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ORIGINAL ARTICLE

POSEIDON classification and the proposed treatment options for groups 1 and 2: time to revisit? A retrospective analysis of 1425 ART cycles

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STUDY QUESTION: Do live birth outcomes differ when Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number (POSEIDON) stratified groups are compared with women with good prognosis (non-POSEIDON group) undergoing ART?

SUMMARY ANSWER: The current study showed no significant difference in the live birth rates (LBRs) per embryo transfer between POSEIDON groups 1 and 2 when compared with women in the non-POSEIDON group undergoing ART.

WHAT IS KNOWN ALREADY: Recently, there has been a lot of focus on the POSEIDON classification for low prognosis women undergoing ART and various management options have been advocated. For POSEIDON groups 1 and 2, low starting dose and gonadotrophin receptor polymorphism have been suggested as possible reasons for a hyporesponse, and increasing the starting gonadotrophin dose, the addition of recombinant LH and dual stimulation have been suggested as treatment options. Most of these treatment options are hypothetical in nature and need validation.

STUDY DESIGN, SIZE, DURATION: In the current cohort study, a total of 1425 cycles were analyzed retrospectively following a single cycle fresh embryo transfer. The study period was from January 2013 to June 2018.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women undergoing ART at a tertiary level infertility clinic were included. Clinical and treatment-related details were obtained from the hospital's electronic medical records. The ART outcomes in a non-POSEIDON group (women with an adequate ovarian reserve *and/or* optimal ovarian response i.e. >9 oocytes retrieved in the previous ART cycle) and a low prognosis group stratified by POSEIDON criteria were compared. We also examined the effectiveness of the modifications made in the current ART treatment protocols among women with an adequate ovarian reserve who had a history of poor/suboptimal response (POSEIDON 1 and 2).

MAIN RESULTS AND THE ROLE OF CHANCE: There was no statistically significant difference in the LBR per embryo transfer in POSEIDON group 1 (32/109, 29%) and group 2 (17/58, 29%) when compared with the non-POSEIDON group (340/1041, 33%) (adjusted odds ratio (aOR) 0.69; 95% CI 0.37–1.27 and aOR 0.93, 95% CI 0.43–1.97, respectively), while significantly lower LBR were observed in POSEIDON groups 3 (17/97, 17.5%) and 4 (12/120, 10%) (aOR 0.49; 95% CI 0.28–0.89 and aOR 0.38, 95% CI 0.19–0.74, respectively). The gonadotrophin dose alone was increased in one-quarter of the cycles and in another 27% the dose was increased along with the protocol change among POSEIDON group 1. In POSEIDON group 2, a change in the dose alone and in combination with protocol change was performed in 5 and 41% of cycles, respectively.

LIMITATIONS, REASONS FOR CAUTION: A limitation of our study is the retrospective nature of the study with an inherent risk of unknown confounders influencing the outcomes. Other limitations are the lack of cumulative live birth data and the relatively small sample within POSEIDON group 2, which could lead to a type II error.

WIDER IMPLICATIONS OF THE FINDINGS: The current study showed no significant difference in the LBR between the POSEIDON groups 1 and 2 when compared with the non-POSEIDON group of women, while groups 3 and 4 had significantly lower LBR. The simple gonadotrophin/protocol changes in groups 1 and 2 resulted in LBRs comparable to women with good prognosis. These findings call for revisiting the proposed treatment strategies for POSEIDON groups 1 and 2.

STUDY FUNDING/COMPETING INTEREST(S): No funding was obtained. There are no competing interests to declare.

Key words: assisted reproduction / ovarian reserve / pregnancy / POSEIDON groups / live birth rates / low prognosis

WHAT DOES THIS MEAN FOR PATIENTS?

Differing definitions of 'poor responders' for women who need help to become pregnant makes it difficult to evaluate improved methods of fertility treatment. Recently, the POSEIDON (which stands for Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number) classification has been introduced to classify women with a low chance of success IVF into four groups based on age, egg count and response in the previous IVF treatment cycle. Treatment options, such as an increase in dose of injectable fertility drugs (the gonadotrophins LH and FSH), additional injections of drugs such as recombinant LH (an injectable fertility drug) and egg pooling have been advocated. Most of these proposed treatment options need further research to prove or disapprove their apparent beneficial effect. Some of these suggested strategies are not patient-friendly and, in addition, raise the treatment cost.

The current study examined whether the live birth rates (LBRs) were different between the POSEIDON groups (low chance of IVF success) and the non-POSEIDON group, which consisted of women with good chance of success in IVF. We found that the LBRs in POSEIDON groups 1 (women aged less than 35 years with a history of IVF with less than 10 eggs obtained) and 2 (women aged 35 years or more with a history of IVF with less than 10 eggs obtained) showed no significant difference when compared to women with good chance of success in IVF, while groups 3 (women aged less than 35 years with low egg count) and 4 (women aged 35 years or more with low egg count) had significantly lower LBRs. Simple modifications, such as an increase in dose of injectable fertility drugs and/or a change in treatment protocols, resulted in success rates following IVF in groups 1 and 2 that were comparable to women with good chance of success in IVF. Our results suggest there is a need to rethink the proposed treatment strategy for women in POSEIDON groups 1 and 2.

Introduction

Across the world, an increasing number of couples are postponing parenthood (Mills *et al.*, 2011). The mean age at which women are delivering their first baby is showing a rising trend (Balasch and Gratacós, 2012). A similar demographic shift has also been observed with an increase in the age at which women first visit a fertility clinic (de Graaff *et al.*, 2011). Due to age-related decline in fecundity, a higher prevalence of infertility is observed in women aged ≥35 years (Maheshwari *et al.*, 2008; Farquhar *et al.*, 2019). Infertile women in their mid-30s may need an expedited fertility treatment algorithm because of an age-related decline in oocyte quality and quantity, which may negatively affect their treatment outcomes. Many infertile women of advanced age or those with diminished ovarian reserve would eventually need ART treatment (Adamson *et al.*, 2018).

Women of advanced age or those with diminished ovarian reserve often end up having a poor ovarian response (development of fewer than four follicles) following standard ovarian stimulation and are commonly categorized as 'poor responders' (Ferraretti *et al.*, 2011). The live birth rate (LBR) after ART in poor responders is low and the cycle cancellation rate is high (Saldeen *et al.*, 2007). The burden of cycle cancellation and low LBR can be quite distressing for the couple and their treating clinician.

Lack of homogeneity in the definition of poor responders makes it difficult to evaluate the effectiveness of the proposed interventions. In an effort to reduce the heterogeneity in the definition of the poor responder, Bologna criteria were introduced (Ferraretti *et al.*, 2011). However, Bologna criteria were critiqued in view of the lack of homogeneity in the population described and for not addressing important factors such as the influence of age on oocyte quality (Younis *et al.*, 2015). Recently, the concept of poor ovarian response has been further refined by the introduction of a 'low prognosis' concept which identifies those women with a low probability of pregnancy following ART and stratifies them based on quantitative and qualitative parameters (Esteves *et al.*, 2018). Documented diminished ovarian reserve and unexpected suboptimal response to standard ovarian stimulation in women with an adequate ovarian reserve are the two different entities captured under low prognosis.

Renewed efforts were made, which led to the more detailed stratification of low prognosis women, and the Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number (POSEIDON) criteria were described, which allowed an individualized approach while treating women with low prognosis in order to improve the LBR (Alviggi *et al.*, 2016a). A study from China evaluated the ART outcomes in different POSEIDON groups and reported higher LBRs in groups 1 and 3 compared to groups 2 and 4 (Shi *et al.*, 2019). Recently, various treatment modalities have been proposed for women belonging to different POSEIDON groups but most of these options are hypothetical in nature and need validation. The proposed treatment options in groups 1 and 2 include increasing the starting dose of gonadotrophin and/or the addition of recombinant LH as well as the use of dual stimulation (duostim) to increase the oocyte yield (Sunkara *et al.*, 2020).

For POSEIDON groups 3 and 4, additional options of adding adjuvants and the use of dual triggers have been suggested (Haahr *et al.*, 2019; Polyzos and Drakopoulos, 2019).

Currently, there is a need to further validate the concept of prognosis-based stratification under POSEIDON classification in different subpopulations across the world owing to ethnicity-related differences in ART treatment outcomes (Armstrong and Plowden, 2012). We planned a study to compare the treatment outcomes following ART among various POSEIDON groups with low prognosis and compared them with women who did not come under the POSEIDON category (non-POSEIDON group). Furthermore, we also planned to investigate the effectiveness of the commonly employed modifications in the ART treatment protocols for women categorized under POSEIDON groups 1 and 2.

Materials and methods

Study population

The current study is a retrospective cohort analysis involving women undergoing fresh ART at a tertiary level infertility clinic between January 2013 and June 2018. Clinical and treatment-related details were obtained from the hospital's electronic medical records. All women undergoing fresh embryo transfer were included. In the POSEIDON groups 1 and 2, some included women had undergone a previous fresh ART cycle, and data from this cycle was also included in the analysis. We excluded women who underwent frozen embryo transfer. All the cycles that were cancelled before stimulation, and those with no oocytes retrieved or had no embryo available for transfer were also excluded. Only data from those women who allowed the use of anonymous data for retrospective studies and gave written informed consent were included in the current study. Ethics approval was obtained from the institutional review board.

ART protocol

Ovarian reserve was assessed by measuring the anti-mullerian hormone (AMH) level and/or antral follicle count (AFC) prior to initiating ART. Serum AMH levels were measured using an Electrochemiluminescence assay (Cobas e602 analyzer, Roche, Germany). The minimum detectable concentration by this assay was 0.01 ng/ml. The intra-assay coefficient of variation was <0.95. AFC was defined as the number of follicles of 2–9 mm in diameter in both ovaries, using transvaginal ultrasound (Voluson e series, GE healthcare, Chicago, IL, USA, probe frequency of 7.5 MHz).

Combined oral contraceptive pills were given prior to stimulation to all women for scheduling purposes. Controlled ovarian hyperstimulation (COH) was performed with recombinant gonadotrophins (Recagon, Merck Sharp & Dohme, NJ, USA and Gonal-F, Merck Serono, Switzerland) starting with a dose ranging between 100 IU and 450 IU based on age, BMI, ovarian reserve and previous cycle response. For some women with a sub-optimal follicular growth (rate of growth $\leq$ 1 mm per day), injectable hMG (Menopur, Ferring pharmaceuticals, NJ, USA) (75–150 IU daily) was added to the recombinant FSH during COH. The GnRH antagonist (flexible) was the most common protocol used. The GnRH antagonist (Ganirelix, Ferring pharmaceuticals, USA or Cetrorelix, Merck Serono, Netherlands) was administered subcutaneously at 0.25 mg per day starting from the day when the lead follicle reached a diameter of 12–13 mm and continued until the day of trigger. In the GnRH agonist long protocol, a daily dose of 0.5 mg of GnRH agonist (Lupride acetate, Sun pharmaceuticals, India) was administered from the mid-luteal phase of the previous cycle and was continued until the day of trigger. In the GnRH agonist ultra-long protocol, downregulation was achieved using between three and four doses of GnRH depot preparation (Lupride depot 3.75 mg, Sun pharmaceuticals, India) prior to initiating COH. In the GnRH agonist short protocol (flare), the GnRH agonist was started on day one of stimulation and continued until the day of the trigger.

Transvaginal oocyte retrieval was planned 35 h after hCG (recombinant hCG-ovitrelle, Merck Serono, Middlesex, UK or urinary hCG 5000 IU, Koragon, Ferring, Wittland, Germany) or GnRH agonist trigger. Luteal support was initiated on the day of oocyte retrieval with progesterone vaginal suppository 400 mg (Naturogest, Zydus healthcare limited, India) twice daily and parenteral progesterone (Gestone, Ferring pharmaceuticals, Switzerland) 100 mg twice weekly, until the pregnancy test (17–18 days after oocyte retrieval). The embryo transfer was carried out at either cleavage stage (Day 2, 3, or 4) or blastocyst stage (Day 5) and between one and three embryos were transferred depending upon the woman's age, number of previous cycles and embryo stage.

Definitions and stratification into groups

The definitions of ovarian reserve, ovarian response and POSEIDON criteria to stratify the low prognosis group have been published previously (Humaidan *et al.*, 2016). Briefly, '*adequate ovarian reserve*' was defined as AFC $\geq$5 *and/or* AMH $\geq$ 1.2 ng/ml. In situations where there was discordance between AFC and AMH level, the adequate ovarian reserve was defined based on AMH level. In women who underwent a previous ART cycle, '*optimal ovarian response*' was defined as the retrieval of more than nine oocytes.

The ART cycles were adjudicated to the '*non POSEIDON group*' if they had an adequate ovarian reserve *and/or* optimal ovarian response ($>$9 oocytes retrieved) in the previous ART cycle. In the current study, the non-POSEIDON population was considered the reference group. '*Low prognosis*' group was defined as a diminished ovarian reserve *or* suboptimal ovarian response ($\leq$9 oocytes retrieved) in the previous stimulation cycle (Humaidan *et al.*, 2016; Alviggi *et al.*, 2016a). Furthermore, a low prognosis group was stratified into POSEIDON groups 1 (women aged $<$ 35 years) or 2 (women aged $\geq$ 35 years) if they had an adequate ovarian reserve, but had an unexpected poor or suboptimal response to standard ovarian stimulation in the previous cycle. The POSEIDON groups 3 (women aged $<$35 years) and 4 (women aged $\geq$ 35) consisted of women with diminished ovarian reserve (Alviggi *et al.*, 2016a).

Outcomes

The primary outcome was *live birth rate (LBR)*, defined as a fetus showing any sign of life beyond 22 weeks gestational age. The LBR was expressed per fresh embryo transfer.

The secondary outcomes were *clinical pregnancy rate*, defined as pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs, and expressed per embryo transfer. The *multiple*

pregnancy rate is defined as multiple pregnancies (more than one gestational sac on ultrasonography) per clinical pregnancy. The *miscarriage rate* was defined as the spontaneous loss of a pregnancy before 22 completed weeks of gestational age. The miscarriage rate was expressed as miscarriage per clinical pregnancy. *Implantation rate* was defined as the number of gestational sacs observed divided by the number of embryos transferred. *Fertilization rate* was defined as the number of zygotes with two pronuclei divided by the number of mature oocytes subjected to fertilization (*in vitro* insemination/ICSI) (Zegers-Hochschild *et al.*, 2017).

We also examined the modifications made in current ART treatment protocols among women with an adequate ovarian reserve who had a history of poor/suboptimal response (POSEIDON 1 and 2) which led to the cancellation of the ART cycle or an unsuccessful attempt. As per clinician's discretion, either the gonadotrophin dosage was altered (mostly increased) or the protocol was changed (mostly antagonist to agonist) or in some cases no changes were instituted in the protocol/gonadotrophin dosage. Modifications suggested by the POSEIDON group, such as adding recombinant LH, dual trigger or dual stimulation, were not used.

Statistical analysis

Continuous variables are expressed as means ± SD and median with interquartile range (IQR), and compared with Student's *t*-test or Mann–Whitney *U* test. Categorical variables are summarized as frequency and percentage. Comparisons between more than two groups for continuous and categorical outcome variables were analyzed using ANOVA or Kruskal–Wallis test, and χ^2 test or Fisher's exact test, respectively. Multivariable analysis was performed by logistic regression analysis by entering clinically important variables associated with live births and the results were expressed as odds ratio (OR) with 95% CI. A two-sided *P*-value of less than 0.05 was considered statistically significant. Statistical analysis was carried out using SPSS (Ver. 21.0, IBM, Chicago, IL, USA) and STATA IC version 16 (StataCorp, College Station, TX, USA).

Results

Baseline and ART characteristics

A total of 2094 cycles were performed between January 2013 to June 2018, and among those 1425 ART cycles with fresh embryo transfer were eligible and analyzed (Fig. 1). The mean age of the overall cohort was 32.2 ± 4.3 years. The most common cause for infertility was combined factor (more than one cause of infertility; 31.8%) and GnRH antagonist protocol was the most commonly used (63.1%). The baseline characteristics of all the cohorts are summarized in Table I. There were significant differences in the indication and ART protocols used between the POSEIDON and non-POSEIDON cohorts, as shown in Table I.

The median total gonadotrophin dose used in the POSEIDON group 2 (2900, IQR 2100–3850), group 3 (3000, IQR 2450–3625) and group 4 (3300, IQR 2700–3975) was significantly higher compared to the non-POSEIDON group (2000, IQR 1500–2700). The majority of embryos were transferred at the cleavage stage (85.7%) (Table II). The median number of oocytes retrieved was not significantly different when non-POSEIDON group (6, IQR 4–9) was compared with POSEIDON group 1 (6, IQR 4–9) and group 2 (6, IQR, 3–8). It was significantly lower in POSEIDON group 3 (3, IQR 2–4) and group 4 (3, IQR 2–4) when compared with non-POSEIDON group. The median number of embryos transferred also was significantly lower in POSEIDON group 3 (2, IQR 1–2) and group 4 (2, IQR 1–2) versus the non-POSEIDON group (2, IQR 2–3).

Outcomes

The LBR per embryo transfer in POSEIDON group 1 (29.4%) and group 2 (29.3%) did not differ significantly when compared with non-POSEIDON group (32.7%) (Table III). However, the LBR per embryo transfer was significantly lower in group 3 (17.5%) and group 4 (10%) versus the reference group. Similarly, the clinical pregnancy rates in the POSEIDON group 1 (40.4%), group 2 (37.9%) were not significantly different when compared to the non-POSEIDON group (41.3%), while the POSEIDON group 3 (21.6%) and group 4 (16.7%) had significantly lower clinical pregnancy rates (Table III). There was no significant difference in miscarriage rates among the POSEIDON groups and the reference group. The multiple pregnancy rates in the non-POSEIDON group (32%), POSEIDON group 1 (36.3%), group 2 (31.8%), group 3 (52.3%) and group 4 (10%) did not differ significantly (Table III).

For the LBR, no significant association was observed with POSEIDON groups 1 and 2 (OR 0.85; 95% CI 0.55–1.32; $P = 0.483$ and OR 0.85, 95% CI 0.47–1.52; $P = 0.596$, respectively) compared to the non-POSEIDON group (Table IV). However, the POSEIDON groups 3 and 4 (OR 0.43; 95% CI 0.25–0.75; $P = 0.003$, and OR 0.22; 95% CI 0.12–0.42; $P < 0.001$, respectively) were associated with significantly lower LBR. After adjusting for important confounders (age, BMI, indication for ART, number of oocytes retrieved, number of embryos transferred, stage of embryo transferred and cycle number) no significant association was observed in POSEIDON groups 1 and 2 (adjusted OR (aOR) 0.69; 95% CI 0.37–1.27; $P = 0.232$ and aOR 0.93; 95% CI 0.43–1.97; $P = 0.847$, respectively), while the POSEIDON groups 3 and 4 were associated with a significantly lower LBR (aOR 0.49; 95% CI 0.28–0.89; $P = 0.019$ and aOR 0.38; 95% CI 0.19–0.74; $P = 0.005$, respectively) (Table IV).

Among various potential confounders, univariate and multivariate analysis showed a significant association of the number of embryos transferred with the LBR. We explored the impact of the number of embryos transferred on LBR in the combined POSEIDON groups 1 and 2 as well as groups 3 and 4. Within the combined population of POSEIDON groups 1 and 2, double embryo transfer was associated with an increase in LBR, although this was not statistically significant (*P*-value = 0.096), while the transfer of three embryos was associated with a significantly higher LBR compared to single embryo transfer (SET) (*P*-value = 0.050). However, transfer of either two or three embryos was not associated with increased LBR in the combined POSEIDON groups 3 and 4 (*P*-value = 0.531and 0.809, respectively) (Table V).

In POSEIDON groups 1 and 2, we analyzed the treatment modifications introduced in the current ART cycle and compared with the previous treatment cycle, which had a poor response/suboptimal response. In POSEIDON group 1, the dose alone was increased in one-quarter of the cycles and in another 27% the dose was increased

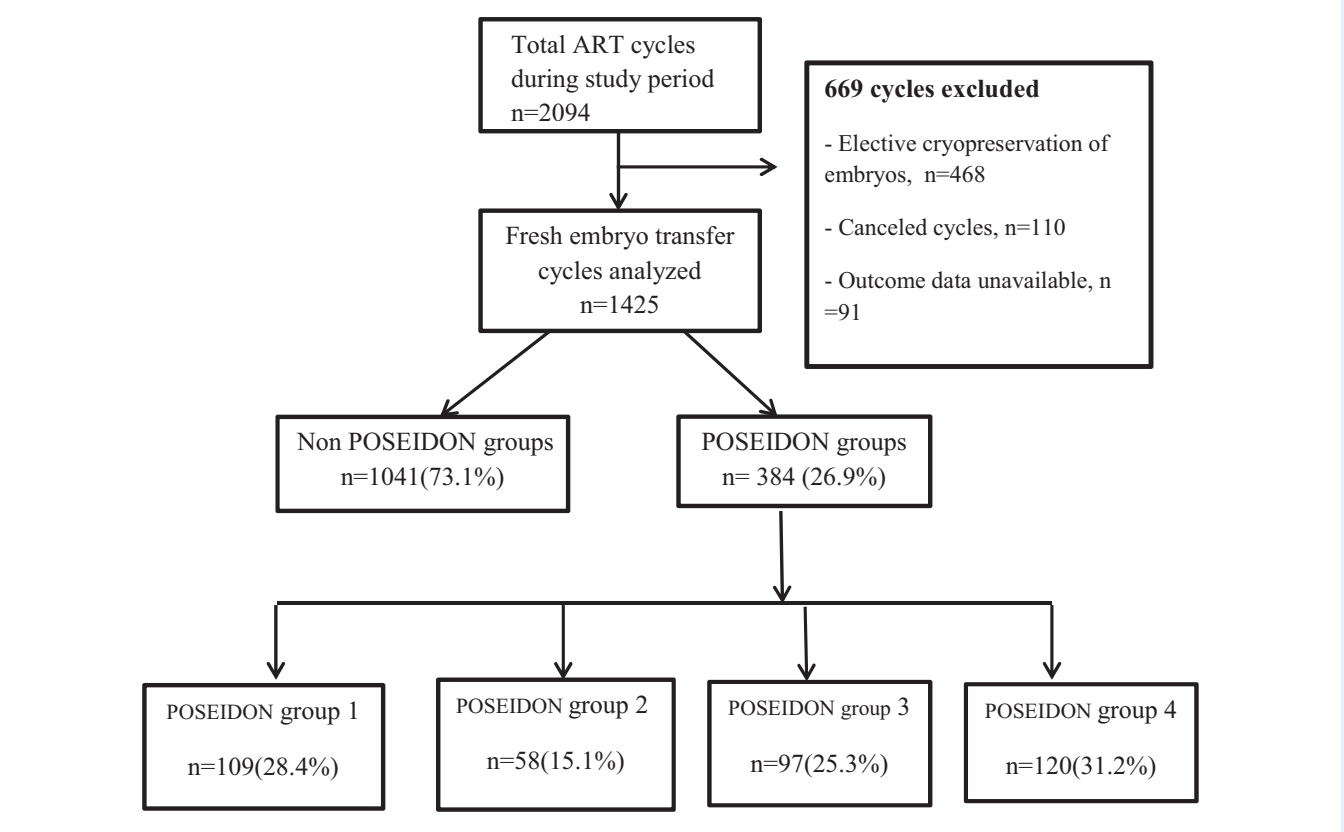

Figure 1. Flowchart of the women who underwent ART. POSEIDON, Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number.

along with a protocol change. In POSEIDON group 2, the dose alone and in combination with a protocol change was carried out in 5 and 41% of cycles, respectively (Supplementary Table SI). The modifications led to a median of one extra oocyte being obtained in both the groups in the current cycle.

Discussion

The current study found no significant differences in LBR per embryo transfer when POSEIDON groups 1 and 2 were compared with a non-POSEIDON group, which mainly consisted of women with a good prognosis. However, the LBR per embryo transfer was significantly lower in the POSEIDON groups 3 and 4 versus the non-POSEIDON group. The increase in gonadotrophin dosage with or without protocol change was the main modification in POSEIDON groups 1 and 2, which resulted in treatment outcomes that were comparable to the non-POSEIDON group.

A retrospective study (n = 18 455 cycles) compared the cumulative live births within the POSEIDON groups (Shi *et al.*, 2019). POSEIDON group 1 was considered the reference group in the study. Within the POSEIDON groups, the highest and the lowest cumulative LBRs were reported in groups 1 and 4, respectively, which is similar to the findings of the current study. Furthermore, these investigators also compared the ART outcomes of POSEIDON group 1 with women with adequate ovarian reserve (≥5 AFC) undergoing their first IVF or those with a previous optimal response (>9 retrieved oocytes): a significantly higher LBR for women with adequate ovarian reserve (≥5 AFC) was found following the first ART cycle compared to POSEIDON group 1. However, no significant difference in the LBR was noted when POSEIDON group 1 was compared with women undergoing ART with previous optimal ovarian response. In the current study, the non-POSEIDON group included a combined population of women with an adequate ovarian reserve and those with previous optimal response to standard ovarian stimulation and this could explain the partial disagreement between our study and that of Shi *et al.* (2019). The other possible reason for the disagreement could be differences in the reported outcomes (cumulative live birth versus live birth following a single cycle) and the difference in reference groups. Another retrospective study by Eftekhar *et al.* (2018) (n = 245) also compared the pregnancy outcomes among the four POSEIDON groups and reported a significantly higher clinical pregnancy rate and LBR in POSEIDON groups 1 and 2 compared to groups 3 and 4, which is in agreement with our study findings. The comparable treatment outcomes between POSEIDON groups 1 and 2 versus non-POSEIDON group was probably achieved in the current study owing to the presence of adequate ovarian reserve in groups 1 and 2, which would have resulted in a higher oocyte yield following an increase in

Table I Baseline characteristics for POSEIDON and non-POSEIDON cohorts.

	Non-POSEIDON (n = 1041)	POSEIDON 1 (n = 109)	POSEIDON 2 (n = 58)	POSEIDON 3 (n = 97)	POSEDON 4 (n = 120)	Total (n = 1425)	*P*-value†
Age (years)#	31.7 ± 4.2	30.9 ± 2.8	36.8 ± 1.9*	31.0 ± 2.8	37.9 ± 2.4*	32.2 ± 4.3	<0.001
BMI (kg/m^2)#	25.9 ± 4.2	25.8 ± 4.8	25.3 ± 4.9	25.7 ± 4.8	26.5 ± 4.4	25.9 ± 4.3	0.450
Infertility, n (%)							
Primary	751 (72.1)	66 (60.6)*	30 (51.7)*	65 (67.0)	69 (57.5)*	981 (68.8)	<0.001
Secondary	290 (27.9)	43 (39.4)	28 (48.3)	32 (33.0)	51 (42.5)	444 (31.2)	
Protocol, n (%)							
GnRH antagonist	726 (69.8)	63 (57.8)*	30 (51.7)*	38 (39.1)*	42 (35.0)*	899 (63.1)	<0.001
GnRH a long	143 (13.8)	19 (17.4)	8 (13.8)	10 (10.3)	7 (5.8)	187 (13.1)	
GnRH a ultralong	127 (12.2)	11 (10.1)	1 (1.7)	18 (18.6)	11 (9.2)	168 (11.8)	
GnRH a short	44 (4.2)	16 (14.7)	19 (32.8)	31 (32.0)	60 (50.0)	170 (11.9)	
Indication, n (%)							
Tubal	128 (12.3)	16 (14.7)	5 (8.6)	17 (17.5)*	15 (12.5)*	181 (12.7)	<0.001
Ovulation disorder	112 (10.8)	10 (9.2)	5 (8.6)	1 (1.0)	4 (3.3)	132 (9.3)	
Endometriosis	97 (9.3)	12 (11.0)	3 (5.2)	23 (23.7)	11 (9.3)	146 (10.3)	
Male factor	283 (27.2)	35 (32.1)	16 (27.6)	10 (10.3)	15 (12.7)	359 (25.2)	
Unexplained	108 (10.4)	5 (4.6)	5 (8.6)	15 (15.5)	20 (16.7)	153 (10.7)	
Combination	313 (30.0)	31 (28.4)	24 (41.4)	31 (32.0)	53 (44.9)	452 (31.8)	

Data are expressed as number of women (percentage) for categorical variables.
#Mean ± SD.
†One-way ANOVA for continuous variables and χ^2 test for categorical variables.
*Pair-wise comparison between Patient-Oriented Strategy Encompassing IndividualizeD Oocyte Number (POSEIDON) and non-POSEIDON group indicates significant difference using Bonferroni multiple comparison test.
GnRH a, GnRH agonist.

Table II ART treatment characteristics of POSEIDON and non-POSEIDON cohorts.

	Non-POSEIDON (n = 1041)	POSEIDON 1 (n = 109)	POSEIDON 2 (n = 58)	POSEIDON 3 (n = 97)	POSEIDON 4 (n = 120)	Total (n = 1425)	*P*-value†
Total Gn dose#	2000 (1500–2700)	2250 (1600–3000)	2900* (2100–3850)	3000* (2450–3625)	3300* (2700–3975)	2250 (1500–3000)	<0.001
Duration of stimulation#	10 (9–11)	10 (9–11)	10 (9–12)	10 (9–12)*	10 (8–11)*	10 (9–11)	<0.001
Semen sample (fresh ejaculate), n (%)	893 (87.0)	93 (86.9)	48 (84.2)	91 (94.8)	112 (93.3)	1237 (88.0)	0.180
Ovary response, n (%)@							
Optimal response (>9 oocytes retrieved)	251 (24.3)	19 (17.8)	11 (19.3)	1 (1.0)	2 (1.7)	284 (20.1)	<0.001
Poor/suboptimal response (≤9 oocytes retrieved)	781 (75.7)	88 (82.2)	46 (80.7)	96 (99.0)*	118 (98.3)*	1129 (79.9)	
Oocytes retrieved#	6 (4–9)	6 (4–9)	6 (3–8)	3 (2–4)*	3 (2–4)*	6 (3–9)	<0.001
Stage of embryo transferred@							
Cleavage, n (%)	856 (83.1)	95 (88.8)	49 (86.0)	93 (95.9)*	116 (96.7)*	1209 (85.7)	<0.001
Blastocyst, n (%)	174 (16.8)	12 (11.2)	8 (14)	4 (4.1)	4 (3.3)	202 (14.3)	
No of embryos transferred#	2 (2–3)	2 (2–3)	2 (2–3)	2 (1–2)*	2 (1–2)*	2 (2–3)	<0.001

Data are expressed as number of women (percentage) for categorical variables.
#Median (interquartile range) for continuous variables.
†Kruskal–Wallis test for continuous variables and χ^2 test for categorical variables.
*Pair-wise comparison between POSEIDON and non-POSEIDON groups indicates a significant difference using Bonferroni multiple comparison test.
@Analysed for available data, ovary response = 1413, embryo stage = 1411.
Gn, gonadotrophin.

Table III ART treatment outcome for POSEIDON and non-POSEIDON groups.

	Non-POSEIDON (n = 1041)	POSEIDON 1 (n = 109)	POSEIDON 2 (n = 58)	POSEIDON 3 (n = 97)	POSEIDON 4 (n = 120)	Total (n = 1425)	*P*-value†
Fertilization rate (%)	76.7	77.3	74.7	84.7*	77.7	77.3	0.004
Implantation rate (%)	27.3	26.2	23.0	19.2	11.4*	25.1	0.039
Clinical pregnancy rate, n (%)	430 (41.3)	44 (40.4)	22 (37.9)	21 (21.6)*	20 (16.7)*	537 (37.7)	<0.001
Miscarriage rate, n (%)	71 (16.5)	10 (22.7)	5 (22.7)	3 (14.3)	8 (40.0)*	97 (18.1)	0.137
Multiple pregnancy rate	138 (32.0)	16 (36.3)	7 (31.8)	11 (52.3)	2 (10.0)	174 (32.4)	0.142
Live birth rate per embryo transfer, n (%)	340 (32.7)	32 (29.4)	17 (29.3)	17 (17.5)*	12 (10.0)*	418 (29.3)	<0.001

Data are expressed as number of women (percentage) for categorical variables; mean and SD for continuous variable.
†One-way ANOVA and χ^2 test.
*Pair-wise comparison between POSEIDON and non-POSEIDON groups indicates a significant difference using Boneferroni multiple comparison test.

Table IV Logistic regression analysis based on the occurrence of live birth.

	No live birth (n = 1007) n (%)	Live birth (n = 418) n (%)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR* (95%CI)	*P*-value
Groups						
Non-POSEIDON	701 (69.6)	340 (81.3)	Reference			
POSEIDON 1	77 (7.7)	32 (7.7)	0.85 (0.55–1.32)	0.483	0.69 (0.37–1.27)	0.232
POSEIDON 2	41 (4.1)	17 (4.1)	0.85 (0.47–1.52)	0.596	0.93 (0.43–1.97)	0.847
POSEIDON 3	80 (7.9)	17 (4.1)	0.43 (0.25–0.75)	0.003	0.49 (0.28–0.89)	0.019
POSEIDON 4	108 (10.7)	12 (2.8)	0.22 (0.12–0.42)	<0.001	0.38 (0.19–0.74)	0.005

*Age, BMI, POSEIDON groups, indication for ART, number of oocytes retrieved, number of embryos transferred, stage of embryo transferred and cycle number were adjusted in the multivariate analysis.
OR, odds ratio.

Table V Logistic regression analysis based on the occurrence of live birth.

	No live births (n = 1007) n (%)	Live birth (n = 418) n (%)	Unadjusted OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value
Groups						
Non-POSEIDON	701 (69.6)	340 (81.3)	Reference			
POSEIDON 1 and 2	118 (11.7)	49 (11.7)	0.11 (0.01–0.81)	0.030	0.12 (0.02–0.97)	0.047
POSEIDON 3 and 4	188 (18.7)	29 (6.9)	0.29 (0.12–0.68)	0.004	0.31 (0.13–0.76)	0.010
Number of embryos transferred						
One	204 (20.5)	43 (10.5)	Reference			
Two	529 (53.0)	243 (59.4)	1.51 (0.99–2.30)	0.051	1.52 (0.99–2.35)	0.056
Three	265 (26.5)	123 (30.1)	1.49 (0.94–2.36)	0.086	1.48 (0.92–2.39)	0.108
POSEIDON groups and number of embryos transferred† (interaction)						
POSEIDON (1 and 2) and TWO embryos transferred	44 (4.4)	20 (4.9)	8.40 (1.02–68.98)	0.048	6.14 (0.72–52.1)	0.096
POSEIDON (1 and 2) and THREE embryos transferred	46 (4.6)	25 (6.1)	10.18 (1.24–83.48)	0.031	8.38 (1.00–70.1)	0.050
POSEIDON (3 and 4) and TWO embryos transferred	79 (7.9)	16 (3.9)	1.39 (0.49–3.91)	0.528	1.40 (0.49–4.0)	0.531
POSEIDON (3 and 4) and THREE embryos transferred	36 (3.6)	6 (1.5)	1.16 (0.33–4.05)	0.812	1.17 (0.33–4.14)	0.809

*Adjusted for age, BMI, indication for ART, number of mature oocytes retrieved, stage of embryo transferred and cycle number in the logistic regression analysis.
†Non-POSEIDON and single embryo transfer as reference group.

gonadotrophin dosage, translating into a higher LBR in the subsequent cycle (Sunkara et al., 2011; Pandian et al., 2013; Leijdekkers et al., 2019). The lower LBR in POSEIDON groups 3 and 4 can be explained by the diminished ovarian reserve and hence the decreased oocyte yield resulting in a lower number of embryos available for transfer (Sunkara et al., 2011; Cohen et al., 2018).

We evaluated the effectiveness of ART protocol modifications which were instituted in POSEIDON groups 1 and 2 as some of the proposed modifications for these groups are not 'patient-friendly' and are likely to increase the cost factor (addition of recombinant LH and duostim). In conventional ART, the gonadotrophin dose for the first treatment cycle is decided based on broad clinical factors, such as age, BMI, ovarian reserve, and presence of polycystic ovary morphology on ultrasound, and the majority of the women will have an optimal response (Olivennes et al., 2011). However, for a subset of the population who have a suboptimal or poor response after receiving the conventional gonadotrophin dose, whether the 'hyporesponse' is the result of inadequate gonadotrophin dose or an underlying 'pathological' cause is unclear. It is quite possible that this subset of women with hyporesponse was simply the outlier in terms of ovarian response (possibly caused by a higher FSH threshold) to standard gonadotrophin stimulation and may not represent any 'abnormal' condition. For women categorized under POSEIDON 1 and 2, low starting dose and gonadotrophin receptor polymorphism have been suggested as possible reasons (Perez Mayorga et al., 2000; Drakopoulos et al., 2018). The cause and effect relationship between FSH/LH polymorphism and hyporesponse is not established, but treatments such as the addition of recombinant LH have been advocated in these subgroups of women with unexpected hyporesponse (Alviggi et al., 2016b, 2018b; Conforti et al., 2019). In a systematic review by Alviggi et al. (2018a), the authors attempted to identify specific subgroups of women undergoing IVF who would benefit from the addition of recombinant LH. The authors reported the addition of recombinant LH resulted in higher oocyte yield and improved implantation rate in women with adequate ovarian reserve with an unexpected hyporesponse (four randomized controlled trials (RCTs)) (Alviggi et al., 2018a). The authors also identified another subgroup of women aged 36–39 years (10 RCTs) in whom they reported an increase in implantation rate but no impact on clinical pregnancy rate. However, the authors did not present a pooled estimate for any of the comparisons. According to a study by Genro et al. (2012), starting with a higher gonadotrophin dose would overcome the FSH receptor gene polymorphism-induced ovarian resistance, which would explain the reasonable LBR reported in the current study (Behre et al., 2005; Genro et al., 2012). The counter-intuitive approach of increasing the starting gonadotrophin dose, which is recommended by the POSEIDON group as well, appears to be beneficial with LBRs in groups 1 and 2 that were comparable to women with good prognosis as shown in the current study (Polyzos and Drakopoulos, 2019; Sunkara et al., 2020). There is a lack of high-quality evidence which indicates a higher prevalence of FSH/LH polymorphism among women categorized under POSEIDON groups 1 and 2. Furthermore, with simple protocol modifications, such as an increase in gonadotrophin dose in the subsequent ART cycle yielding reasonable LBR, the need for routine gonadotrophin polymorphism testing appears limited. The inclusion of women who underwent ART with 5–9 retrieved oocytes under POSEIDON groups 1 and 2 may have introduced heterogeneity in terms of prognosis (≤4 vs 5–9 retrieved oocytes). The decision to use a cutoff based upon retrieved oocyte number (i.e., nine, which distinguishes POSEIDON groups 1 and 2 and women within the non-POSEIDON group) needs further discussion as, practically, the prognosis for women with 8 versus 10 retrieved oocytes may not vary substantially.

We explored the impact of transferring multiple embryos on the primary outcome, LBR, within the combined POSEIDON groups 1 and 2 as well as groups 3 and 4. While SET is ideal and well supported by randomized trials, the evidence is mostly applicable to younger women with a good prognosis (Kamath et al., 2020). The treatment outcome following the transfer of multiple embryos among women with a low prognosis is less well studied. A study by Vega et al. (2016), (n = 7 13) evaluated the impact of the transfer of multiple embryos in women with poor prognosis (women with previous failed IVF cycles and no embryos available for cryopreservation). They reported that the transfer of multiple embryos showed an improved clinical pregnancy rate in women with poor prognosis (Vega et al., 2016). The authors suggested considering the transfer of multiple embryos in older women and those with poor prognosis to increase the likelihood of live birth. The current study findings suggest the transfer of two or three embryos versus SET was associated with a higher LBR following fresh ART in the combined POSEIDON groups 1 and 2, while in the combined POSEIDON groups 3 and 4 no such association was seen. Overall, based on the current evidence, the benefit of increased live birth is offset to some extent by the rise in multiple pregnancies (Kamath et al., 2020).

The current study is the first study evaluating the ART treatment outcomes in the POSEIDON groups among the South Asian population. Most of the previous studies have compared the POSEIDON group 1 with other groups, while in the current study the non-POSEIDON group consisting of women with good prognosis was the reference group, which perhaps helps in contextualizing the POSEIDON classification within the ART population. The current study is the first one to evaluate the effectiveness of conventionally employed modifications while treating POSEIDON groups 1 and 2. The additional findings related to the association of the number of embryos transferred and LBR need cautious interpretation as these findings are only applicable to a single fresh ART cycle outcome. A cumulative LBR as an outcome would be a more appropriate outcome to evaluate the effectiveness of different embryo transfer strategies in the ART population and the non-availability of such cumulative data was one of the limitations of our study (Smith et al., 2015). Another limitation is the retrospective nature of the study with an inherent risk of unknown confounders influencing the outcomes even though we have adjusted the results for potential confounders. The relatively small sample within POSEIDON group 2 could lead to a possible type II error. The inclusion of women with an adequate ovarian reserve who had lower oocyte yield (<10) during their first ART cycle in the non-POSEIDON group could have negatively influenced the LBR for the group and affected the comparisons with other POSEIDON groups.

Conclusion

The current study found no statistical difference in LBR per embryo transfer following ART between POSEIDON groups 1 and 2 and

women with a good prognosis, while the LBR were significantly lower in POSEIDON groups 3 and 4. Simple modifications, such as an increase in gonadotrophin dosage and/or a protocol change, resulted in treatment outcomes that were comparable between POSEIDON groups 1 and 2 and the non-POSEIDON group. The current findings need further investigation and call for revisiting the proposed treatment strategies for POSEIDON groups 1 and 2. There is an urgent need for robust evidence backed up by high-quality trials focusing on investigating the effectiveness of proposed interventions for POSEIDON groups before they can be advocated in routine ART practice.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

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Authors' roles

M.S.K. conceived the hypothesis. B.A. and C.P. performed the analysis. C.P. drafted the manuscript along with A.T.K. and M.S.K. M.S.K. and A.T.K. appraised the manuscript and participated in the discussion.

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Conflict of interest

None declared.

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