnormal ovarian reserve test (antral follicle count (AFC) less than 5) and anti-Müllerian hormone (AMH) less than 1.2 nanograms/cc. The fourth group includes patients older than 35 with abnormal ovarian reserve test, AFC less than 5, and AMH less than 1.2 ng/cc [3, 4, 7, 8].

Studies show that about 50% of cancellation of cycles in ART occurs in POR people [9, 10]. Also, the number and quality of oocytes retrieved during ovarian stimulation are low. Therefore, a few transferable embryos could be obtained [4]. The pregnancy rate in these people is lower despite IVF [10, 11].

Several ovarian stimulation protocols are applied in POR patients, but the best protocol is not defined [9, 12]. One of the protocols used in POR patients is the "double stimulation in the same ovarian cycle" (DuoStim) protocol. During the DuoStim protocol, both follicular and luteal phases of an ovarian cycle are stimulated [13–15]. It allows us to retrieve oocytes twice in one ovarian cycle. Therefore, more oocytes are obtained in less time [9].

Also, the quality of the embryos obtained in the DuoStim protocol is better than the conventional ovarian stimulation methods [16]. In the current study, ovarian stimulation in follicular and luteal phases in an ovarian cycle (DuoStim) in patients with poor ovarian response is compared.

2 Methods

2.1 Study design

This study was conducted as a clinical trial (irct.ir identifier: IRCT20221001056068N1) on 26 infertile patients with poor ovarian response (POR) referred to the infertility center of Shahid Beheshti hospital, Isfahan, Iran. The study was approved by the research ethics committee of the Alzahra Research Centers (IR.ARI.MUL.REC.1401.183), and written informed consent was obtained from all patients.

2.2 Study participants

All patients with POR were assessed to include in the study, regardless of age. Inclusion criteria were: patients with poor response to ovarian stimulation according to POSEIDON 3 and 4 diagnostic criteria and undergoing the necessary treatment protocols for IVF.

Patients with partners suffering from azoospermia, a history of uterine surgery, severe endometriosis, hydrosalpinx, endocrine disorders, repeated abortions, recurrent implantation failures (≥ 3 times), and a body mass index of more than 30 were excluded.

2.3 Oocyte retrieval and fertilization

In the conventional method, ovarian stimulation is performed in just one ovarian phase (follicular or luteal). In the DuoStim protocol, ovarian stimulation is performed in both ovarian phases. From the third day of menstruation (the third day of the follicular phase), patients underwent ovarian stimulation with gonadotropins at a daily dose of 300 units-150 units of human menopausal gonadotropin (HMG) and 150 units of recombinant follicle-stimulating hormone (FSH). When the follicle diameter was higher than 14 mm, daily gonadotropin-releasing hormone (GnRH) antagonist (0.25 mg cetrorelix) was prescribed. Whenever at least two follicles with a diameter of 17 mm were observed through vaginal ultrasound, a single dose of 0.25 mg of human chorionic gonadotropin (hCG) was used to trigger the oocyte growth. Then transvaginal ultrasound-guided oocyte retrieval is performed. On the same day, a semen sample was taken from the partner. Eligible sperms were retrieved and microinjection was performed by an embryologist. Three days later (the third day of the luteal phase), a vaginal ultrasound was performed to check the ovaries for the presence of follicles, and the same drugs were prescribed.

Then transvaginal ultrasound-guided oocyte retrieval is performed. On the same day, a semen sample was taken from the partner. Eligible sperms were retrieved and microinjection was performed by an embryologist.

2.4 Outcomes

The outcomes included fertilization rate, number of 2 pro-nuclei cells, follicles >14 mm in diameter, total and metaphase II oocytes retrieved, gonadotropin dosage, stimulation duration, and quality of embryos in follicular and luteal phases. The quality of 3-day-old embryos was determined by an embryologist. Embryo quality parameters are the number of blastomeres (0, 1, 2, 3, and so on) and morphological characteristics in terms of the degree of fragmentation as 0, ≤10, 11–20, 21–50, more than 50% or completely fragmentation [17].

2.5 Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences for Windows (SPSS, Version 16.0, SPSS Inc., Chicago, IL, USA). Numerical variables were described with mean and standard deviation, and qualitative variables were described with frequency and percentage. Two-mode qualitative variables in two phases were compared using McNemar’s test. Numerical variables were checked for normality by Shapiro–Wilk test and then compared according to the result of normality in two phases using paired t-test or its non-parametric equivalent, Wilcoxon. The significance was accepted for $P < 0.05$ (Fig. 1).

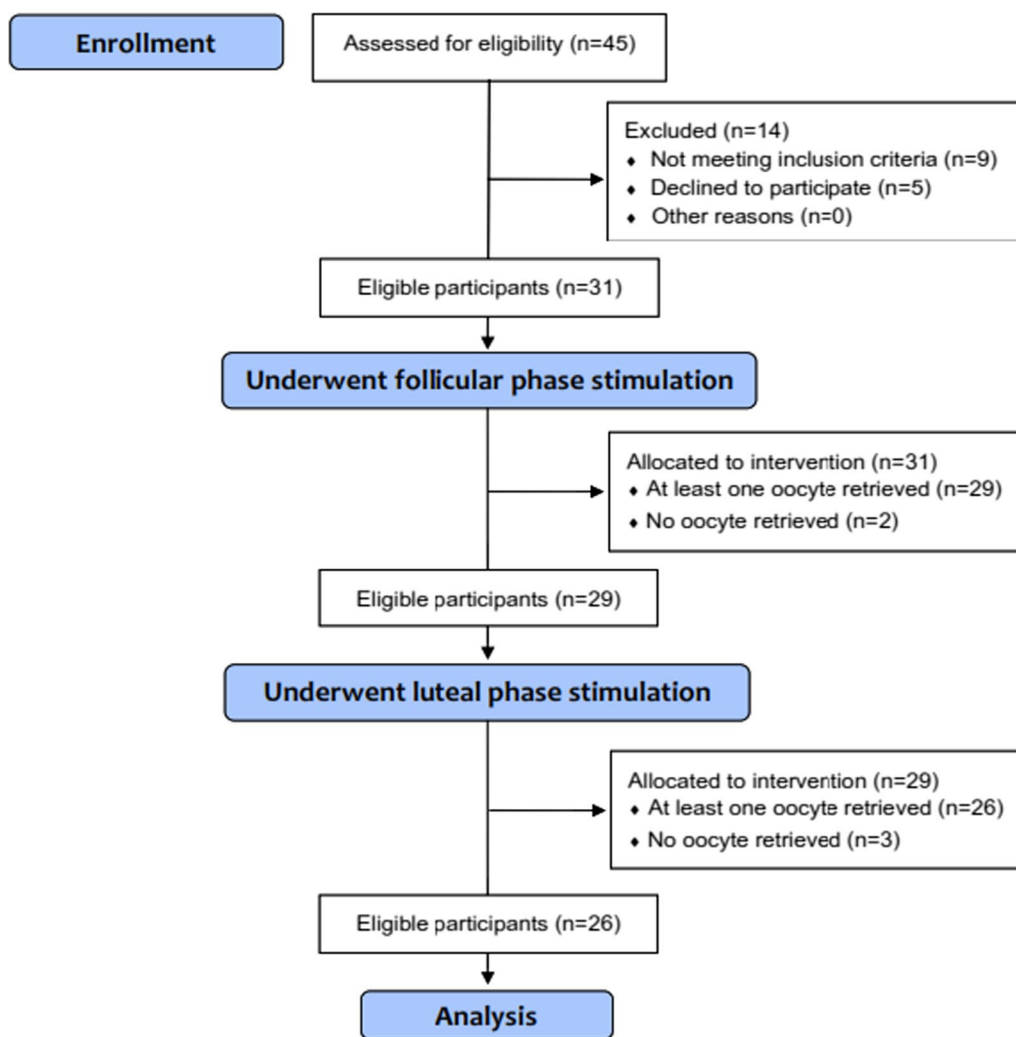


Fig. 1 Flow chart of study

3 Results

In this study, 26 women with POR underwent the DuoStim protocol. They had a mean \pm standard deviation (SD) age of 36.3 ± 3.15 years. The baseline characteristics of patients are presented in Table 1.

Approximately, luteal phase stimulation (LPS) was two days longer than follicular phase stimulation (FPS) (11.5 ± 1.6 days vs. 9.7 ± 1.8 days; $p_{\text{value}} = 0.003$). The total dose of gonadotropin was significantly higher in LPS than FPS (2052 ± 395 IU vs. 1793.1 ± 272.1 IU; $p_{\text{value}} = 0.013$). In FPS, the mean number of follicles

bigger than 14 millimeters in diameter was lower than in LPS (3.5 ± 1.4 vs. 4.5 ± 1 ; $p_{\text{value}} = 0.002$). On average, significantly fewer total oocytes were collected after FPS than after LPS (2.7 ± 1.5 vs. 3.9 ± 1.1 ; $p_{\text{value}} = 0.001$).

There were no statistically significant differences in fertilization rate among both ovarian phases stimulation. Additionally, the number of metaphase II oocytes and top-quality day-3 embryos were similar in both FPS and LPS (Table 2).

Table 1 Basic characteristics of poor ovarian responders undergoing DuoStim protocol

Parameters		Units	Patients (n = 26)
Age (mean \pm SD)		Years	36.3 ± 3.15
BMI (mean \pm SD)		Kg/m ²	24.9 ± 1.4
AMH (mean \pm SD)		ng/ml	0.73 ± 0.34
Basal FSH (mean \pm SD)		IU/L	7.12 ± 1.46
Antral follicle counts (n, percentage)	2 follicles		6 (23.1%)
	3 follicles		6 (23.1%)
	4 follicles		8 (30.8%)
	5 follicles		5 (19.2%)
	6 follicles		1 (3.8%)
Previous IVF attempts (n, percentage)	None		20 (76.9%)
	One attempt		5 (19.2%)
	2 attempts		1 (3.8%)
Infertility causes (n, percentage)	Male factor		2 (7.7%)
	Tubal factor		10 (38.5%)
	Unknown factor		14 (53.8%)

BMI, body mass index; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; IVF, in vitro fertilization; SD, standard deviation; IU, international unit.

Table 2 Cycle characteristics of poor ovarian responders undergoing DuoStim protocol

Parameters		FPS (n = 26)	LPS (n = 26)	P_{value}
Fertilization rate (%)		78.1 ± 30	72.9 ± 29.4	0.540
2 pro-nuclei cell (n)		2.1 ± 1.3	2.3 ± 1.1	0.605
Stimulation duration (days)		9.7 ± 1.8	11.7 ± 1.6	0.003
Gonadotropin dosage (IU)		1793.1 ± 272.1	2052.9 ± 395	0.013
Follicles > 14 mm in diameter (n)		3.5 ± 1.4	4.5 ± 1.0	0.002
No. of total oocytes retrieved (n)		2.7 ± 1.5	3.9 ± 1.1	0.001
No. of metaphase II oocytes (n)		2.4 ± 1.3	2.6 ± 1.3	0.523
Metaphase II to total oocytes ratio		0.87 ± 0.29	0.67 ± 0.23	0.012
No. of Day 3 embryos (n)	Null	8 (30.8%)	5 (19.2%)	0.881
	1 embryo	4 (15.4%)	6 (23.1%)	
	2 embryos	8 (30.8%)	10 (38.5%)	
	3 embryos	4 (15.4%)	3 (11.5%)	
	4 embryos	1 (3.8%)	2 (7.7%)	
	5 embryos	1 (3.8%)	0 (0.0%)	
No. of top-quality Day 3 embryos (n)		0.6 ± 0.7	0.7 ± 0.9	0.249

The significance was accepted for $p_{\text{value}} < 0.05$

Data are presented as the mean \pm standard deviation or percentage

4 Discussion

For those at risk of losing or rapidly declining fertility, time is a critical factor. Women with malignancy who require gonadotoxic treatment or removal of gonads have a brief time frame to preserve suitable oocytes. The time frame is as limited in patients with poor ovarian response. Nevertheless, more stimulation cycles are required to obtain the appropriate number of oocytes and subsequently top-quality embryos.

In our study, ovarian stimulation was performed by DuoStim protocol in one ovarian cycle in people with poor ovarian response. The duration of ovarian stimulation was shorter and the dose of gonadotropins was lower in the follicular phase than in the luteal phase ($p_{\text{value}} < 0.05$). Also, the total amount of oocytes retrieved and the number of follicles with a diameter greater than 14 mm in the luteal phase were significantly higher than in the follicular phase ($p_{\text{value}} < 0.05$). Significantly, the number of mature oocytes to the total number of oocytes ratio is higher in the follicular phase than in the luteal phase ($p_{\text{value}} < 0.05$). However, fertilization rate, number and quality of the retrieved embryos, and the number of mature oocytes were similar in both ovarian phases.

In a retrospective case–control study, Liu et al. [18] found that the total dosage of gonadotropin and duration of ovarian stimulation was more in FPS than in LPS. However, the differences were not significant. Regarding the duration of ovarian stimulation and the total dose of gonadotropins, previous studies [9, 19, 20] were consistent with our survey. Induction of the similar dosage of GnRH agonist may make the FSH surge and luteinizing hormone (LH) surge much higher in the first trigger than in the second trigger. Notably, the ovarian sensitivity to the HMG stimulation is reduced during the luteal-phase ovarian stimulation. It could be inquired in further studies.

Due to previous stimulation in the follicular phase, patients had higher estrogen and progesterone levels in the luteal phase. Therefore, a significant difference was expected in the total number of retrieved oocytes and the ratio of metaphase 2 oocytes to total oocytes. In studies with stimulation of merely one ovarian phase, due to the absence of cumulative effect of estrogen and progesterone, the number of oocytes in two ovarian phases did not differ significantly. Also, participants in follicular and luteal phase groups were not adjusted for age and ovarian reserve [9, 12]. The number and quality of the embryos and the overall fertility rate of previous studies had similar findings [9, 12, 19, 20].

5 Limitations

Since 26 people participated in this research, it seems that more reliable results would have been obtained if more people participated. In case of follow-up of patients and examination of the number of live fetuses and the rate of abortion, more comprehensive results could be obtained about the effects of Dostim during pregnancy. Considering that all participants were evaluated in the embryology center by a unique embryologist, the data is more reliable than the data of multi-center studies.

6 Conclusion

Utilizing the DuoStim strategy in people with poor ovarian response in this study caused more oocytes to be retrieved in a shorter time frame and patients who needed ovarian stimulation were not placed in repeated ovarian cycles. This study supports the putative benefits of LPS in infertile women with POR. In addition, using GnRH antagonists prevented the effects of premature LH surge and the follicles grew more optimally.

Abbreviations

POR	Poor ovarian response
IVF	In vitro fertilization
DuoStim	Double stimulation in the same ovarian cycle
FPS	Follicular phase stimulation
LPS	Luteal phase stimulation
POSEIDON	Patient-oriented strategies encompassing individualized oocyte number
AFC	Antral follicle count
AMH	Anti-Müllerian hormone
hMG	Human menopausal gonadotropin
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
hCG	Human chorionic gonadotropin
SPSS	The Statistical Package for the Social Sciences for Windows
SD	Standard deviation
BMI	Body mass index
IU	International unit

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission. E.N. conceived of the presented idea. E.N. and R.A.H. designed the study. N.H. and S.S. performed the measurements and gathered the data. V.S. and E.N. were involved in planning and supervised the work. N.B. processed the experimental data, performed the analysis. V.S. designed the tables. All authors discussed the results and commented on the manuscript. S. Salehi took the lead in writing the manuscript with support from E.N. and N.H. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are not publicly available due to hospital ethics policies but are available from the corresponding author on reasonable request.

Declarations**Ethics approval and consent to participate**

The local Institutional Review Board deemed the study exempt from review. The study was approved by the research ethics committee of the Alzahra Research Centers (IR.ARI.MUI.REC.1401.183). Written informed consent was obtained from all individuals participating in this study.

Consent for publication

Not applicable.

Competing interests

Authors state no competing interest.

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