

Sequential Ovarian Stimulation for POSEIDON Group 3-4 Poor Responders

POSEIDON 3-4 Düşük Over Rezervi Olgularda Ardışık Kontrollü Ovarian Stimülasyon (KOS)

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ABSTRACT

Objective: The most important issue in both the follicular and luteal phases of the same ovarian cycle (DuoStim) is determining the most appropriate stimulation protocol for the 1st OPU (follicular phase) and 2nd OPU (the luteal phase). We analyzed the POSEIDON 3-4 groups undergoing sequential stimulation treatment to investigate the effects on oocyte number, oocyte maturation, fertilization, and blastocyst rate. **Material and Method:** POSEIDON 3-4 groups were scheduled for a sequential stimulation protocol in a private fertility center. Ovarian stimulation was started with a fixed dose of 300 IU/day of a combination of gonadotropins (150 IU recFSH+75 IU rec LH along with an additional 75 IU rec FSH). Medroxy progesterone acetate 10mg/day using. Final oocyte maturation was induced with 0.2 mg triptorelin. Two days after the first retrieval, the second stimulation was initiated with the same protocol and daily dose. Ovulation was induced with a bolus of GnRH-a with rec hCG (250mcg). One or two cleavage or blastocyst stage embryos were calculated. **Results:** 49 POSEIDON 3-4 patients were included in the final analysis, representing 98 cycles. The mean age was 34.4 ±6 years, and the serum AMH level was 0.85 ±0.2 ng/ml. The duration of stimulation (8.8±3.1 vs 8.2±3.6 days) and the total dose of gonadotropin used (2829±840 vs 1846±421 IU) were comparable. The mean number of oocytes retrieved (1.9 vs 2.1) and M2 oocytes (1.4 vs 1.7) were comparable. Mean oocyte maturation rates per cycle were also comparable (75.6% vs. 77.5%, respectively, p=0.8). Mean fertilization rates per cycle did not differ for oocytes collected (84.7% vs. 68.6%, p=0.06). The mean blastocyst rate per cycle was 63% and 69% respectively (p=0.8). At least 1 embryo was retrieved after the 1st and 2nd cycle or both in 79.6% of patients after DuoStim. **Conclusion:** DuoStim can be an effective method to reduce the time to achieve embryo and blastocyst, especially in POSEIDON 3-4. Also, with the sequential stimulation protocol, we can obtain the same number of oocytes and blastocyst rates in both cycles, and this methodology can save patients time. Further large-scale randomized controlled trials are recommended, especially for the POSEIDON 3-4 groups.

Keywords: Sequential ovarian stimulation; POSEIDON group 3-4; poor responders

ÖZET

Amaç: DuoStim (double ovarian stimulation) en önemli kısım kontrollü ovarian stimülasyon için overlerden oosit toplanması (OPU) (foliküler faz) ve 2. OPU (luteal faz) için en uygun stimülasyon protokolünün belirlenmesidir. Çalışmamızda ardışık stimülasyon tedavisi etkisi, toplanan oosit sayısı, oosit matürasyonu, fertilizasyon ve blastosist oranlarını POSEIDON 3-4 gruplarında analiz edilmesi planlandı. **Gereç ve Yöntem:** POSEIDON 3-4 grup hastalara özel bir merkezde ardışık KOS protokolü uygulanması planlandı. Ovarian stimülasyonu, gonadotropin kombinasyonunu 300 IU/gün fiks doz (150 IU recFSH+75 IU rec LH ile ek 75 IU rec FSH ile birlikte) ile başlandı. Medroksi progesteron asetat 10mg/gün kullanılarak antagonist etki sağlandı. Son oosit matürasyonu 0.2 mg Triptorelin ile indüklendi. İlk oosit toplanmasından iki gün sonra aynı protokol ve doz ile ikinci stimülasyon başlandı. Ovulasyon, rec hCG (250 mcg) ile bolus GnRH-a ile indüklendi. Klivaj ve blastosist embriyo sayıları hesaplandı. **Bulgular:** 98 siklusu oluşturan 49 POSEIDON 3-4 hastası çalışmaya dahil edildi. Ortalama yaş 34.4 ±6 yıl ve serum AMH düzeyi 0.85 ±0.2 ng/ml idi. 1. ve 2. siklus oosit stimülasyon süresi (8,8±3,1'e karşı 8,2±3,6 gün) ve kullanılan toplam gonadotropin dozu (2829±840'a karşı 1846±421 IU) benzerdi. Alınan ortalama oosit sayısı (1.9'a karşı 2.1) ve M2 oositlerin (1.4'e karşı 1.7) benzerdi. Ortalama oosit matürasyon oranları da benzer idi. (Sırasıyla %77.5; %75.6, p=0.8). Oositler için siklus başına ortalama fertilizasyon oranları farklılık göstermedi (%84.7'ye karşı %68.6, p=0.06). Siklus başına ortalama blastosist oranı sırasıyla %63 ve %69 idi (p=0.8). DuoStim sonrası hastaların %79.6'sında 1. ve 2. siklustan sonra veya her ikisinden sonra en az 1 embriyo elde edildi. **Sonuç:** Özellikle oosit elde etme olasılığı az olan hastalarda embriyo ve blastosist elde etme süresini kısaltmak için ardışık KOS etkili bir yöntem olabilir. Ayrıca ardışık stimülasyon protokolü ile hem 1. hem de 2. siklusta aynı sayıda oosit ve blastosist oranları elde edilebilmekte ve bu yöntemle hastalara zaman kazandırabilmektedir. Özellikle POSEIDON 3-4 grupları için daha büyük ölçekli randomize kontrollü çalışmaların yapılması önerilir.

Anahtar Kelimeler: Ardışık ovarian stimülasyon; POSEIDON grup 3-4; düşük over rezervi

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Patients in POSEIDON groups 3-4 have a poor ovarian reserve (POR), and a low chance of live birth, disregarding age.¹ Overall, POSEIDON groups 1-4 account for approximately 47% of patients undergoing assisted reproductive technology therapy (ART).¹ Because of the increasing delay in childbearing nowadays POSEIDON group 4 creates over 50% of the total POSEIDON population.² Both POSEIDON group 3 and 4 patients need certain concentrations of the ideal pretreatment strategy, ovarian stimulation, and triggering plan to improve the possibility of obtaining at least one euploid blastocyst for one transfer.²

Treating women with POSEIDON 3-4 with assisted reproductive technology (ART), it is difficult to obtain a sufficient number of oocytes yield.³ Specifically, in this group, the number and the quality of oocytes are critical and the spending of time and cost on embryonic blastocyst strains the success of ART.³ Although studies suggest that there are multiple waves of follicular growth, the efficacy of controlled ovulation stimulation (COS) in both the follicular and luteal phases of the same ovarian cycle (DuoStim) has not yet been demonstrated in women. The most important issues in DuoStim are determining the most appropriate stimulation protocol for the 1st OPU (follicular phase) and 2nd OPU (the luteal phase) for COS, an applicable drug to use for the antagonist and LH trigger, the ideal number of days for an interval between 1st OPU and 2nd OPU, and determining fertilization and cumulative live-birth rate (CLBR).

In this study, we aim to evaluate the feasibility of sequential stimulation drug protocol (1st and 2nd stimulation in terms of oocyte number, oocyte maturation, fertilization, blastocyst rate, and available transferable embryos for POSEIDON group 3 and 4.

MATERIAL AND METHODS

This retrospective study was conducted between 2020-2022 in a private fertility center. An electronic database was used to detect eligible cases. Ethics committee approval was not obtained because our study was a retrospective data analysis. The study was conducted in accordance with the principles of the Declaration of Helsinki. Inclusion was based on the following criteria:

patients 18-42 years with poor ovarian reserve pre-stimulation parameters, namely, AFC <5 and/or AMH <1.2 ng/ml defined as POS 3-4 group and being scheduled to undergo a sequential stimulation protocol during the study period. The sequential protocol was offered as an alternative protocol in this IVF center mainly based on physicians' discretion. Data were collected from those who consented to the sequential protocol. The exclusion criteria were women who had not completed the 1st stimulation or started the 2nd sequential stimulation, and patients already included in another ovarian stimulation protocol, severe male factor, severe endometriosis, hydrosalpinx, and a history of any endocrine disorders.

SEQUENTIAL OVARIAN STIMULATION

After the scan and basal assessment of the ovaries, ovarian stimulation was started with a fixed dose of 300 IU/day combination of gonadotrophins (150 IU recFSH+75 IU reach along with an additional 75 IU of rec FSH). Follicular growth was monitored on day 6 and then every 2-3 days. Medroxy progesterone acetate 5 mg tablets PO (Tarlusal, Deva ilaç, Turkey) twice daily (10mg/day) was started when the leading follicle was 12 mm. Final oocyte maturation was triggered with 0.2 mg triptorelin sc bolus (Gonapeptyl, Ferring, Switzerland) when at least one follicle reached 17-18 mm in diameter. Oocyte retrieval was performed after 36 h, using a standard procedure. All follicles larger than 11-12 mm were punctured. Two days after the first retrieval, the second stimulation was initiated with the same protocol and daily dose, regardless of the number of antral follicles visible with the ultrasound scan (Figure 1). Ovulation was triggered using bolus GnRH-a (0,2 mg triptorelin) with rec hCG (250 mcg). The second oocyte retrieval was performed after 36 h. The procedures for denudation, intracytoplasmic sperm injection, blastocyst culture, vitrification, warming, and frozen transfer procedures have been previously described.⁴

RESULTS

A total of 478 patients were defined as POSEIDON group 3-4 during this period. All 49 patients fulfilled the criteria and were included in the final analysis, accounting for 98 cycles. Basic demographics were

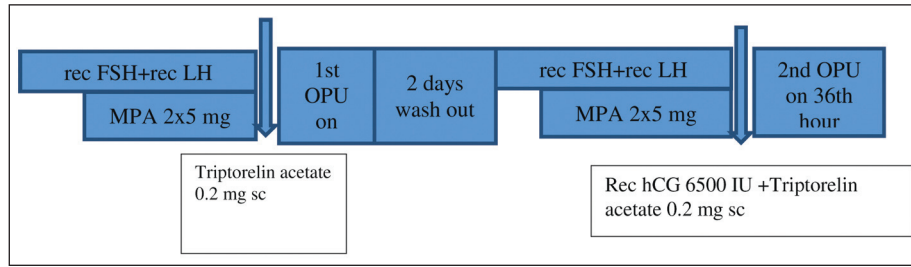


FIGURE 1: Schematic view of the sequential protocol.

TABLE 1: Demographic outcomes of the patients.

Age (years)	34.4 ±6.6
AMH (ng/ml)	0.85 ±0.2
AFC (N)	3.1 ±0.7
Previous failed IVF cycle(s)	2.7
Mean starting gonadotropin dose (IU/day)	326

given in Table 1. The mean women's age was 34,4±6 years and the mean serum AMH level was 0.85±0.2 ng/ml. The duration of stimulation (8.8±3.1 vs. 8.2±3.6 days) and the total dose of gonadotrophin used (2829±840 vs. 1846±421 IU) in the 1st and 2nd stimulation were comparable ($p>0.05$).

Mean collected no of oocytes (1.9 vs 2.1) and M2 oocytes (1.4 vs 1.7) were comparable in 1st and 2nd stimulation groups respectively. Mean oocyte maturation rates per cycle are also comparable among groups as 75.6% vs 77.5% in the 1st and 2nd stimulation groups respectively ($p=0.8$). Mean fertilization rates per cycle did not differ with the oocytes obtained in the 1st and 2nd stimulation cycles (84.7% vs. 68.6%, respectively, $p = 0.06$). The mean blastocyst rate per cycle is 63% and 69% respectively in the 1st and 2nd stimulation cycles ($p=0.8$) (Table 2).

A total of 20 patients revealed at least 1 transferrable embryo following 1st stimulation cycle but no embryos after 2nd stimulation cycle. Likewise, only 9 patients revealed at least 1 transferrable embryo only after 2nd stimulation cycle. A total of 10 patients revealed embryos in both cycles and 10 patients failed to reveal any transferrable embryos in both cycles (Table 3).

DISCUSSION

In the present study, we did not observe significant differences in terms of gonadotrophin consumption, duration, oocyte, and blastocyst yield between 1st and 2nd cycles. Moreover, one-half of all patients revealed transferrable embryos following 2nd stimulation. Therefore, sequential stimulation offers reasonable oocytes and embryos in POSEIDON 3-4 patients in a short time frame.

From a clinically practical point of view, the incorporation of age, oocyte yield, and ovarian reserve into the POSEIDON classification allow for the distinction of two main categories, namely the "expected" (groups 3 and 4) and the "unexpected" PORs (groups 1 and 2). In the POSEIDON population,

TABLE 2: Ovarian stimulation and embryologic outcomes following sequential stimulation.

	1 st stimulation	2 nd stimulation	p value
Duration (days)	8.8	8.2	0.3
Gonadotropin dose (IU)	2829 ± 1183	2820±1440	0.9
Collected no of oocytes	1.9	1.7	0.7
M2 oocytes	1.4	1.4	0.8
Mean maturation per cycle (%)	75.6	77.5	0.6
Mean fertilization per cycle (%)	84.7	68.6	0.06
Mean blastocyst formation per cycle (%)	63	69	0.7

TABLE 3: Overall frozen embryo data.

Available transferrable embryo (N)	
After 1 st cycle	20
After 2 nd cycle	9
In both cycles	10
Neither cycles	10

around 55% fall into group 4 and 10% into group 3.⁵ However, with the increasing age at first maternity wish, the percentage of patients among POR who fall into group 4 can be up to 76% overall studies in the POSEIDON 3-4 groups, the rate of LBR per cycle was 5-7%, and the main factor affecting LBR was underlined the number of oocytes.^{6,7} In this context, double stimulation in the same ovarian cycle (DuoStim) has been proposed.^{8,9} It combines follicular phase stimulation (FPS) with luteal phase stimulation (LPS) and can be considered a valuable option in patients with DOR.¹⁰ This strategy led to reports of an ongoing pregnancy rate per DuoStim cycle that reach 20.7% in POSEIDON group 4 patients.¹⁰ Duo stimulations include stage one (follicular phase) and stage two (luteal phase) stimulations, defined according to the Shangai protocol to raise the number of oocytes retrieved and avoid time loss, the chance of retaining transferable embryos which is crucial for patients with POR and were applied consecutively in the same one menstrual cycle.^{8,9,11-13} Also, Ubaldi's study showed that more oocytes were retrieved during DuoStim and that the number of viable embryos cryopreserved (2.57 ± 1.99) was significantly higher than in those who received only one stimulation (1.17 ± 1.34).⁸ In our study, we find the mean collected no of oocytes (1.9 vs 2.1) and M2 oocytes (1.4 vs 1.7) and blastocyst rate per cycle is 63% and 69% respectively ($p=0.8$) were similar in 1st and 2nd stimulation groups.

In fact, the patient drop-out rate between repeated cycles is a crucial issue for any ART treatment, which for POR becomes critical. In particular, the failure of the first attempt by itself accounts for 25% of the reported causes of patient drop-out and it is second only to the financial burden of ART (62.5%).^{14,15} Accumulating more and more embryos

obviously necessitates more collected mature oocytes and for this purpose, more stimulation cycles can be needed. At this point, treatment duration should also be reasonable to reduce patient drop-out rates. In our study, a mean of 8 days was required to complete the 2nd stimulation cycle and this duration seems reasonable. Likewise, the time frame between the 1st and 2nd oocyte retrieval was nearly 10 times lesser in the sequential stimulation group compared to 2 conventional stimulations ($p<0.05$), in the study of Vaiarelli et al. with comparable pregnancy rates.¹⁰

There have been many different combinations suggested for both stimulations, down-regulation, and wash-out periods for sequential protocols. Besides gonadotropins, we chose progestin primed ovarian stimulation protocol (PPOS) over standard antagonists basically due to reducing the costs. Since short-antagonist protocol and PPOS reveal comparable LBRs, sequential stimulation can be carried out using the PPOS strategy to reduce the overall costs.¹⁶ As frozen embryo transfer is inevitable in sequential regimens, PPOS may not have been attributed as a limitation for fresh embryo transfer in POR patients.

Another important issue with DuoStim is the level of appropriate gonadotropin dose (GN) dose and the differences in use between the 1st and 2nd cycle. In contrast to previous studies that showed that the average GN dose increased significantly with the second stimulation compared to the first stimulation.^{9,17,18} In our study, there was no significant difference in GN dose between 1st and 2nd stimulation (2829 ± 840 vs. 1846 ± 421 IU); similar to the studies by Liu et al. (1882 ± 958 IU vs. 1728 ± 937 IU). The reason could be attributed to the wash-out period in our protocol as a relatively shorter period was chosen over longer periods such as 4-5 days as in the study of Ubaldi et al. Nevertheless, more comparative studies are needed to conclude the proper wash-out period.⁸

Our analysis have some major limitations; retrospective design, small sample size, and lack of pregnancy and lives birth data. However, there have been few data published in the literature using different wash-out periods and stimulation protocols. Further large-scale RCTs are needed, especially for the POSEIDON 3-4 groups.

CONCLUSION

In the POSEIDON 3-4 group, at least 1 embryo was retrieved after the 1st and 2nd cycle or after both cycles in 79.6% of patients after DuoStim. Psychological factors prevent factors that play an important role in patients who drop out of IVF treatment. The oocyte stimulation protocols at DuoStim appear to be an alternative to conventional follicular phase stimulation and reduce the risk of cancellation. It may be an effective method to shorten the time to achieve embryo and blastocyst, especially in a patient profile with a low probability of obtaining oocytes. Further large-scale randomized controlled trials are recommended, especially for the POSEIDON 3-4 groups.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that pro-

vides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Emre Göksan Pabuçcu, Recai Pabuçcu; **Design:** Emre Göksan Pabuçcu, Ece Garipoğlu Dalgin, Recai Pabuçcu; **Control/Supervision:** Emre Göksan Pabuçcu; **Data Collection and/or Processing:** Emre Göksan Pabuçcu, Ece Garipoğlu Dalgin, Recai Pabuçcu; **Analysis and/or Interpretation:** Emre Göksan Pabuçcu; **Literature Review:** Nilüfer Akgün, Emre Göksan Pabuçcu; **Writing the Article:** Emre Göksan Pabuçcu, Nilüfer Akgün; **Critical Review:** Emre Göksan Pabuçcu, Nilüfer Akgün; **References and Fundings:** Emre Göksan Pabuçcu, Recai Pabuçcu.

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