Original Article

Luteal Phase Stimulation in the Same Cycle Is an Effective Strategy to Rescue POSEIDON Poor Responders with No Embryos after the First Follicular Stimulation

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Abstract

Background: Poor responders may benefit from recruiting a 'second wave' of antral follicles within the same cycle. This concept forms the basis of double stimulation which has been named as 'DuoStim'. This protocol involves ovarian stimulation in both follicular and luteal phases with egg retrieval in each phase, respectively, to increase the number of oocytes and embryos in one menstrual cycle. This can be considered a potentially valuable option for women with poor ovarian reserve/response to maximise the number of oocytes retrieved in a single ovarian cycle in the shortest possible time. **Aims:** The aim of this study was to evaluate the efficacy of the DuoStim protocol in women classified as POSEIDON poor responders undergoing *in vitro* fertilization by comparing the embryological outcomes between the follicular and luteal phase stimulations in the same menstrual cycle. **Settings and Design:** This was a retrospective cohort study of 131 patients who enrolled to undergo DuoStim cycles from January 2021 to Sept. 2022, at a IVF center in a tertiary care hospital. **Materials and Methods:** The follicular phase stimulation used a standard antagonist protocol for the first oocyte retrieval. Thereafter, the luteal phase stimulation was started 3 days after the first retrieval, with the same dose of gonadotropin along with a daily 10 mg medroxyprogesterone acetate tablet, followed by a second oocyte retrieval. Blastocysts produced in both the phases were subsequently vitrified. **Statistical Analysis Used:** The paired *t*-test was used for comparing means and 95% confidence intervals (CIs) for different parameters. McNemar's test was used to compare paired proportions. The analysis was conducted using R statistical environment 4.2. **Results:** The mean number of oocytes retrieved and the mean number of utilizable blastocysts frozen per stimulation cycle were found to be significantly higher in the luteal phase as compared to the follicular phase (5.71 ± 3.95 vs. 4.87 ± 2.79, $P = 0.02$, and 1.43 ± 1.22 vs. 0.95 ± 1, $P = 0.001$, respectively). However, the mean fertilization rate and the mean blastocyst utilization rate were found to be similar between both the phases. The length of stimulation was found to be approximately 3 days longer in the luteal phase (12.63 ± 2.43 vs. 9.75 ± 1.85, $P = 0.001$). Overall, the odds of obtaining a usable blastocyst in the luteal phase was found to be significantly higher than in the paired follicular phase (73.9% vs. 57.7%, $P = 0.012$, odds ratio: 2.286

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[95% CI: 1.186–4.636]). Also importantly, the luteal phase stimulation was able to rescue 68% (32/47) of patients where no blastocysts were formed in the follicular phase. **Conclusion:** Our data demonstrate that in women with poor reserve, the addition of luteal stimulation could increase the chances of achieving a pregnancy by significantly increasing the number of eggs and transferable embryos per menstrual cycle compared to follicular stimulation alone. Furthermore, luteal phase stimulation in the same cycle proved to be an effective strategy to rescue POSEIDON poor responders with no embryos after the first stimulation.

Keywords: *Dual stimulation, DuoStim, luteal phase stimulation, poor responder, POSEIDON*

Introduction

Traditionally, an *in vitro* fertilization (IVF) cycle requires a woman to undergo a single round of controlled ovarian stimulation (COS) followed by egg retrieval in one menstrual cycle. It has now been proven that the number of oocytes retrieved after COS greatly influences clinical outcome in terms of cumulative live birth per started IVF cycle.[1] In fact, oocyte numbers have become one of the most important independent variables prognosticating IVF outcomes, with no detrimental effect of increasing oocyte numbers on oocyte or embryo quality.[2] Rather, it has been observed that age-adjusted cumulative live birth rate (LBR) significantly increases with the number of oocytes retrieved.[3] Therefore, COS in assisted reproductive technology is the first step which directly influences the likelihood of achieving success.

Despite the recent advances in techniques of COS, the estimated prevalence of poor responders, according to the POSEIDON criteria, is around 40%.[4] The success rates for IVF in this group of poor responders remain dismally low, and the search for an ideal protocol for COS continues. Due to poor prognosis for these patients, treatment dropout rates are also relatively high. Pooling of oocytes and embryos has been shown to reduce dropout rates and improve LBR.[5] On the other hand, increasing gonadotropin dose has been unable to compensate for the absence of antral follicles in the ovary and does not improve the IVF outcomes for patients with poor reserve.[6] Many protocols have been adopted in the past to treat such patients, yet the search for an ideal stimulation strategy is ongoing. Often, these women end up accepting donor oocytes to realise their dream of parenthood.

There is enough evidence now to suggest that multiple waves of folliculogenesis occur during one menstrual cycle challenging the age-old theory of single antral follicular recruitment.[7,8] In fact, three theories of antral follicle recruitment were postulated by Baerwald *et al*. where the third theory describes two or three waves of follicular recruitment in each menstrual cycle.[8] Therefore, unlike traditional IVF protocols, where patients undergo a single round of COS followed by egg retrieval in one cycle, poor responders may benefit from recruiting a 'second wave' of antral follicles within the same cycle.[9-11] This concept forms the basis of double stimulation which has been named as 'DuoStim'. This protocol involves ovarian stimulation in both follicular and luteal phases with egg retrieval in each phase, respectively, to increase the number of oocytes and embryos in one menstrual cycle. This can be considered a potentially valuable option for women with poor ovarian reserve/response to maximise the number of oocytes retrieved in a single ovarian cycle in the shortest possible time. Most studies so far have suggested that luteal phase stimulation may yield more oocytes leading to more embryos formed as compared to follicular phase stimulation.[11-15]

In the DuoStim protocol, embryos obtained in the follicular and luteal phases are required to be frozen before they can be transferred later in another cycle. The rapid development of cryopreservation technology and the implementation of 'freeze-all' policies across the world to improve cumulative LBR have allowed the DuoStim protocol to effectively pool embryos and achieve more favourable results. The high survival rates associated with vitrification allow the implementation of 'freeze-all' policy for embryos obtained via DuoStim.

The present study was undertaken to evaluate the efficacy of the DuoStim protocol in different categories of poor responders based on the Poseidon classification wherein embryological outcomes including the mean number of oocytes retrieved and utilizable blastocysts obtained were compared in the paired follicular phase and luteal phase stimulation cycles.

Materials and Methods

Study design and setting

This was a retrospective cohort study conducted at a tertiary care IVF centre after seeking ethical approval (EC approval number-EC/09/22/2129) from the Institutional Ethics Committee of the hospital. Data from 131 DuoStim cycles undertaken between January 2021 and September 2022 were included in the study. All patients gave written informed consent before entering the DuoStim cycles,

and all principles of the Helsinki Declaration (2013) were adhered to while conducting the study.

Patient population

DuoStim was offered to infertile women in the age group of 21–45 years with the following indications: (1) women with an expected poor response in view of poor ovarian reserve evaluated on the basis of Anti-Mullerian hormone (AMH) and antral follicle count (AFC) (AMH <1.2 ng/mL and AFC <5), (2) women with normal ovarian reserve (AMH ≥1.2 ng/mL and AFC ≥5) but an unexpected poor response with ≤9 oocytes retrieved and/or poor embryo conversion in previous cycle (s) and (3) DuoStim was also offered to women if an unexpected poor response was encountered in the ongoing follicular phase stimulation.

Patients who voluntarily withdrew from undergoing the second luteal phase stimulation after enrolling for DuoStim, due to either adequate embryos formed in the follicular phase or a desire to undergo a fresh embryo transfer (ET), were excluded from the study. In addition, patients with medical conditions warranting cessation of COS or oocyte retrieval in the luteal phase were also excluded.

Materials and methods

The follicular phase stimulation was started from day 2/3 of the menstrual cycle following the conventional antagonist protocol with a daily dose of 300–375 IU of recombinant follicle-stimulating hormone (rec-FSH) (Foligraf, Bharat Serums and Vaccines, India; Folisurge, Intas, India; or Gonal-F, Merck, Germany) based on the patients' anticipated response. GnRH antagonist 0.25 mg (Cetrotide, Merck, Germany) was started either in a fixed dose regimen from day 6 of stimulation after ultrasound monitoring and serum estradiol and luteinizing hormone (LH) assessment or later as per flexible dose regimen if the ovarian response appeared to be very poor. The gonadotropins were continued till the day when at least one follicle reached 17 mm in diameter, following which a final trigger was administered by GnRH agonist 0.2 mg (Decapeptyl, Ferring, Switzerland) and/or recombinant human chorionic gonadotropin (hCG) 250 µg (Ovitrelle, Merck, Germany) and oocyte retrieval was performed subsequently at 35 h post-trigger.

After 72 h of oocyte retrieval, rec-FSH was started in the luteal phase in the same doses as that of the follicular phase. Tablet medroxyprogesterone acetate (MPA) 10 mg (Meprate, Serum Institute, India) was also started once daily orally after 72 h of oocyte retrieval and was continued with the gonadotropin till the final trigger day. The first ultrasound monitoring in the luteal phase was done after 8 days of stimulation, and ovulation trigger was given with recombinant hCG and/or GnRH agonist and oocyte retrieval was scheduled 35 h later.

In both the phases, the cohort of oocyte cumulus complexes (OCCs) retrieved from the follicular fluid was transferred to GIVF culture media (Vitrolife, Sweden) at 37°C in 6% CO_2 and 5% O_2. OCCs were subjected to conventional IVF or intracytoplasmic sperm injection according to the centre's established protocol. Fertilization was checked the next morning at 16–18 h post-insemination or injection. Embryo culture was undertaken in sequential culture media (Vitrolife, Sweden) wherein 2pn embryos were cultured in groups of 6–8 embryos in 0.7 mL of G1P under oil (Vitrolife, Sweden) from day 1 to day 3 and G2P under oil (Vitrolife, Sweden) from day 3 to day 6. On day 5 or day 6 of culture, blastocysts were graded based on morphology using the Gardner and Schoolcraft scoring system, following which blastocysts with expansion grade 3 or higher were vitrified after removing the blastocoel fluid, using RapidVit Blast vitrification media (Vitrolife, Sweden) and the Cryotech device (Cryotech, Japan).

End points

The mean number of oocytes retrieved per stimulation cycle and the mean number of blastocysts utilized (or frozen) per stimulation cycle were set as the primary end points of the study. The secondary end points included mean fertilization rate, mean blastocyst utilisation rate, length of stimulation, and total dose of gonadotrophins used. The blastocyst utilisation rate was calculated by dividing the number of usable blastocysts frozen with the number of normally fertilised embryos for each patient. Then, the mean utilization rate was calculated by taking the mean of the blastocyst utilisation rate for each patient.

Statistical analysis

The minimum required sample size required to detect a significant change in the mean number of oocytes retrieved, from 1.7 in the follicular phase to a mean number of 3.5 in the luteal phase in the same cycle,[12] was calculated to be 54, with a two-sided alpha of 0.05 and power = 80%. The common standard deviation for both the phases was taken as 3 for the purpose of estimation. The final sample size was taken as 60 participants after rounding off and accounting for 10% loss to follow-up. The sample size estimation was done using Stata 16.1.

The data in the study were paired, i.e. the same patient was observed during both the follicular and luteal phases of DuoStim. A descriptive and inferential analysis was performed. For descriptive analysis, categorical

variables were expressed as a proportion (95% confidence interval [CI]) and continuous variables were presented as mean and standard deviation or median and range (minimum and maximum). Inferential analysis was done through paired *t*-test for comparing means and 95% CIs for different parameters amongst participants during the two phases of the study. McNemar's test was used to compare paired proportions. $P < 0.05$ was considered significant for all analyses. The analysis was conducted using R statistical environment 4.2.

Results

One hundred and thirty-one women with poor response were recruited to undergo DuoStim. Out of these, 13 women discontinued treatment after the first oocyte retrieval by choice and instead opted to undergo fresh ET due to the availability of enough embryos in the follicular phase itself. The remaining 118 women underwent dual stimulations in paired follicular and luteal phases of the same cycle coupled with blastocyst vitrification. Six patients recovered no oocytes after follicular phase stimulation while ten patients had luteal phase cancellations due to very poor response. The baseline characteristics of these women are listed in Table 1.

The embryological outcomes obtained in the follicular phase versus the luteal phase stimulation cycles are given in Table 2. The total number of oocytes retrieved in the follicular phase and luteal phase was 575 and 674, respectively. The mean number of oocytes retrieved per stimulation cycle was found to be significantly higher in the luteal phase as compared to the follicular phase (5.71 ± 3.95 vs. 4.87 ± 2.79, $P = 0.02$). Consequently, the mean number of 2pns obtained per stimulation cycle was also significantly higher in the luteal phase (3.7 ± 3 vs. 2.85 ± 2, $P = 0.001$) even though the fertilization rate per cycle was not significantly different between both the arms. The total number of blastocysts frozen (or utilized) in the follicular and luteal phases was 112 and 169, respectively, and the mean number of blastocysts frozen per cycle was found to be significantly higher in the luteal phase as compared to the follicular phase (1.43 ± 1.22 vs. 0.95 ± 1, $P < 0.001$). The mean blastocyst utilization rate per cycle showed a higher trend in the luteal phase (42.65 ± 30.92 vs. 35.32 ± 33.85, $P = 0.13$), but the difference was not statistically significant. The length of stimulation was found to be approximately 3 days longer in the luteal phase (12.63 ± 2.43 vs. 9.75 ± 1.85, $P = 0.001$) and consequently the total dose of gonadotrophin used was also significantly higher in the luteal phase (mean difference [95% CI]: 963.56 IU [806.52–1120.6 IU]; $P = 0.001$).

Table 1: Baseline characteristics of women undergoing DuoStim (*n*=118)

Parameters	Mean±SD
Age (years)	35.9±4.56
Median (min-max)	36 (26–43)
Duration of infertility (years)	4.3±3.4
Median (min–max)	3 (0.3–20)
Number of previous IVF cycles	0.66±0.93
Median (min–max)	0 (0–6)
BMI (kg/m^2)	26.4±4.53
Median (min–max)	25.7 (17.1–44.1)
AMH (ng/mL)	1.14±0.95
Median (min–max)	0.94 (0.14–6.8)
AFC	6.53±3.05
Median (min–max)	5 (3–14)

BMI=Body mass index, IVF=*In vitro* fertilization, AMH=Anti-Mullerian hormone, AFC=Antral follicle count, SD=Standard deviation

Next, patients were segregated based on their ovarian response in the luteal phase compared to that of the follicular phase stimulation. In 72 patients (61%), an increased number or equal number of eggs were retrieved in the luteal phase, whereas in the remaining 46 patients (39%), fewer eggs were retrieved. On comparing embryological outcomes in these patients who retrieved fewer eggs [Table 3], it was observed that despite the significantly lower number of eggs, the number of blastocysts utilized in the luteal phase was equivalent to that in the follicular phase (1.13 ± 1.15 and 0.87 ± 1, respectively, $P = 0.23$). This may have been observed because of an improved mean blastocyst utilization rate per retrieval in the luteal phase; however, the difference was not statistically significant.

Out of the total 118 patients included in the study, 111 patients fulfilled the POSEIDON criterion and were thus segregated into different POSEIDON groups, as given in Table 4. The remaining 7 patients underwent DuoStim due to poor embryo conversion in the previous cycles and therefore were excluded from further analysis. Interestingly, our data showed that while groups 1 and 2 (unexpected poor responders) showed a similar response in terms of the proportion of patients who produced utilizable blastocyst (s) in both the phases, in Poseidon groups 3 and 4, i.e. patients with an expected poor response, DuoStim appeared to be more effective with a significantly higher proportion of patients producing utilizable blastocyst (s) in the luteal phase compared to the follicular phase (73.2% vs. 53.7%, $P = 0.009$).

Overall, the luteal phase yielded utilizable blastocyst(s) in 82/111 (73.9%) patients, while in the follicular phase, usable blastocysts were formed in 64/111 (57.7%) cycles presented in Table 5. Hence, the odds of obtaining a

Table 2: Embryological outcomes in the paired follicular and luteal phase stimulation cycles (*n*=118)

Variable	Follicular phase	Luteal phase	Mean difference (95% CI)	*P*
	Mean±SD			
Number of eggs retrieved[a]	*n*=575 4.87±2.79	*n*=674 5.71±3.95	0.84 (0.12–1.56)	0.02*
Number of 2pns[a]	*n*=336 2.85±2.0	*n*=437 3.7±3	0.86 (0.28–1.43)	0.001*
Mean fertilization rate[a,c] (%)	60.07±27.74	66.18±25.67	5.87 (−0.43–12.18)	0.07
Number of utilized blastocysts[a]	*n*=112 0.95±1	*n*=169 1.43±1.22	0.48 (0.22–0.74)	0.001*
Mean blastocyst utilization rate[a,c] (%)	35.32±33.85	42.65±30.92	7.01 (−2.16–16.19)	0.13
Total dose of gonadotropin[a] (IU)	3088.98±855.92	4052.54±878.1	963.56 (806.52–1120.6)	0.001*
Days of stimulation[a]	9.75±1.85	12.63±2.43	2.87 (2.42–3.32)	0.001*

*Difference was considered significant when $P<0.05$, [a]Per stimulation cycle, [b]Cycles with no oocytes retrieved were excluded while calculating mean fertilization rate, [c]Cycles with no 2pns were excluded while calculating mean blastocyst utilization rate. SD=Standard deviation, CI=Confidence interval, IU=International units

Table 3: Embryological outcomes of patients with fewer number of eggs obtained in the luteal phase compared to the follicular phase stimulation cycles (*n*=46)

Variable	Follicular phase	Luteal phase	Mean difference (95% CI)	*P*
	Mean±SD			
Number of eggs retrieved[a]	*n*=299 6.5±2.55	*n*=174 3.78±2.92	−2.72 (−3.2–−2.23)	0.0003*
Number of 2pns[a]	*n*=176 3.83±2.1	*n*=108 2.35±1.96	−1.48 (−2.04–−0.91)	0.0001*
Mean fertilization rate[a,b] (%)	59.04±24.46	64.29±26.45	4.31 (−6.01–14.63)	0.40
Number of utilized blastocysts[a]	*n*=52 1.13±1.15	*n*=40 0.87±1	−0.26 (−0.7–0.17)	0.23
Mean blastocyst utilization rate[a,c] (%)	30.14±31.05	37.89±36.76	8.97 (−7.05–25)	0.26
Total dose of gonadotropin[a] (IU)	3030.98±765.11	4019±882.41	988.04 (750.37–1225.72)	0.001*
Days of stimulation[a]	9.74±1.88	12.35±2.09	2.61 (1.99–3.23)	0.001*

*Difference was considered significant when $P<0.05$, [a]Per stimulation cycle, [b]Cycles with no oocytes retrieved were excluded while calculating mean fertilization rate, [c]Cycles with no 2pns were excluded while calculating mean blastocyst utilization rate. SD=Standard deviation, CI=Confidence interval, IU=International units

Table 4: Number of patients with utilizable blastocysts in follicular versus luteal phase stimulation cycles in different POSEIDON groups (*n*=111)

	Patients with utilizable blastocyst (s) in follicular phase, *N/n* (%)	Patients with utilizable blastocyst (s) in luteal phase, *N/n* (%)	*P*
POSEIDON 1 (*n*=14) + POSEIDON 2 (*n*=15)	20/29 (69)	22/29 (75.9)	NS
POSEIDON 3 (*n*=28) + POSEIDON 4 (*n*=54)	44/82 (53.7)	60/82 (73.2)	0.009*

*Difference was considered significant when $P<0.05$. NS=Not significant

Table 5: Number of patients who produced utilizable blastocysts (positive) versus those who did not (negative) in the paired follicular and luteal phase stimulation cycles (*n*=111)

	Follicular phase (positive), *N/n* (%)	Follicular phase (negative), *N/n* (%)	Total luteal phase, *N/n* (%)
Luteal phase (positive)	50	32	82/111 (73.9)
Luteal phase (negative)	14	15	29/111 (25.4)
Total follicular phase	64/111 (57.7)	47/111 (42.3)	111

McNemar's test was used to compare the paired proportions, $P<0.05$ was considered to be statistically significant. Odds of producing utilizable blastocysts in luteal phase was significantly higher than that of the paired follicular phase (73.9% vs. 57.7%, $P=0.012$, OR: 2.286 (95% CI: 1.186–4.636). OR=Odds ratio, CI=Confidence interval

usable blastocyst in the luteal phase was found to be significantly higher than that of the paired follicular phase (73.9% vs. 57.7%, P = 0.012, odds ratio [OR]: 2.286 [95% CI: 1.186–4.636], McNemar's test). No blastocysts were obtained in both follicular and luteal phase stimulations in 15 (13.5%) patients.

Of note, the luteal phase stimulation was able to rescue 68% (32/47) of patients where no blastocysts were formed in the follicular phase. On average, the mean number of blastocysts obtained in the luteal phase for this subset of patients was 1.8 ± 1.2. On the other hand, there were 21.9% (14/64) of patients who failed to make any blastocyst in the luteal phase after making one or more blastocysts in the follicular phase.

Discussion

The present study sought to evaluate the efficacy of the DuoStim protocol by comparing the embryological outcomes in the follicular versus luteal phase of the same cycle. The matched case–control analysis showed that the mean number of oocytes retrieved per cycle and the mean number of blastocysts frozen per oocyte retrieval were significantly higher in the luteal phase as compared to the follicular phase. Furthermore, the luteal phase stimulation was able to rescue ~70% of cycles in which no embryos were formed in the follicular phase.

A significant increase was observed in the luteal phase in the mean number of oocytes retrieved (5.71 ± 3.95 vs. 4.87 ± 2.79, P = 0.02), mean number of 2pn embryos (3.7 ± 3 vs. 2.85 ± 2, P = 0.001) and mean number of blastocysts frozen per stimulation cycle (1.43 ± 1.22 vs. 0.95 ± 1, P = 0.001). However, the mean fertilization rate and the mean blastocyst utilization rate per retrieval were found to be comparable between both the phases suggesting similar competence of both cohorts of oocytes retrieved. Similar observations were made previously, where the luteal phase yielded equivalent or higher numbers of blastocysts compared to the follicular phase.[10,11,13,15-18] Furthermore, implantation potential and clinical outcomes in terms of clinical pregnancy rate and LBR were shown to be similar between embryos derived from both the phases.[14] These authors concluded that the addition of luteal stimulation in patients with poor reserve could increase the chances of achieving a pregnancy by significantly increasing the number of eggs and transferable embryos per menstrual cycle compared to follicular stimulation alone. It appears that the higher number of oocytes obtained in the luteal phase could be a result of recruitment of a more synchronised cohort of antral follicles at the start of luteal phase stimulation due to high levels of estradiol and GnRH antagonist administration during the stimulated follicular phase.[19,20] In addition, the continuous exposure to progesterone after follicular phase oocyte retrieval may further add to a better cohort formation of antral follicles. Higher levels of follicular estradiol may also sensitise FSH receptors in the granulosa cells of the new follicular cohort, therefore leading to a better luteal stimulation response.[21] Only one study, in poor responders, failed to show any significant improvement in the luteal phase.[22] However, as opposed to standard stimulation regimens, the strategy adopted in this study involved mild ovarian stimulation with clomiphene, letrozole and human menopausal gonadotropins and hence warrants further investigation.

We observed that ~42% (47/111) of POSEIDON poor responders failed to produce a transferable blastocyst after the first stimulation. However, the subsequent luteal phase stimulation was able to rescue 68% (32/47) of these cycles wherein the mean number of transferable blastocysts formed was almost two per stimulation. On the other hand, there were 21.9% (14/64) patients who failed to make any blastocyst in the luteal phase after making one or more blastocysts in the follicular phase. In total, at the end of one menstrual cycle, 96/111 (86.5%) patients had at least one transferable blastocyst available after one DuoStim cycle, thereby effectively reducing the proportion of cycles which failed to produce a utilizable embryo from 42% to 13% (15/111). Overall, our data demonstrated a higher likelihood of producing an utilizable blastocyst in the luteal phase as compared to the follicular phase (73.9% vs. 57.7%, P = 0.012, OR: 2.286, 95% CI: 1.186–4.636). Our results are in concordance with previous studies which also demonstrated that luteal phase stimulation added to the number of embryos in cycles where there was no embryo conversion in the follicular phase.[13,23]

Our results also showed that the length of stimulation in the paired luteal phase was longer than the follicular phase stimulation by almost 3 days (mean difference [95% CI]: 2.87 [2.42–3.32], P = 0.001) with a proportionate increase in gonadotropin dosage of approximately 1000 IU (4052.54 ± 878.1 vs. 3088.98 ± 855.92, P = 0.001) per luteal stimulation. A plausible explanation for this could be the fact that we started the second stimulation just 3 days after the first retrieval, whereas other groups began stimulation 5 days after the first pickup.[11,13,15] However we believe that a true luteal phase would be constituted within 3 days of egg collection. If we were to start stimulation after 5 days, especially after a GnRHa trigger, the patient would be highly likely to menstruate by the 5th–7th day, and then, the second stimulation could be simply considered equivalent to a second follicular

phase. However, we aimed to study the value of a true luteal phase stimulation. Hence, we decided to start gonadotropin along with MPA on day 3 after the first retrieval. A longer luteal stimulation before the second oocyte retrieval has been reported in a few other studies also.[13,24,25] However, compared to previous studies, our ovarian stimulation approach was unique since despite the use of the conventional antagonist protocol for follicular phase stimulation, we used MPA in place of GnRH antagonist for LH suppression in the luteal phase. This afforded a huge benefit in terms of reducing the number of injections and the associated cost by eliminating the need for administering antagonist during the second stimulation. This perhaps could be considered some compensation for the longer gonadotropin stimulation otherwise. Progesterone-primed ovarian stimulation (PPOS) was first reported by Kuang *et al*., to effectively suppress LH surge in ovarian stimulation cycles in freeze-all IVF cycles,[26] and has now become a popular protocol of COS in cycles where one does not plan a fresh transfer, such as donor cycles, oocyte/embryo cryopreservation for oncology or social reasons or cycles requiring pre-implantation genetic testing. However, PPOS has been used scarcely in DuoStim protocols to suppress LH surge in the luteal phase.[24] The use of PPOS for luteal phase stimulation in our study not only improved patient compliance by reducing the number of injections to 1/day, but it also eased out the need for frequent ultrasound monitoring and hormonal monitoring in the second phase. Gonadotropins were started 72 h after the first retrieval and given for 8 days before the first ultrasound monitoring was scheduled. This gap of 11 days after the first retrieval allowed most of the corpus lutea to regress before the first ultrasound was performed which minimised the interference in assessing the ovary for fresh follicles being recruited. Most importantly, PPOS helped in postponing menstruation and thus avoiding the risk of any infection on account of a retrieval during menstruation. None of the 118 women who underwent PPOS had menstrual bleeding while being stimulated in the luteal phase.

On the other hand, using the standard antagonist protocol in the follicular phase allowed the patient to have an option of a fresh transfer in case adequate embryos were available, especially in women who fall under POSEIDON groups 1 and 3 (<35 years) where even a single blastocyst may be sufficient to achieve a live birth. In our study, 9.1% (12/131) of women opted out of DuoStim for the same reason. This is also why most of our patients received a recombinant hCG or dual trigger instead of a GnRH agonist trigger in the follicular phase unlike other studies which only used a GnRH agonist trigger to ensure rapid luteolysis.

In this study, 111 out of 118 women who underwent DuoStim belonged to the POSEIDON group [Table 5]. It was interesting to note that in POSEIDON groups 3 and 4, i.e., patients with AMH <1.2 ng/mL and AFC <5, DuoStim proved to be more effective with a significantly higher proportion of patients producing utilizable blastocyst(s) in the luteal phase compared to the follicular phase (73.2% vs. 53.7%, $P = 0.009$). This brings forth the possibility that if only one COS must be planned, then possibly a luteal phase stimulation could favour a better outcome. A possible explanation for this would be a more synchronised cohort of the newly emerging follicles resulting from elevated progesterone levels immediately following natural ovulation or during early luteal phase stimulation that would cause enough suppression of the pituitary gonadotropins to not select follicles daily.

We have observed that in women with poor ovarian response, dropout rates from IVF treatment are likely to be higher after the failure of a single conventional follicular IVF-ET cycle, especially when no eggs or embryos are obtained. Accordingly, we recognised that patients perhaps found it psychologically less stressful to undergo two back-to-back stimulations within the same cycle with all embryos frozen. The fact that DuoStim was able to rescue 68% of cycles, in which no eggs or embryos were obtained in the follicular phase, showed that this strategy proved to be decisive for majority of patients who otherwise faced a risk of a potential dropout after the first failure.

One of the limitations of the DuoStim protocol was the significant lengthening of luteal stimulation, along with a definite possibility of cancellation of oocyte retrieval for some patients (~22%) due to no response. This has been a universal concern and thus measures are being tried to reduce the length of stimulation in the luteal phase. It is known that letrozole helps increase follicular sensitivity of FSH receptors by increasing concentrations of intra-ovarian androgen, thereby reducing the stimulation duration.[27] Therefore, the addition of letrozole to luteal stimulation, an approach that has been employed once previously,[12] could prove to be beneficial but needs to be further investigated for its clinical efficacy in poor responders (this work is in progress).

Conclusion

Our data demonstrate that in women with poor reserve, the addition of luteal stimulation could increase the chances of achieving a pregnancy by significantly increasing the number of eggs and transferable embryos per menstrual cycle compared to follicular stimulation alone. Furthermore, the luteal phase stimulation was

able to rescue ~ 70% of cycles in which no embryos were formed in the follicular phase. Our findings were especially significant since the DuoStim protocol showed a statistically significant increase in the proportion of patients producing utilizable blastocyst (s) who belonged to the POSEIDON 3 and 4 groups, i.e. true poor responders with low ovarian reserve. The addition of PPOS to the luteal stimulation made it patient-friendly and cost-effective, but we need to explore methods to further reduce the length of luteal phase stimulation. Our data prove that DuoStim is a promising protocol that could be introduced into routine clinical practice for women with low ovarian reserve and potentially help them realise their dream of having their own genetic offspring.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data are available on request due to privacy/ethical restriction.

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