The Journal of Obstetrics and Gynecology of India (January–February 2023) 73(1):57–61
https://doi.org/10.1007/s13224-022-01703-1

ORIGINAL ARTICLE

Oocyte Quality and Blastocyst Formation Rate with Dual Stimulation in Patients Belonging to POSEIDON Groups 3 and 4: A Retrospective Comparative Study

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Received: 17 March 2022 / Accepted: 22 August 2022 / Published online: 21 October 2022

Abstract

Aim To evaluate the oocyte retrieval rate and blastocyst formation rate with DuoStim protocol in patients belonging to POSEIDON groups 3 and 4.

Methods This observational, retrospective, single-center study including 90 patients belonging to POSEIDON groups 3 and 4 was conducted at a tertiary care hospital from October 2017 to March 2020. Patients were allocated into two groups based on POSEIDON classification criteria: group A (POSEIDON group 3) and group B (POSEIDON group 4). DuoStim protocol was performed with human menopausal gonadotropin (hMG) at 225 IU and 300 IU in groups A and B, respectively. Study groups were again subdivided by considering the phase in which stimulation had been done [follicular phase stimulation (FPS) and luteal phase stimulation (LPS)], and then, inference was made accordingly in terms of oocytes retrieval rate and blastocysts formation rate. Data were compiled and analyzed using statistical software SPSS version 20.

Results The baseline characteristics of two groups were compatible with POSEIDON groups 3 and 4. A significant difference was found between study groups with respect to age and anti-mullerian hormone levels ($p < 0.05$). Significantly, a greater number of oocytes and blastocysts were obtained in LPS stage, substantially more in group A (3.69 ± 3.4 vs. 4.52 ± 4.3 and 1.36 ± 0.65 vs. 3.17 ± 1.84) than group B (2.2 ± 1.36 vs. 3.6 ± 4.5 and 0.41 ± 0.8 vs. 1.29 ± 2.04). A greater blastulation rate (50 vs. 66.7% and 33.3 vs. 50%) and 100% oocyte maturity rate were observed in LPS stage of both the study groups.

Conclusion In patients belonging to POSEIDON groups 3 and 4, the number of oocytes retrieved and blastocyst formation rate were greater in LPS stage when compared to FPS with DuoStim protocol.

Keywords Luteal phase · Oocyte retrieval · Follicular phase · Cryopreservation · Reproductive technology

Introduction

Infertility is the third most serious health concern of the twenty-first century, and about 60 to 80 million couples suffer from infertility globally due to delayed marriages and delayed age of pregnancy since people are occupied with career and other commitments. Aging is directly proportional to ovary's inability to produce high-quality eggs [1]. In addition, patients belonging to POSEIDON groups 3 and 4 are a challenging cohort in the field of assisted reproduction technology (ART). The POSEIDON strategy has improved the comparability and homogeneity of clinical trials and can potentially help physicians to provide better therapeutic strategy for these low-prognosis patients. A good quality and higher number of oocytes have not been obtained in this cohort [2]. With the evolution of follicular wave theory, controlled ovarian stimulation (COS) protocols

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have been updated with the protocols of luteal phase stimulation (LPS) and, in recent times, the DuoStim protocol (follicular phase stimulation [FPS] and LPS during the same menstrual cycle). Employment of DuoStim strategies in patients belonging to POSEIDON groups 3 and 4 is recent developments [3].

However, limited literature is available on DuoStim protocol in patients belonging to POSEIDON groups 3 and 4 that might be due to its limited application at clinical settings, as it is time-consuming and expensive [2, 4]. Though DuoStim protocol does not show significant changes in all study parameters, majority of the studies comparing DuoStim and COS protocols have reported better reproductive outcomes with DuoStim. Here are some previous studies that compared the oocytes obtained after the FPS and the LPS stage in DuoStim protocol reported that stimulation during the LPS stage tends to have a higher number and good quality of oocytes [5–8]. However, results were weak or controversial. In addition, it is essential to discuss about the good quality oocyte retrieval rate from DuoStim protocol at different doses of gonadotropin. All these considerations motivated in designing the current research that aimed to evaluate the oocyte quality and blastocyst formation rate with DuoStim protocol in patients belonging to POSEIDON groups 3 and 4.

Methods

This hospital-based, retrospective, comparative study was conducted at a tertiary care hospital from October 2017 to March 2020, after obtaining ethical clearance from the ethical board (ECR/162/2017-10 P2017). A written informed consent form was obtained from all the study participants to use their data in scientific publications. This research was conducted in accordance with the principles of the Declaration of Helsinki.

Data were extracted from the hospital's repository database. A vigorous analysis was done to retrieve missing information and review the quality of the data. Accuracy and integrity were double-checked to assure appropriateness.

The study included 90 patients belonging to POSEIDON groups 3 and 4 with normal uterus, no cervical factor infertility, no pathology in fallopian tubes, no ultrasonography (USG) features of endometriosis and no history of previous ovarian surgery; hormonal parameters were measured prior to FPS in order to categorize the study participants into groups [9]. All these patients underwent intracytoplasmic sperm injection (ICSI) to rule out sperm abnormalities. Patients with uterine abnormalities, deranged sperm parameters, pathology in fallopian tubes, such as hydrosalpinx and cervical factor infertility, adenomyosis, endometriosis, pelvic adhesions and history of ovarian surgery were excluded. In addition, patients who require testicular sperm aspiration (TESA) and percutaneous epididymal sperm aspiration (PESA) were also excluded from the study.

Patients were allocated into two groups based on POSEIDON group 3 and 4 criteria: group A [POSEIDON group 3: age < 35 years, anti-mullerian hormone (AMH) < 1.2 ng/ml and antral follicular count (AFC) ≤ 5] and group B (POSEIDON group 4: age ≥ 35 years, AMH < 1.2 ng/ml and AFC ≤ 5). In the study, human menopausal gonadotropin (hMG) had been administered in all the patients to rule out FSH polymorphism at the dose of 225 IU and 300 IU in groups A and B, respectively. (Same dose of gonadotropin with fixed days was used in both phases: FPS and LPS.) All the study participants had received a pre-treatment with 75 mg dehydroepiandrosterone and testosterone gel 1.6% daily for 3 months to enhance the quality of oocytes.

DuoStim protocol was followed in all the study participants as shown in Fig. 1. In both follicular and luteal phases, ovum pickup (OPU) was done only when follicles reached 18–20 mm in diameter. A vaginal USG was done after five days of first OPU to make a note of follicular size. In patients with follicular size of minimum 10 mm, LPS was done with hMG followed by cetrorelix. There were no dropouts after FPS as all the study participants had been informed and counseled about need of dual stimulation as a part of study procedure and consent was obtained for the same. In FPS, smaller follicles were not aspirated in order to achieve positive response in LPS. Study groups (A and B) were again subdivided by considering the phase in which stimulation had been done (FPS and LPS), and then, inference was made in terms of oocyte maturity (MII phase oocyte) and blastocyst formation rate.

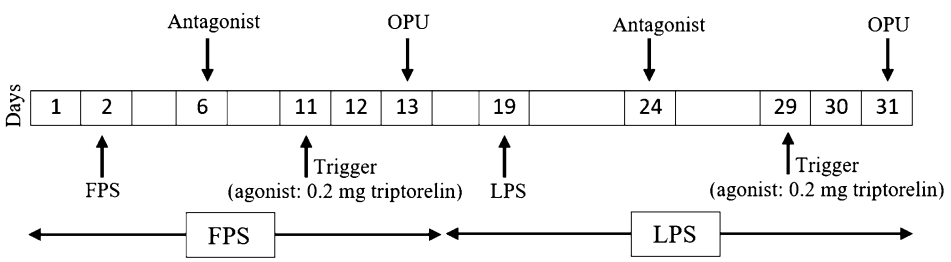

Fig. 1 Ovarian stimulation with DuoStim protocol. *FPS* follicular phase stimulation, *LPS* luteal phase stimulation, *OPU* ovum pickup

Statistical Analysis

Data were compiled and analyzed using statistical software SPSS version 20 (IBM Corp, Armonk, USA). Kolmogorov–Smirnov test was used to check the normality of the data. Categorical variables were represented by frequency tables and analyzed using Chi-square test. Continuous variables were represented in the form of mean ± standard deviation and analyzed using Student's *t* test. $p < 0.05$ was considered statistically significant.

Results

The baseline characteristics including women's age, BMI, AMH levels and AFC are presented in Table 1. A significant difference was found between study groups with respect to age and AMH levels ($p < 0.05$). Table 2 summarizes the findings related to cycle parameters and IVF laboratory outcomes. A greater number of oocytes and blastocysts were found in LPS when compared to FPS in both the study groups, substantially more in group A (Table 2). A 100% oocyte maturity rate was observed in LPS stage of both study groups (Table 2).

Discussion

The current study evaluated the oocyte retrieval rate and blastulation rate with DuoStim strategy in patients belonging to POSEIDON groups 3 and 4. Findings from the current study have shown better reproductive outcome with DuoStim strategy. The POSEIDON group emphasized the importance of obtaining at least one euploid embryo after COS as novel primary outcome in IVF [2]. Despite higher dosage of gonadotropins administered and the prolonged ovarian stimulation, the number of oocytes retrieved and blastocysts obtained was substantially greater in LPS stage.

In the current study, the number of oocytes retrieved and blastulation rate during the LPS stage are significantly higher than those during the FPS stage with DuoStim protocol. All these findings are in concordance with previous studies [4, 7, 10, 11]. Embryos obtained during LPS stage favor better reproductive outcomes because of higher estrogen and progesterone levels at LPS stage and tend to have more synchronous follicular development [12, 13]. In group B patients, additional oocyte and MII oocytes after LPS were obtained compared to LPS in group A patients. These findings are in concordance with previous studies that have highlighted the superiority of the LPS in these patient categories, irrespective of the stimulation regimen adopted [4]. In a retrospective cohort study conducted by Tian et al., there was a direct proportion of high AMH levels with an increased availability of good embryos in patients belonging to POSEIDON groups 3 and 4. The current study observations support Tian et al.'s conclusion [2]. Moreover, the LPS has demonstrated to be more reproductive comparable to FPS [4, 14, 15].

The current study findings suggests that the LPS increases the probability of obtaining at least one blastocyst formation during the DuoStim protocol as the mean number of blastocysts derived from the LPS was significantly higher compared to the FPS. However, there is a dearth in the literature on superiority of DuoStim approach over an isolated LPS after conventional stimulation in the same ovarian cycle [4]. Standing evidence is suggesting that the DuoStim protocol connecting FPS to LPS could be a promising option in managing patients belonging to POSEIDON groups 3 and 4 and patients found to be POR [16].

According to our knowledge, this study is the first to compare follicular and luteal ovarian stimulation of the same ovarian cycle in POSEIDON groups 3 and 4

Table 1 Baseline characteristics of patients

Variables	Group A ($n=55$)	Group B ($n=35$)	*P*
Age (years)	32.3 ± 1.42	37.4 ± 1.62	0.0001
BMI (kgs)	23.8 ± 1.94	24 ± 2.92	0.717
AFC (n)	4.3 ± 1.62	4.1 ± 0.81	0.864
AMH (ng/ml)	0.9 ± 0.36	0.7 ± 0.23	0.002

AFC Antral follicle count, *AMH* anti-mullerian hormone

Table 2 Cycle parameters and IVF laboratory outcomes

Parameters	Group A		*P* value	Group B		*P* value
	FPS	LPS		FPS	LPS	
Number of oocytes	3.69 ± 3.4	4.52 ± 4.3	0.000	2.2 ± 1.36	3.6 ± 4.5	0.000
Number of mature oocytes (MII)	1.8 ± 1.6	4.4 ± 3.8	0.000	1.3 ± 0.81	2.6 ± 2.8	0.000
Oocyte maturity rate	75%	100%	0.000	66.7%	100%	0.000
Blastulation rate (per fertilized oocyte)	50%	66.7%	0.027	33.3%	50%	0.001
Blastocyst number (per cycle)	1.36 ± 0.65	3.17 ± 1.84	0.000	0.41 ± 0.8	1.29 ± 2.04	0.000

AFC antral follicle count, *AMH* anti-mullerian hormone

Table 3 Number of retrieved oocytes and MII oocytes obtained with DuoStim protocol from the established literature

Studies	Number of oocytes retrieved		Number of MII oocytes	
	FPS	LPS	FPS	LPS
Zhang et al. (2017) [17]	2.2 ± 1.6	3.3 ± 2.6	2.03 ± 1.53	3.16 ± 2.55
Zhang et al. (2018) [18]	1.3 ± 0.9	1.8 ± 1.1	–	–
Madani et al. (2019) [19]	1.5 ± 1.1	1.5 ± 2	1.4 ± 1.0	1.2 ± 1.6
Rashtian et al. (2017) [8]	1.6 ± 0.2	1.9 ± 0.2	1.6 ± 0.2	1.6 ± 0.2

patients. A recent systematic review with a meta-analytical approach reported that a higher number of retrieved oocytes (MD = − 0.52, 95% CI = − 1.10, 0.05) and MII oocytes (MD = − 0.63, 95% CI = 1.18, − 0.09) were observed in the LPS stage with DuoStim protocol in the mixed population subgroup [16]. Table 3 represents the number of retrieved oocytes and MII oocytes obtained with DuoStim protocol from the established literature. With this study, the approach of DuoStim protocol suggesting the phenomenon that women exhibit multiple (two or three) waves of folliculogenesis during the same ovarian cycle demonstrates a beneficial effect in either patients belonging to POSEIDON groups 3 and 4 or patients with advanced maternal age.

However, a recent literature reported that a DuoStim protocol has many weaknesses based on SWOT analysis; a greater number of stimulations canceled in the luteal phase, no cost-effectiveness analysis or randomized clinical trials has been conducted till date evaluating the usage of DuoStim protocol, a freeze-all approach is mandatory, and it has been performed only in patients with poor prognosis [20].

Limitations and Recommendations

However, this research has its own limitations in being a single-center retrospective study with limited data. There were no data found with respect to hormonal parameters measured prior to FPS and LPS stage. By taking all these considerations into account, long term randomized controlled trials with a large sample size are needed to confirm the current study findings and to develop expert consensus as well.

Conclusion

In patients belonging to POSEIDON groups 3 and 4, the number of oocytes retrieved and blastocyst formation rate were significantly greater in LPS stage when compared to FPS with DuoStim protocol.

Acknowledgements None.

Funding This research received no specific grant from any funding agency in the public, commercial or any profit sectors.

Declarations

Conflict of interest There are no conflicts of interest to declare.

Ethical Clearance An ethical clearance was obtained from Ethical and Research Committee (ECR/162/2017-10 P2017).

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